Ravindra Soni
Deep Chandra Suyal
Lourdes Morales-Oyervides
Mireille Fouillaud *Editors*

Current Status of Marine Water Microbiology

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Preface

In recent years, marine microbes are gaining much more attention and are being investigated exponentially in search of novel species and natural products. The novel microbes and natural bioactive compounds have a high demand in pharmaceuticals, food, cosmetics, textiles, and other industries. Moreover, the metabolites of marine microorganisms are also being investigated for their role in green chemistry and sustainable development. However, much of the research is still needed in this field as most of the marine microbes are not easily accessible due to the ocean depths and culturing limitations. If such microbes can be isolated from the deep ocean, then novel research will pave the way for drug discovery to treat those ailments that are not possible to date.

The present book is a novel attempt to compile several aspects of marine water microbiology including diverse habitats, associated microorganisms, their adaptations, ecological interactions, biogeochemical cycling, and industrial applications. It comprises 21 chapters that are arranged into four major parts, namely "General Considerations," "Communities of Special Interest," "Marine Microorganisms and Environmental Bioremediation," and "Others Applications and Perspectives." Each chapter includes in-depth knowledge of marine microorganisms along with the latest innovations, and technological advancements in the field. We have a firm belief that besides helping scientists, researchers, and students, this book will also be useful for those who are interested in marine environments and ecology.

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About the Editors

Ravindra Soni is an assistant professor in the Department of Agricultural Microbiology, I.G.K.V., Raipur, CG, India. He has more than 13 years of experience in teaching and research. He is well experienced in the fields of agricultural microbiology, microbial ecology, molecular biology and biotechnology, especially with reference to higher altitudes. He has published several research articles and chapters in well-reputed international journals and books. He also edited 8 books with international publishers.

Deep Chandra Suyal is an assistant professor in the Department of Science, Vidyadayini Institute of Science, Management and Technology, Bhopal, Madhya Pradesh, India. He has been actively engaged in research for 12 years and has experience in the fields of agricultural microbiology, molecular biology and biotechnology, and microbial ecology. He is currently investigating the genomics and proteomics of cold-adapted microorganisms. He has published several research articles, book chapters, and edited books with reputed international journals and publishers.

Lourdes Morales-Oyervides obtained her PhD in Engineering from University College Cork, Ireland (2015). Later on (2015–2016), she collaborated as a postdoctoral researcher at the Dairy Processing Technology Centre (Limerick, Ireland). Currently, she is a professor at the Autonomous University of Coahuila (México), and she is recognized as a member of the National System of Researchers (SNI) from CONACyT (México) since 2016. Her research interests are focused on the design, optimization, and scale-up of sustainable bioprocesses to produce food additives, enzymes, biopolymers, and other added-value compounds.

Mireille Fouillaud is a senior lecturer at the biology department of the University of La Reunion and a researcher in the Laboratory of Chemistry and Biotechnology of Natural Products (ChemBioPro). She graduated in 1990 as an industrial microbiology engineer. Following a PhD in Cell Biology and Microbiology from the University of Aix-Marseille (France), defended in 1994, she was recruited in the biology department of the Faculty of Sciences and Technology of the University of La Reunion. Between 2009 and 2020, she joined the food engineering department of ESIROI (Ecole supérieure d'Ingénieurs Réunion Océan Indien), where she taught biology and microbiology applied to agribusiness. In 2020, she joined the Faculty of Sciences of the University of La Réunion again to teach microbiology and project management. Her main research interests range from the microbial diversity of ecosystems and organisms to the biotechnological production of metabolites of interest to industries. Some years ago, she decided to focus on pigmented metabolites obtained from marine-derived filamentous fungi, with potential applications in foods or dyeing industries. She also leads research studies on actinomycetes producing specialized bioactive metabolites.

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Part I

General Considerations

1

Impact of Physical and Chemical Processes on Marine Environment

Navneet Kishore, Manjul Gondwal, Ravindra Soni,
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and Bhanu Pratap Singh Gautam

Abstract

The marine and coastal environment is a highly productive zone made up of several subsystems, including seagrass beds and coral reefs. It is a diverse habitat with a vast variety of living organisms that includes simple primitive to highly developed organisms. The famous marine ecosystems in which humans have invaded include Atlantic, Pacific, Indian, Arctic, and Antarctic Ocean basins. Since the origin of life on Earth, the seas have been responsible for preserving a healthy balance in the ecosystem of the entire planet. The waste products that are produced as a result of human activities eventually end the ocean ecosystem continuously. There are various chemical pollutants frequently drained in to the coastal and marine habitats. The ocean occupied a total area of about 360 million square kilometres, which is accounting for 71% of the Earth's surface area. The most significant means of commercial transportation for import and export is sea route. The ocean along with the ocean ecosystem is a very significant part of our everyday lives. It can be said that the ocean has become the most important part of people's life around the world. Hence, the state of the marine environment at the moment is not encouraging, and marine pollution has emerged as a major

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issue in modern civilisation. Moreover, the chemical and oil leakage incidents also affect the entire ecosystem. Thus, it is today's urgent need to recover the maritime environment.

Keywords

Chemical contaminants · Physical processes · Chemical processes · Anthropogenic contaminants · Microorganisms

1.1 Introduction

It is a great experience, if we were to stand on a beach that was free from disturbance, we could be able to observe the beautiful colour of the water, hear the wind and waves interacting with one another, and smell the salty air. Divers who spend time underwater have long been fascinated to the marine flora and fauna; nevertheless, those who do not dive but nonetheless appreciate the beauty of nature have also been drawn to these elements. Since the origin of life on Earth took place in the oceans, the seas have been responsible for preserving a healthy balance in the ecosystem of the entire planet and providing food for its inhabitants. Everyone desires to bequeath to future generations a sea that is not only beautiful but also healthy and abundant in its resources. All of the waste products that are produced as a result of human activities will eventually end up in the ocean, which is the original home of life on Earth. It is now common knowledge that human activity poses a hazard to the environment in the form of environmental contamination.

Chemical contaminants are common, and their presence can have an effect on the way an ecosystem works (McMahon et al. 2012). In the field of conservation research, pollution in general is one of the stressors that receives the least amount of attention, despite the fact that the risk of contamination is high and has been increasing over time (Crain et al. 2008; Lawler et al. 2006; Kumar et al. 2021). There is a large variety of commonly used chemical pollutants that can be found in coastal and marine habitats. Some examples of these pollutants are metals, herbicides, and hydrocarbons. However, a study of the impacts within different groups of contaminants may reveal various patterns of impact on the structure and functioning of the organism. However, the dangerous effects of chemicals tend to manifest themselves in certain ways. Although a similar synthesis has not yet been performed for the effects of chemicals on the functioning of ecosystems, Johnston and Roberts (2009) were able to perform one for the effects of contaminants on marine biodiversity. They found that harmful chemicals were associated with significant diversity losses (Hector and Bagchi 2007).

Humans are dependent, both socially and economically, on the services provided by ecosystems, such as the production of food, and on the regulatory functions provided by ecosystems, such as the maintenance of hydrological cycles (Liquete et al. 2013; TEEB 2010). According to Costanza et al. (2014), terrestrial ecosystems produce around US $12 trillion worth of goods and services annually. However, marine habitats, including those in the intertidal zone and open ocean, are thought to produce between US $14 and US $21 trillion (Harley et al. 2006). The provision and maintenance of these services are facilitated by the processes occurring within an ecosystem, which give rise to the movement of carbon, water, and mineral nutrients, as well as the exchange of energy and matter between different trophic levels and the surrounding environment. In order to ensure the continued provision of these services, it is imperative to conserve the biological processes responsible for their delivery.

1.2 Physical and Chemical Properties of Seawater

There are currently a significant number of marine species that are on the edge of extinction, and even their continued existence is uncertain. There is no way for us to know in advance whether or not other people will make the same mistake. The quantity of chemical compounds that are allowed to be present in marine waters has been subject to a predetermined cap that has been set. This decision was made with human safety in mind. Despite the fact that polychlorinated biphenyl (PCB) contamination is higher in the terrestrial environment than in the marine environment, the typical amounts of PCBs found in human and Orcinus orca lipids are approximately one and five hundred parts per million (ppm), respectively. Cetaceans inherit an inability to produce P450, which results in a significant buildup of PCBs in their bodies. However, the maximum allowable amount should be stated while also taking into account the overall risk to the ecosystem. This is because the biological buildup of chemical compounds is another major concern for our environmental management, and the catalytic activity of organisms varies depending on the species (Yokota and Matranga 2006).

1.2.1 Chemical Processes

It should come as no surprise that water plays a significant role in sedimentary environments. In addition to its role as a physical medium for physicochemical reactions that take place within pore fluids (the water that is contained within the pore spaces of sediment), it also plays the role of a medium for ecological processes and primary geochemical processes that ultimately result in the direct precipitation of mineral matter (e.g. evaporites).

1.2.1.1 A Eh and pH Defined

An efficient method of illustrating the effects of oxidation–reduction potential, acid and base, complexing ligands, temperature, and pressure for an aqueous system is an Eh-pH diagram, often known as a Pourbaix diagram. It has applications in a variety of scientific disciplines, including corrosion science, geo- and solution chemistry, hydro- and electrometallurgy, and (Huang 2016) Geochemists continue to use Eh-pH diagrams as one of their primary tools for understanding naturally occurring water-bearing systems. It is generally known how to create these diagrams, but as additional thermodynamic data becomes available, the diagrams' visual representations evolve. For any project, care should be taken to build fresh figures using high-quality, current thermodynamic data. The application and understanding of Eh-pH graphs are discussed in this entry.

Eh-pH diagrams are used often in a variety of contexts, including those in which an aqueous system is impacted by oxidation–reduction and/or acid–base processes, ligand complexation, temperature, or pressure (Lin et al. 2021).

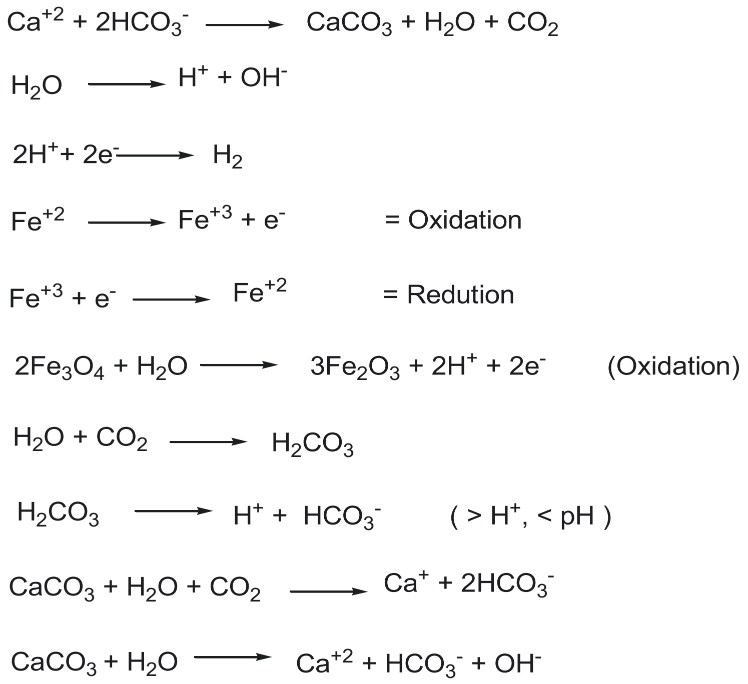
Acidity and alkalinity are defined by pH, which is the "activity" of hydrogen ions in a solution.

- An ion's "activity" is essentially the same as its effective concentration in solution.
- A pure substance, such as water or a solid mineral, has an activity of 1.0 by definition.
- Activity and concentration are more closely correlated the lower the overall ion concentration in the solution is.
- The activity of a given ion is typically higher than its concentration when the concentration of dissolved ions increases, for example, in saltwater.
- Activity explains how other electrostatically charged ions in solution interfere with certain ions' ability to react.
- Common ion activity coefficients found in "typical" seawater Cl^- is equal to 0.63, Mg^{2+} is 0.25, SO_4^- is 0.068, and CO_3^- is 0.021.
- pH is equal to the negative log base 10 of a solution's hydrogen ion activity:
- H activity = 10^{-4} pH = −Log 10 (10^{-4}) = 4 (acidic).
- H activity equals 10^{-14}. pH = −Log 10 (10^{-14}) = 14 (basic).
- Oxidation–reduction potential is how "Redox" potential is defined. A gauge of a geochemical system's capacity to support, respectively, oxidation and/or reduction reactions. The process of oxidation involves chemical processes in which the interacting elements lose orbital electrons, increasing their valence numbers, shown in Fig. 1.1.
- Reduction: Chemical processes in which the involved elements gain orbital electrons, resulting in "lower" valence numbers.

Eh is measured as a solution's electron concentration, hence Eh is also measured as a solution's "potential" (i.e. volts) across a standard hydrogen electrode.

1.2.2 Miscellaneous Chemical Reactions and Mineral Formation

Phosphorite (calcium carbonate-phosphate, or apatite), and manganese nodules are examples of marine chemical products with unknown origins (manganese oxides). Phosphorite, also known as calcium carbonate-phosphate or apatite, and Manganese Nodules are marinechemical compounds whose origins remain uncertain, specifically in the case of Manganese Oxides. This phenomenon is associated with periods



$$Ca^{+2} + 2HCO_3^- \longrightarrow CaCO_3 + H_2O + CO_2$$

$$H_2O \longrightarrow H^+ + OH^-$$

$$2H^+ + 2e^- \longrightarrow H_2$$

$$Fe^{+2} \longrightarrow Fe^{+3} + e^- \qquad = \text{Oxidation}$$

$$Fe^{+3} + e^- \longrightarrow Fe^{+2} \qquad = \text{Redution}$$

$$2Fe_3O_4 + H_2O \longrightarrow 3Fe_2O_3 + 2H^+ + 2e^- \qquad (\text{Oxidation})$$

$$H_2O + CO_2 \longrightarrow H_2CO_3$$

$$H_2CO_3 \longrightarrow H^+ + HCO_3^- \qquad (> H^+, < pH)$$

$$CaCO_3 + H_2O + CO_2 \longrightarrow Ca^+ + 2HCO_3^-$$

$$CaCO_3 + H_2O \longrightarrow Ca^{+2} + HCO_3^- + OH^-$$

Fig. 1.1 Oxidation and reduction of metals in marine system

characterized by significantly reduced rates of terrigenous sedimentationand calm water conditions. Clastic deposits generally impede the process of chemical precipitation.

(a) It is believed that phosphates are linked to marine circulation patterns and the “upwelling” of cold, nutrient-rich bottom waters that are abundant in phosphate ions (PO_4^{2-}).
(b) Manganese nodules discovered in lacustrine and marine environments. Origin might be connected to volcanic or microbiological activity beneath the surface.

Mn-Fe oxides precipitate around a grain nucleus, building up into a nodule shape with concentric internal “growth” structures. The precipitation mechanism involves oxidising Mn^{2+} to Mn^{4+}.

1.3 Main Factor Responsible for the Physiochemical Process

Pollutants made by chemicals are everywhere around us, and their presence can alter the way an ecosystem works (McMahon et al. 2012). In the field of conservation science, pollution is typically one of the stressors that receives the least amount of attention, these pollutants come from the widespread use of chemicals. Because there are so many different substances that are now in use, it is hard to predict the effects that will be caused by any one pollutant. However, each chemical has its own specific mode of harmful action, and by comparing the effects of many classes of pollutants, it may be feasible to identify distinct patterns in the way that these pollutants damage the structure and functionality of the organisms they come into contact with.

1.3.1 Transport of Pollutants

In aquatic systems, pollutants are distributed through advection and mixing. Although eddy diffusion and molecule-level mixing both take place in real systems, eddy diffusion is the main topic of attention. The efficient dispersion of contaminants from a wastewater outfall into a lake, coastal area, or estuary depends on mixing. High departure velocities, coastal currents, or river flow velocities can help in dispersal. The establishment of thermal fronts, which are especially common during the spring warming period in near shore regions lakes, may prevent horizontal mixing in lakes (Göransson et al. 2021). When nearshore water that has been warmed to a higher temperature meets lake water that has been chilled to 4 °C to form a vertical barrier of 4 °C water, the thermal front is created. As spring warming advances, this barrier extends into the lake and loses some of its distinctiveness (Sharma and Ahmad 2014).

Both surface waters and groundwaters may include dissolved or particulate forms of pollutants that can travel down the water column. In surface waterways, soil particles may be carried downstream in bed-load transport by rolling, sliding, or saltation. Alternatively, they can be deposited in streams and carried downstream in particulate form. This transport is influenced by the flow rate, the turbulence, as well as grain parameters such as size, shape, and density. Particulate transport in groundwaters is less apparent and only occurs for particles with extremely tiny grain sizes. The movement of pollutants that are dissolved in water is very important because it is intimately connected to more easily accessible types of contaminants that (Heyman et al. 2016) provide a greater risk to the environment. In addition to this, by doing so, poisons are able to spread farther away from their point of origin, perhaps reaching other sites or environmental compartments. Leaching processes, also known as the process by which pollutants are released from the solid phase into the liquid phase under the effect of their dissolution and desorption from their support phases, are the primary mechanisms that are implicated in this phenomenon. This is dependent on a number of variables, including soil pH, redox conditions, biotic activity, and the amount of water penetrating the soil, which will carry the contaminants to surface or groundwater repositories, as was previously indicated. Soil pH, redox conditions, and biotic activity are all examples of these variables. The aerobic conditions of surface waters and the anaerobic conditions of groundwaters have the potential to have a considerable impact on the dissolved transport. This influence may ultimately result in the precipitation of pollutants as a result of changes in the redox state.

1.3.2 Sedimentation Processes

In lakes or seas, sediments often act as a nearly ultimate deposit for particle-associated contaminants. This suggests that sediments may still be polluted from previous discharges even after contamination from diverse point and nonpoint sources has been reduced. Pollutants in the sediments may be discharged back into the water column under the right conditions, endangering both people and the biota. Clean sediment may build up on top of polluted material when the rate of sedimentation is rapid and sediment mixing is minimal, thereby isolating the contamination. If natural degradation of organic pollutants, such as polychlorinated biphenyls (PCBs), is conceivable and water depth for navigation is not a concern, the situation is often left unchecked as it is assumed that natural cleaning has already taken place. If not, some kind of corrective action may be needed, such as capping with clean sediment, injecting oxidizers, or performing hydraulic dredging and land disposal. Due to the recalcitrant nature of buried pollutants and the expense associated with cleaning up contaminated sites, sediment pollution can be quite challenging to manage. However, the presence of these contaminants can also present a chance to identify sources through geochronology, chemical mass balance modelling, and related methodologies. Evaluation of the effects of contaminants released into the environment as a consequence of the industrial revolution has been especially fruitful when using sediment dating based on ^{210}Pb and ^{137}Cs (Chapman and Wang 2001).

Marine sediments are classified and arranged into four main categories based on the origin, size, and location of their grain origins. Lithogenous, biogenous, hydrogenous, and cosmogenous are the four categories. All of them are distinct from one another in various ways, yet they all have a propensity to accumulate along the ocean bottom as a result of several natural processes including weathering, erosion, and collision.

1. **Lithogenous sediments** are made up of tiny fragments of weathered rocks and oceanic volcanoes and are created by the weathering process. They often come into being when silicate and metal ions bind. Terrigenous and “red clay” are two distinct forms of lithogenous deposits, and their differences stem from the processes that gave rise to them.
2. **Biogenous sediments** are made up of things such as bones and teeth that cannot dissolve in water. In many places with shallow water, most of these sediments are made up of corals and shells or pieces of shelled sea creatures.
3. **Hydrogenous sediments** are created when minerals in the ocean’s water precipitate or when the ocean’s water reacts chemically with sediments already present on the ocean bottom to create a new mineral. Because of the interactions that occur, this sedimentary process is intriguing chemically. For instance, dissolved ions may be found in the water of the seas on Earth. The region might

become saturated with salt residues from this process when evaporation takes place and significant volumes of these ions are left behind.

4. **Cosmogenous sands** are extraterrestrial in origin and resemble small meteorites in most cases. These deposits are the byproducts of major space debris strikes (such as comets and asteroids). As one would expect, they are not very common to discover and are made of silicates and combinations of various metals.

1.3.3 Atmospheric Interactions

Many of the chemicals that are found in water come from the atmosphere. Water partially dissolves all of the elements that make up air, including carbon dioxide (CO_2), nitrogen (N_2), oxygen (O_2), and other minor elements. Water quality, aquatic life, and a number of chemical processes that take place in water depend on dissolved oxygen (DO). Although certain gases, such as N_2 and Ar, are inert and do not interact with water, others, including CO_2, SO_2, and NO_x, may change the properties of the water they come into contact with. In addition, the atmosphere serves as a significant long-distance transporter for chemical contaminants that are volatile and semi-volatile in nature. For an overview of the subject, see Wei and Li (2010). Atmospheric pollutants may exist as gaseous molecules or as attachments to different particulate matter dispersed in the air. They may get into aquatic habitats through partitioning from the gas phase, wet or dry deposition of the particle, or other methods. However, certain hydrophobic organic contaminants (HOCs) from lakes (Nelson et al. 1998) or estuaries are heavily eliminated by volatilisation to air. Because the Henry's law constant is larger in the summer and favours the gas phase, volatilization is more prominent.

1.3.4 Chemical Pollutants

High NO_x levels in the atmosphere have been shown to sometimes cause aquatic contamination. For instance, it has been estimated that up to 35% of the nutritional nitrogen imports into Chesapeake Bay originate from the atmosphere (Howarth 2008). This is an important input as nitrogen is a scarce resource in Chesapeake Bay. Except for algae that fix nitrogen, naturally occurring nitrogen in the atmosphere exists as the inert gas N_2, hence the NO_x pollution from automobiles in the Washington, DC, and Baltimore region is the issue. Similar atmospheric nitrogen fluxes are seen in other eastern estuaries. Small, inadequately buffered seepage lakes in the eastern United States are particularly acidic due to the deposition of NO_x and sulphate. However, this issue seems to have become less significant with the development of superior sulphur reduction equipment for coal-fired power plants. Although atmospheric HOCs and metals have a higher effect on interior lakes that are phosphorus-restricted, nitrogen imports to coastal estuaries are a serious problem. According to Howarth (2008), the atmospheric contribution to Cd, Cu, and Mn in the mid-Atlantic bight is below 50%. However, the majority of Ni, Zn, Fe, Al, and

Pb in the same region are obtained from atmospheric sources. Significant quantities of mercury are deposited into inland lakes in Wisconsin from atmospheric sources. Mercury is present in fly ash derived from coal-fired power plants.

1.3.5 Pathogens

Both drinking water and recreational waterways should be free of pathogens. Bacteria, protozoa, and viruses are the three main types of pathogenic microorganisms. The infectious dosage for bacteria, such as toxic *Escherichia coli* or campylobacter, may be quite high, up to 106 or more, as can be shown in Table 8.1. In contrast, viruses and the protozoa *Giardia lamblia* or *Cryptosporidium*, which may sometimes be found in water treatment facilities, only need to be present in extremely small quantities to cause illness. It is obvious that knowledge of survival tactics used inside the water distribution system is required to appropriately estimate the hazards from waterborne illness. An organism that is both viable and culturable (VNC) is more likely to transmit illness than one that is neither viable nor culturable. Salmonella, shigella, toxigenic *Escherichia coli*, and campylobacter are the most prevalent of the bacteria on the list (Dash et al. 2021). *Giardia lamblia* and *Cryptosporidium parvum* are the two most common protozoa. In many respects, the group of organisms responsible for transmitting aquatic diseases, viruses, is the least known. Despite the minimal infectious dosage, there are a lot of individuals that are impacted (6,500,000). Hepatitis A, rotaviruses, and Norwalk or Norwalk-like viruses are a few of the most prevalent viruses found in water. Numerous poliovirus varieties, coxsackieviruses, echoviruses, reoviruses, adenoviruses, and hepatitis E viruses are among the other watery viruses.

1.3.6 Metals

As a consequence of air inputs, metal contamination of sediment occurs in remote places (Hermanson 1993; Lockhart et al. 1993), it also happens in industrialised areas, as shown, for instance, by Cahill and Unger (1993) and Tuncer et al. (1993). The generation of hydrogen ions, or acidic circumstances, may result from the oxidation of sulphur, iron, and nitrogen. This is significant because pH levels may decrease during dredging operations to remove polluted anaerobic silt from ports and ship canals, potentially leading to the discharge of metals like Cd and Zn (Calmano et al. 1993). The biota may suffer from both a drop in pH and an increase in the concentration of dissolved metals. In aquatic systems, buffers, notably $CaCO_3$, Al_2O_3, and Fe_2O_3, help to control pH changes. However, pH decreases may be substantial when dredged material is dumped on land. The organic-sulphide phase (OSP) often drops during oxidation, whereas the proportions of the easily reducible phase (ERP) and moderately reducible phase (MRP) frequently rise.

1.3.7 Tracers

Tracers are helpful in identifying the channels and patterns of pollution dispersion in natural streams. For instance, bacteria in sewage treatment plant effluent may prompt authorities to restrict beaches. When there are several potential contributing outfalls, it is preferable to identify the infections' most probable source and the extent of the potential harm. Due to their sporadic appearance and challenging quantification, assessing the amounts of pathogens in the outfall plume may not be the optimal strategy for this goal. Instead, another unique component of the effluent, such as turbidity or chloride, may be used to identify outfall plumes. As an alternative, the effluent may be mixed with a synthetic tracer, such as a regular or fluorescent dye, such as rhodamine. Following that, the level of this tracer is determined either via field sampling or remote sensing. This will provide a snapshot of the plume in a transient or steady state and show any compounds in the effluent that could have an effect, such as pathogens, whose movement is comparable to that of the tracer. A tracer should have the following desirable characteristics: (a) it should be simple to detect, especially at low concentrations; (b) it should be chemically inert, with no precipitation or chemical reactions; and (c) it should be non-hazardous with little to no radioactivity, if any, and short-lived. The Milwaukee Cryptosporidium outbreak is an example of a pathogen in sewage effluent or urban runoff getting into the drinking water supply (Fox and Lytle 1994; Christensen et al. 1997). In a significant portion of the population, the parasite protozoan cryptosporidium caused the gastrointestinal disease known as cryptosporidiosis. Over 400,000 people were impacted, with 100 deaths mostly among immune-compromised individuals. In this instance, the two water intakes, notably the southern one for the Howard Avenue Filtration Facility, show some overlap with the outfall plume from the river flows and the Jones Island wastewater treatment plant. The Howard Avenue intake pipe has been expanded by 1.3 km (0.8 miles), dual media filters are being installed in lieu of sand filters, and ozone disinfection is being added to chlorination to enhance the quality of the intake water. The pollutant plume was calculated for the design of the intake pipe extension using tracer investigations, two-dimensional hydrodynamic modelling, and water quality modelling.

1.4 Sea-Based Activities Resulting in the Release of Contaminants

The majority of anthropogenic pollutants enter the marine environment directly from land-based sources; nevertheless, there are instances in which anthropogenic contaminants are either released or re-mobilised inside the marine environment itself.

1.4.1 Accidental Spillage

Hazardous and noxious substances (HNS) are defined as any substance other than oil that, if introduced into the marine environment, is likely to pose risks to human health, damage amenities, harm living resources and other marine life, or interfere with other legal uses of the sea. Shipping is the most important mode of transportation for a significant number of HNS (IMO 2000). The seaborne commerce in chemicals is predicted to exceed 215 million tonnes by 2015, with an estimated 2000 distinct chemicals used by humans being routinely carried by sea, either in bulk or packaged form (Parnell et al. 2008).

1.4.2 Emissions from Antifouling Paints

Antifouling paints have typically used biocides to stop the development of potential fouling organisms since the build-up of organisms on ship hulls may decrease performance and increase fuel consumption. However, the biocide discharge from a ship's hull may be detrimental to species that are not the intended targets. Organotin tributyltin (TBT), which has an endocrine-disrupting impact, is a long-used active ingredient in antifouling paints that is especially harmful to shellfish (Dafforn et al. 2011). TBT paints are no longer allowed to be used globally, although their effects on particular specific European coastal habitats remain of concern (Sousa et al. 2009). In many antifouling coatings, biocides such as copper (I) salts, primarily in the form of copper oxide (Cu_2O) and copper thiocyanate (CuCHNS), have become the primary substitutes for TBT. According to OSPAR (2010a), copper losses at sea from coatings of moving ships are regarded as a substantial and growing anthropogenic source of copper to the aquatic environment. Copper is a danger to the marine ecology while being less toxic than TBT and having the potential to harm species at concentrations beyond those required for physiology (Karlsson et al. 2010; Ytreberg et al. 2010).

1.4.3 Mariculture

The European Commission intends to encourage the expansion of the aquaculture business as a method of satisfying the growing demand for seafood in the foreseeable future, as well as a potential source of jobs and economic growth (EC 2012). Even though substantial headway has been achieved to enhance the environmental performance of aquaculture, the fast development of this industry may still have a major influence on the ecosystem in the surrounding area. Antibiotics are used to manage illness, pesticides are used to control parasites and algae, and antifoulants are used to prevent algae from growing on boats. Farmers utilize a broad variety of chemicals to boost production and growth (Guardiola et al. 2012). The intense production of finfish, primarily salmonids, as well as sea bass and sea bream, is related with the largest levels of chemicals used in aquaculture. These are also the areas of the industry that have witnessed the fastest growth rate over the last few years.

1.4.4 Medicinal Products

To preserve animal health, fish producers need to have access to a range of legally permitted medications. In Europe, only 14 pharmaceuticals are completely authorized and permitted for use in marine farming (amoxicillin, azamethiphos, bronopol, cypermethrin, emamectin benzoate, florfenicol, flumequine, hydrogen peroxide, oxolinic acid, oxytetracycline, sarafloxacin, sulfadiazine:trimethoprim, teflubenzuron, and tricaine methane sulphonate). Due to the expensive research and licencing costs for such a little market in comparison to other markets for pesticides and medicines, the number is constrained. In addition, the list of drugs that have been licenced differs greatly across nations. Despite the potential regulatory issues, it seems that a number of chemicals are still freely accessible, and even if they lack complete licences, they may still be used off-label (Daniel 2009; Rodgers and Furones 2009).

1.4.5 Offshore Activities

The oil and gas sector often carries the biggest risks of chemical exposure owing to offshore operations. The primary sources of pollutants from routine offshore oil and gas activities are thought to be formation water brought up with the hydrocarbons (produced water) and rock cuttings from drilling (drill cuttings) (Bakke et al. 2013). A priority has been identified as improving chemical transparency and understanding the potential environmental effects of offshore effluents due to the inadequate monitoring of volumes and subsequent inputs to marine ecosystems (Roose et al. 2011). Given that the European oil and gas sector is moving to deeper ocean locations, where there is less knowledge about the impacts on the species that live there and where appropriate regulatory frameworks to avoid environmental harm might be more challenging, this becomes more pertinent (Science for Environment Policy 2012).

1.4.6 Other Sea-Based Activities

Among the various potential sources of pollution and contamination in marine environments, there are additional sea-based contributors. There have been several marine incidents resulting in fatalities, both in actual occurrences and in hypothetical scenarios. According to the International Atomic Energy Agency (IAEA 2015), there have been documented instances of radioactive material escaping. The maritime environment may also be exposed to artificial radionuclides. Artificial radionuclides may also be present in the maritime environment. The study conducted by Benn et al. (2010) examines the phenomenon of nuclear weapons testing conducted underwater. In addition, both current and historic shipwrecks that may contain pollutants pose a threat to the maritime ecology (Alcaro et al. 2007). It has been estimated that between 2.5 and 20.4 million metric tonnes of oil include materials that might potentially be distributed as Shipwrecks degrade with time (Landquist et al. 2013). Regression and the corrosion of aging structures may also result in the leakage of other materials. The presence of many hazardous substances, such as arsenic, cadmium, copper, chromium, lead, mercury, zinc, polychlorinated biphenyls (PCBs), asbestos, biocides, polyvinyl chloride (PVC), and radioactive waste, has been documented in the literature (Alcaro et al. 2007; Annibaldi et al. 2011; Sprovieri et al. 2013).

1.4.7 Munitions and Chemical Weapons

Chemicals that are created using components that are suitable for use in weapons might potentially leak. The locations where waste is dumped into bodies of water and then spreadout farther areas. Recent data reveals that rusty bombs may be releasing potentially dangerous compounds into landfills in the Baltic region (Missiaen et al. 2010; Baršienė et al. 2014) and Adriatic Sea (Amato et al. 2006; Torre et al. 2013). There is a forecast that corrosion will lead to Torre et al. (2013), and there are expectations that corrosion will result in Roose et al. (2011) found that the highest leakage occurrences occurred in the middle of the twenty-first century. In addition to this, there has been a rising interest in aquatic activities include things like pipelines and wind farms located offshore, as well as changes to fishing regulations methods generate additional complications as they have the potential to impact ammunition that has not been used. During the twentieth century, there were around 70 different chemicals that were used or kept for use in military conflict (CHEMSEA 2014). There is not a comprehensive list since, in many instances, it is impossible to determine what kinds of goods were deposited there (Beddington and Kinloch 2005). It is believed that the majority of the material that has been dropped consists of conventional weapons, such as nitroaromatic explosives such as TNT (2,4,6-trinitrotoluene) and DNT (2,4 dinitrotoluene), as well as nitramines explosives such as RDX. These types of explosives can be found in nitroaromatic explosives (hexahydro-1,3,5-trinitro-1,3,5-triazine). With the exception of the explosive component, conventional ammunition is almost completely composed of various types of metals, the most common of which are copper, iron, nickel, tungsten, tin, lead, aluminium, and zinc. In addition to that, it consists of plasticizers and stabilisers such as nitroglycerin and nitrocellulose nitrate esters as well as propellants and plasticizers. It is also common knowledge that a large quantity of incendiary weapons, including those containing white phosphorus, were often discarded at sea. There is evidence that incendiary devices have contributed to the pollution of white phosphorus in a number of coastal areas across the world, including the Baltic Sea, the OSPAR maritime area, and the Gulf of Mexico (Amato et al. 2006; OSPAR 2010b; HELCOM 2013).

1.4.8 Anthropogenic Contaminants

Due to human activity in these locations, many contaminants may reach coastal areas. However, these locations are impacted by more than just the things that happen there. According to some research, land-based activities are responsible for up to 80% of the pollution in seas and oceans. There has been much investigation about the pollution of chemical contaminants of different types on coastal ecosystems. A research by Berne et al. (1980) that examined the effects of the hydrocarbon contamination brought on by Amoco Cadiz's 1978 oil leak on the Brittany coast is one example. Furthermore, the existence and consequences of developing contaminants in the environment have been a rising source of scientific concern in recent decades (Richardson 2009; Deblonde et al. 2011; Gavrilescu et al. 2015; Geissen et al. 2015). These pollutants are characterised as compounds that exist naturally and artificially but are not tracked by monitoring or surveillance systems and are thus not covered by environmental laws. However, it is believed that these contaminants have harmful impacts on ecosystems or human well-being Although the existence of emerging contaminants (ECs) in the environment is not necessarily new, it has only recently been feasible to detect many of them thanks to the development of high resolution and very sensitive analytical approaches (Richardson 2009; Deblonde et al. 2011; Gavrilescu et al. 2015). In addition, since chemical compounds are continuously developed, their applications and disposal result in new sources of developing contaminants (Geissen et al. 2015).

1.5 Plastic Pollution and Ocean Environment

In the coastal and marine ecosystems all around the globe, plastic pollution is acknowledged as a serious anthropogenic problem. Any aquatic ecosystem's structure, functioning, and therefore, services and values are directly and/or indirectly disrupted by the unprecedented and ongoing buildup of rising plastic pollution from human sources. The main sources of these pollutants entering the ocean via diverse ways are land- and sea-based sources. Different types of plastic pollution, including megaplastic, macroplastic, mesoplastic, and microplastic, are found across ecosystems. The water, sediment, and biota of the marine and coastal ecosystems show a broad dispersion of microplastics in their primary and secondary forms. Different aquatic settings across the globe have microplastic levels that vary from 0.001 to 140 particles/m^3 in the water to 0.2–8766 particles/m^3 in the sediment. The rate of microplastic build-up in marine and coastal animals ranged from 0.1 to 15,033 counts. Accordingly, plastic pollution has a variety of adverse impacts in addition to having an impact on the environment and society. Significant ecological consequences with escalating risks to biodiversity and trophic connections include entanglement, toxicological effects from ingesting plastics, asphyxia, hunger, dispersion, and rafting of creatures, supply of new habitats, and introduction of invading species. Loss of ecosystem values and services is linked to the degradation (changes in the status of the ecosystem) and alteration of marine systems. As a result, this developing pollutant has a detrimental influence on shipping, fishing, tourism, and public health, which in turn affects socioeconomic factors. Practical methods for tackling the problem of plastic pollution include preventing sources of accumulation, the 3Rs (Reduce-Recycle-Reuse), raising capacity and awareness, and producer/manufacturer accountability. The reduction of plastic trash in the marine and coastal zones is greatly helped by the policies, laws, regulations, and initiatives that are already in place or that have been established at the international, regional, and national levels. The creation of recommendations or solutions for important research gaps may pave the way for a fresh approach to this environmental problem that is also scientifically sound. To raise awareness of a blue ocean free of plastic and in good health in the near future, this study concludes by demonstrating the present state of plastic pollution in the marine environment (Thushari and Senevirathna 2020).

Because aquatic ecosystems and the terrestrial environment are intertwined, changes in one system have an effect on the other. The coastal and marine ecosystems have been under stress for decades due to a variety of reasons, including human activity (Adams 2005; Richmond 2015). The environment is being physically destroyed and pollution are two examples of these stressors. Due to unsustainable development and building operations, debris or litter collection is one of the serious concerns that humans have posed to marine and coastal systems. Plastic litter is persistent in ocean basins compared to other types of trash such as glass, cloth, paper, food waste, metal, rubber, medical/personal hygiene items, smoking/firework items, and wood (Nualphan 2013; Rosevelt et al. 2013). This is because plastics have special properties, such as the potential for ready transportation by water current and wind due to their long shelf-lives. Due to incorrect garbage disposal, there are 5 trillion pieces of plastic debris floating in the world's oceans, totalling more than 260,000 tonnes (Eriksen et al. 2014). Regardless of wealthy or poor areas of the globe, plastic pollution has recently increased to the point that it affects practically all ocean basins.

1.6 Role of Mercury in Chemical Process

The ocean plays a crucial role in the biogeochemical cycle of mercury (Hg) (Mason and Sheu 2002; Sunderland and Mason 2007; Strode et al. 2010). The amount of mercury in ocean waters is estimated by Sunderland and Mason (2007) to be 1750 mmol (3:5108 kg), whereas the amount in the atmosphere is 28 mmol (5:6106 kg). Approximately 30–40% of the present Hg intake to the atmosphere comes through ocean emissions, which also include evasion from soils, hydrothermal vent activity, and volcanic eruptions (Sunderland and Mason 2007). However, the majority (90%) of the mercury in the seas comes through wet and dry deposition from the atmosphere (Mason et al. 1994; Andersson et al. 2011).

When mercury is released into the ocean, it undergoes a number of biogeochemical transformations. These include association and dissociation with ligands, precipitation and dissolution as minerals (like mercury sulphide), oxidation and reduction reactions, adsorption and desorption methylation and demethylation, to

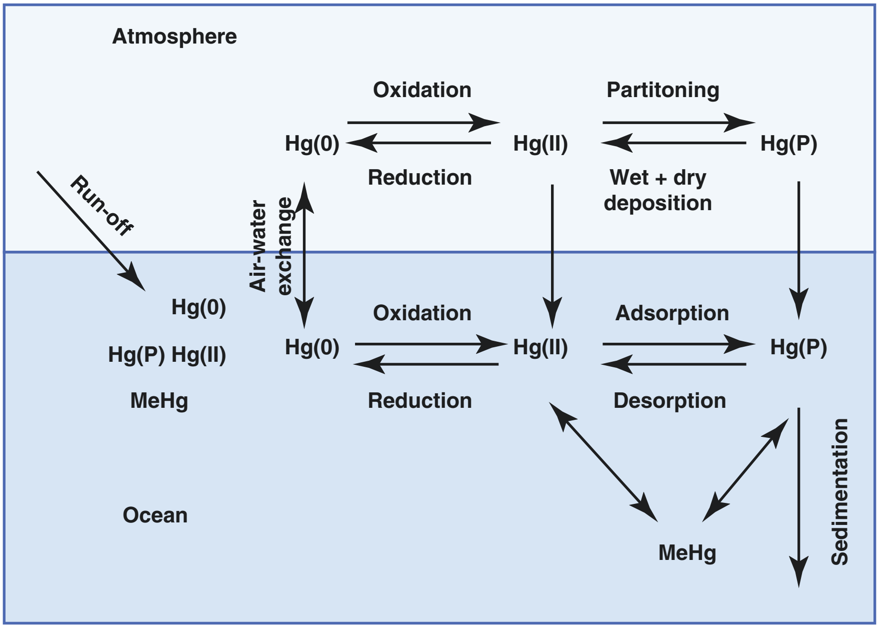


Fig. 1.2 Generalized diagram of oceanic mercury conversions (Batrakova et al. 2014)

suspended particulate matter (SPM), sedimentation and resuspension, leaching and transport, sedimentation and resuspension to groundwater, and uptake by aquatic biota (Liu et al. 2012).

One of the most important sources of mercury in marine ecosystems is wet and dry deposition of mercury from the atmosphere. For instance Zagar et al. (2014) calculated that the Mediterranean Sea receives around 45% of its total mercury influx through atmospheric deposition. This percentage is larger on a global scale, and atmospheric deposition's contribution may reach over 75% (Sunderland and Mason 2007; Amos et al. 2013). Mercury comes from river systems in particular coastal zones.

For the fate of mercury in the ocean, adsorption of Hg(II)/and methylmercury onto suspended particles and sediments is crucial. The bioavailability, toxicity, and destiny of mercury in the water are influenced by its phase speciation and size distribution. The majority of the mercury particles has been reported to be dissolved in organic suspensions. The amount of organic matter in bottom sediments was measured in several locations throughout the globe (Boszke et al. 2002). Figure 1.2 depicts the mercury cycle in the ocean schematically.

1.7 Impact of Physiochemical Process on Human Health and Marine Organisms

The idea that marine resources are unbounded by human endeavours has been around for a while. Huxley (1883) in fact had no doubts about the eternal nature of oceanic resources However, humanity became aware of the finite nature of marine resources in the twentieth century. It has been noted and is widely understood that human actions jeopardize both the global environment and human existence. Marine pollution, manmade chemicals, rising ocean temperatures, increased UV radiation, and other factors have an impact on marine species. For a very long time, the impacts of chemical contaminants have been of great interest. It is now well-accepted that exposure to chemical pollutants makes organisms more vulnerable to radiation, illnesses, thermal changes, and infections. When the acute impacts of pollutants become obvious, it is easy to take convenient measures to combat them. However, because chronic pollution impacts take time to manifest, the damage they cause may not be apparent until we are aware of them. As a result, prolonged poisoning brought on by pollutants typically causes malformation of the sexual organs, which is followed by a decline in the organism's reproductive abilities.

Although most organic synthetic substances are created and eaten on land, effluents and sewage allow them to enter the water because of massive industrial zones on the oceanfront, a number of pollutants frequently enter the water. The majority of international transportation is also maritime. Then, the release of ship ballast waters and chemical elution may pollute the oceans. The majority of marine animals used as fishing bait live in the littoral zone, where anthropogenic activities can readily change the environment. Marine species are therefore readily exposed to the effects of synthetic organic compounds.

Petroleum makes up a significant portion of the energy sources we currently use. Accidental oil spills often result from the transport of petroleum. Due to the fact that crude oil contains a number of dangerous organic compounds, oil spills from tankers have a considerable impact on marine contamination. With the exception of spills brought on by acts of war, the amounts of oil spilled from tankers were 1176, 1140, and 146 kt in the 1980s, 1990s, and 2000–2004, respectively.

The majority of marine invertebrates go through planktonic larval phases, hence abnormalities caused by UV light exposure have been documented (Lesser and Barry 2003; Bonaventura et al. 2005; Schroder et al. 2005). When chlorofluorocarbons are released into the environment, marine fauna, which is one of our key protein sources, reproduce less frequently.

Depending on how vulnerable the population is, changes in the ecosystem caused by human pressure on the marine environment have an impact on humans. To ensure the stability and homeostasis of the ocean, significant modifications to past and present human progress are required. In addition, it is crucial to comprehend the dynamic of marine processes better in order to help reduce the hazards connected with human exposure. It entails the creation of a system that can produce data on a variety of complex environmental processes that should be used to stop impacts on humans and biodiversity. Fisheries, harmful alagal blooms (HABs), viruses, and pollutants are just a few of the environmental elements that climate change and other anthropogenic influences can affect. Environmental models are necessary to both mitigate the effects of changing ecosystems and to better understand the dynamics of the ocean and ecosystems and their impact on climate change. To set up procedures to investigate and stop the effects of ocean changes on human health, indicators must be developed. It is strongly recommended that marine ecosystems be preserved, especially those that appear to have undergone minimal changes.

Through ecosystem services, as a source of discoveries in pharmacology and biomedicine, for cultural values, and simply because of the harmony of healthy seas and their steady biodiversity, the oceans have a vital link with human wellness. The stability of the coast, control of nutrients and climate, management of pollutants, energy resources, and natural goods with value for biomedicine, tourism, and recreation are only a few of the services provided by the marine ecosystem. As a result, ocean quality is crucial for maintaining the integrity of the species that exists in this biome as well as producing positive impacts and being crucial for the preservation and stability of terrestrial ecosystems for human welfare and health (Fleming and Laws 2006; Moura et al. 2011; Allen 2011). The coastal areas provide a significant natural setting for human recreation, which helps both the body and the mind. Access to natural surroundings has shown to boost health and wellness, prevent sickness, and hasten the healing process after illness, according to medical research. Activities including swimming, surfing, coastal walks, and beach sports are encouraged by coastal settings (Allen 2011). These physical and mental workouts may help prevent cancer and obesity as well as cardiovascular disorders (Allen 2011). In addition, the leisure activities may aid in the prevention or treatment of several mental health conditions, including stress reduction. The complicated economic values of environmental services and natural resources have undergone extensive analysis. The preservation of the ecosystem is often seen as being more economically beneficial than the economic benefits associated with the acquisition and use of its resources, which frequently result in significant environmental liabilities (Costanza and Farley 2007; Costanza et al. 1997). Constanza et al. have shown that although coastal regions make up just 8% of the world's geographical area, they provide almost 43% of the ecosystem services with a total estimated value of 12.6 trillion dollars.

1.8 Future Prospective

The impact of human-induced changes in the marine ecosystem on humans varies depending on the population's vulnerability. In order to ensure the stability and equilibrium of the ocean, substantial alterations are necessary to sustain human advancement. Understanding the intricacies of marine systems is of utmost importance in mitigating the risks associated with human exposure. The task at hand entails the development of a comprehensive system capable of generating data pertaining to diverse, intricate environmental processes. This data is intended to be utilized in efforts aimed at mitigating human activities that pose threats to biodiversity and its associated ecosystems. Climate change and other anthropogenic factors havethe potential to impact various environmental components, including fisheries, harmful algal blooms (HABs), viruses, and pollution. There is a pressing requirement for the advancement of contemporary enhancedenvironmental models in order to effectively address and alleviate the impacts of evolving ecosystems. It is imperative to consider the intricate dynamics of ocean ecosystems and their impact on climate change through thorough analysis during the development process. There exists a pressing imperative to establish indicators and protocols for the purpose of investigating and mitigating the impacts of oceanic changes on human well-being. The current human activities affecting ocean ecosystems are anticipated to give rise to significant challenges in the future. Numerous activities can be discerned and mitigated according to their necessity in order to minimize the detrimental impact on the ocean ecology. Therefore, it is highly advisable that expedited research be conducted in order to save the maritime ecosystems.

1.9 Conclusions

The world's oceans are split into five major oceans and several seas based on differences in history, culture, geography, science, and size. The Atlantic, Pacific, Indian, Arctic, and Antarctic ocean basins are among the most well-known marine ecosystems that humans have colonised.

The use of traditional methods to maintain the cleanliness of environmental ecosystems is still very prevalent today. Hence, they cannot be considered as ineffective to upgrade it. There is undeniable evidence that human activities such as overfishing, the loss of habitat, and pollution alter marine ecosystems. The ocean and the resources contained within it seem to have no limits despite it directly pointed a danger to the long-term productivity of the oceans. As a result of declining yields in several fisheries and the deterioration of valuable marine ecosystems like coral reefs, there has been an increase in interest in the formulation of an all-encompassing strategy. A network of marine protected areas (MPAs) has been established with the aim of enhancing the management of marine resources through the designation of specific zones for heightened protection. As a result, there is an immediate need to investigate how marine-protected areas (MPAs) might be used as instruments to assist particular conservation requirements of marine and coastal waters in both the United States and worldwide. It is possible for harmful chemicals to seep into the coastal and marine environment, where they may cause a negative impact on ecosystems, biodiversity, and human health. Most of these contaminants have little to no records and also our understanding of their impacts and where they go is limited at best. These pollutants produced polluting effects in marine habitats which arises a difficult task for administrators, authorities, and investigators. The development of cost-effective and sustainable techniques to mitigate the negative impacts on ocean ecosystem is required after the identification of anthropogenic pollutants.

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2

Microbes in the Recycling of Carbon in the Arctic Regions: A Short Review

R. Ushasri, G. Charumathi, and Sraya Saseendran

Abstract

The thermal factors of Arctic Oceans permit the growth of single-celled algae produced by utilizing certain growth factors. Algae grow using sun sources as light energy and/or organic compounds as carbon sources and convert them into simpler compounds. The metabolites produced by microbes kill them leading to the degeneration of microbes in Oceans. Algae degrade the leftover food deposited into seas Arctic Ocean in the Arctic areas. The Permafrost melting converts carbon to CO_2 in the presence of sunlight, degrading the growth of the aerobic microbial population. Recent research showed that microbes require genetic methods and resolution chemistry to recycle carbon. Microscopic cultural and molecular tests characterized the Arctic microbes. Fifty percent of carbon is found in the Arctic Ocean accumulated from the soil. The seasonal variation had led to the release of green gases by the conversion of organic compounds to inorganic compounds, which affects global warming. Microbes undergo nitrification during winter. It was found that there are 2500 species of bacteria namely proteobacteria, Bacteroidetes, and firmicutes. Snow algae are found in polar and Arctic areas. The objective of this review study is to determine the role of Arctic microbes in the recycling of carbon. The carbon dioxide is released into the atmosphere, which is required by plants for photosynthesis. The plants take up carbon dioxide and release oxygen for the survival of human beings. Global warming is sustained by maintaining the carbon dioxide and oxygen ratio in the atmosphere.

Keywords
Arctic microbes · Cryonites · Global warming · Carbon dioxide · Recycling

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2.1 Introduction

Microbes in the Arctic Ocean play an important role in the recycling of carbon derived from carbon-rich sea foods. The thermal factors of Arctic Oceans permit the growth of single-celled algae produced by utilizing certain growth factors. Algae grow using sun sources as light energy and/or organic compounds as carbon sources and convert them into simpler compounds. The metabolites produced by microbes kill them leading to the degeneration of microbes in Oceans. Apart from Algae, bacteria and fungi also play an important role in the recycling of carbon-based fud products by breaking down dead cells into biomass (Bar-On et al. 2018). The microbes survive in ice for hours of duration with the help of organic substances in a frozen ocean at optimum conditions of growth. Based on a review of the literature, it was found that the Arctic sea contains 100 billion microbes that act as a base of food web microbes that act as Arctic Ocean supports and provide oxygen supply to most of the aquatic fauna algae are regarded as primary producers. They synthesize their own food by means of photosynthesis zooplankton secondary consumers feed on algae. Algae degrade the leftover food deposited into seas Arctic Ocean in the Arctic areas, utilizing carbon dioxide produced by microbes like aerobic bacteria and fungi (Burkert et al. 2019).

2.2 Reduce, Reuse, and Recycle: Carbon Dioxide

The fungi change their shape depending on the environment. In arctic regions, fungi exhibit spherical cells. The recycling of the waste in melting ice by microbes is critical hence during summer, the microbe's waste and microbial debris settle down for years by a mechanism called carbon draw down which has many advantages such as global warming (Coumou et al. 2018).

2.3 Microbial Role in the Recycling of Carbon Dioxide

The accumulation of carbon dioxide in Arctic Oceans was found to be due to human activity and microbial biomass production aerobic microbes contribute to the production of mass CO_2. Arctic microbes play an important role in changing the diversity of evolution. The microbes in Arctic Oceans make an era for understanding the fundamentals of Microbiology and Arctic biology. Microscopic cultural and molecular tests characterized the Arctic microbes. Fifty percent of carbon is found in the Arctic Ocean accumulated from the soil. The seasonal variation had led to the release of green gases by the conversion of organic compounds to inorganic compounds, which affects global warming (Colatriano et al. 2018). The microbial utilization of organic carbon from frozen sea releases carbon dioxide into the atmosphere. The Permafrost melting converts carbon to carbon dioxide in the presence of sunlight, degrading the growth of the aerobic microbial population. Recent research showed that microbes require genetic methods and resolution chemistry to recycle carbon. The microbial population is most predominant in cold regions and becomes most resistant to frozen temperatures (Suyal et al. 2018). The metabolic activity of psychrophiles is very low compared to thermophiles. These microbes acclimatize to depletion of nutrients greater saline content, much UV radiation, and oxidative stress at greater altitudes and deeper oceans. The lack of liquid phase is a critical factor for slow metabolism of arctic microbes although multiplication occurs at low temperatures such as −20 °C. The reproduction of arctic microbes depends on unicellular and multicellular organisms. Vitrification is an important factor where a glass-like transparent sheet is formed on the surface of arctic microbes. And liquid phase is transformed into solid phase. The authors of this review study stated that vitrification is a form of desiccation based on the production of metabolites and exchange of gases. Vitrification was found to be not harmful and reoccurrence of solid state to liquid state takes place during the summer season. The arctic microbes acclimatize to extremes of low temperature by means of genomic, metatranscriptronic, and proteomic analysis (Cook et al. 2020).

Microbes in cold regions are most susceptible to climatic changes (Kumar et al. 2019). Emerging and outstanding technologies played a crucial role in understanding the role of different types of microbes in reflecting carbon. Based on the review of the literature, it was found that 90% of ocean temperature is below 5 mm with the existence of psychrophiles including diatoms, bacteria, and fungi. The Arctic region includes sea ice, lake ice, snow ice, and glacial environment cold water lakes. The microbes migrated from the different regions of the world to cryosphere. Based on metagenomics data recovered from microbes across the world, it was found that the majority of Arctic microbes play a significant role in the conversion of organic carbon to carbon dioxide. The study of microbiology is an emerging field of life sciences involving proteomes and metagenomics after isolation and identifying Arctic microbes, namely Stable Isotope Probing Flouresence in-situ Hybridization (SIP, FISH). Psychrophilic adaptation psychrophiles in cold habitats come across various challenges, namely depletion of nutrients high salinity low activity of water, UV radiation oxidation stress, and high pressure exerted at different altitudes (Dinasquet et al. 2018). Microbial growth occurs below 20 °C followed by physiological activity below 30°. The life cycle of unicellular organisms at low temperatures is higher than multicellular organisms. Microbes undergo nitrification during winter. It was found that there are 2500 species of bacteria namely proteobacteria, Bacteroidetes, and firmicutes. Snow algae are found in polar and Arctic areas. Proteobacteria produce biomass from organic carbon and are converted into carbon dioxide by other microorganisms such as heterotrophic bacteria, namely cyanobacteria through photosynthesis. Heterotrophic microorganisms in Himalayan and other cold environments exhibited a greater capacity to reduce organic compounds to carbon dioxide along with metabolic enzymes. Microorganisms in cryoconite convert organic compounds into inorganic nutrients and carbon dioxide. Based on the review of the literature, it was found that diatoms such as *C. gelidius* grow at 2° in the closed batch system for 12 days under conditions of specific growth factors such as nitrogen and carbon. The levels of particulate organic carbon particulate nitrogen, and carbon dioxide were measured at different days of growth, and finally carbon, the nitrogen ratio was estimated. Diatoms play an important role in the blooming of phytoplankton in the Arctic Sea the high penetration of light into the sea decreases the rate of photosynthetic activity in ice. The change in irradiance might affect the carbon levels. The consistently low Arctic sea temperature increased the acidification process by converting carbon to carbolic acid (Cook et al. 2020).

The low pH value with an increase in high carbon levels effect the microbial growth in ocean. The variability of high carbon levels has a great impact on carbon metabolism with respect to the carbon cycle the dissolved carbon dioxide level that is greater than 1% in Arctic Ocean bicarbonate ions in oceans contributes to 98% of carbon source. The carboxylating enzyme degrades the substrate and releases carbon dioxide. The rise in carbon dioxide level is due to the conversion of bicarbonate by the enzyme. Arctic diatoms play an important role in the acidification of ocean levels and exhibited high plasticity at high levels of carbon dioxide concentration. The pH ranges from 7.2 to 8.1 showing high physiological activity. *C gelidius* has a greater capacity to absorb carbon sources from bicarbonate ions under variable conditions such as 50–100 μm and 240–900 micro atoms of carbon dioxide (Coumou et al. 2018).

2.4 Conclusion

Understanding the role and life cycle of arctic microbes has made a pavement for research scholars and scientists in the field of aquatic frozen microbiology. The frozen microorganisms are isolated and identified by advanced techniques in collaboration with conventional methods. Biotechnology plays an important role in the detection of genes that code for adaptation of arctic microbes to low temperatures. The review of literature stated that emerging and empirical technologies would definitely assist research scholars to find out facts about arctic microbial communities and their role in recycling carbon dioxide to atmosphere. The authors of this study had stated that the interaction between arctic microbes and flora and fauna of the arctic region plays an important role in recycling the carbon dioxide. Based on the review of the literature, it was concluded by the authors of this review study that the acidification arctic sea is the most challenging and problematic concern for Phyto and zooplankton under different seasons. The diatom is the most *active* unicellular organism in maintaining carbon dioxide and oxygen levels. Arctic microbes are vital in the Arctic ecosystem. They provide food supplements to zooplankton and provide oxygen supply to arctic aerobic microbes and zooplankton. They derive carbon dioxide by degrading bicarbonates accumulated in the deep ocean. The carbon dioxide is released into the atmosphere that is required by plants for photosynthesis. The plants take up carbon dioxide and release oxygen for the survival of human beings. Global warming is sustained by maintaining the carbon dioxide and oxygen ratio in the atmosphere.

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Microbial Symbiosis in Marine Ecosystem

3

Pragati Srivastava, Manvika Sahgal, and Hemant Dasila

Abstract

Symbioses between eukaryotic hosts and microorganisms are significant at all scales, from the individual organism to the entire ecosystem, and they support the health of the planet's most endangered marine ecosystems. In spite of progression in study of microorganisms that are associated with hosts, ranging from single microbial symbionts to consortia of highly relevant taxa, little is known about how these bacteria interact with the vast bulk of marine host species. Expanding our knowledge of how microbial symbionts affect host performance and adaptation is crucial as microbial symbionts are present in almost all living things. To improve our understanding of host–microbiome interactions and how they affect marine ecosystems, we list research priorities. We contend that these developments in knowledge will guide future management plans by allowing us to forecast how species, communities, and ecosystems will react to disturbances brought on by human activities.

Keywords

Symbioses · Ecosystem · Marine microorganisms · Algae

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3.1 Symbiotic Nature in Marine Environment

Marine environment harbours about two million species (Mora et al. 2011) and compromises of different body designs that vary from simple sponges to organ possessing vertebrate such as mammals. Marine animals are considered as key members of marine ecosystem. The marine environment also possess vast diversity of microorganisms that includes protists, archaea, fungi and virus (Eakins and Sharman 2010). Main role of these microorganisms is to produce oxygen, maintain nutrient cycle, organic matter degradation, and other critical function in the ocean and earth (Arrigo 2005; Falkowski et al. 2008). Microorganisms that shows association with marine animals are considered part of animal microbiome. It is believed that a few microorganisms that compromise the microbiomes of marine animals seem to originate surrounding supply of seawater-associated cells. Integrative microbiology approach unfolds many new secrets of animal microbiomes (McFall-Ngai et al. 2013).

Symbiotic relationship between marine animals and microorganisms has been studied by using an integrative approach (Smith 2001; Douglas 2010; Bhatt et al. 2022). Some reports of diverse bacteria, protists, archaea, and virus that are associated with corals provide insights of diverse function performed by microorganisms via associating with corals niches (Thompson et al. 2015; Bourne et al. 2016). Focus has been shifted towards the identification of "core" microbiome members of the microbiome (Shade and Handelsman 2012). Generally targeting of small subunits of ribosomal RNA gene of microorganisms has been done to understand the physiology and behaviour of the microorganisms (Ezenwa et al. 2012; McFall-Ngai et al. 2013). The marine environment has been consistently changing with the change in environment and this causes a serious change in the microbial community that is associated with marine environment.

Host microbiome system generally falls into two main categories (a) symbiosis, in which organisms are involved in metabolic interaction and (b) dysbiosis, study has been majorly concerned with humans and humanised models (Hamdi et al. 2011; Nicholson et al. 2012; Scharschmidt and Fischbach 2013) and many of these models are good fit for organisms in the sea (Egan and Gardiner 2016).

Normal microbiome–animal relationship in the marine is generally referred to as symbiotic state but, however, exact nature of this relationship varies with cell, for example, cells that reside within the gut cavity of an animal are physically associated and do not share specific association and this type symbiotic association are influenced by variation in light and temperature, especially association of microorganisms with photic zone animals. Some reports suggest that bacteria, virus, protists, and archaea were in association with corals (Thompson et al. 2015; Bourne et al. 2016). These microorganisms provide many insights into biochemical and genetic interactions that contribute to animal behaviour, ecology, and health (Thompson et al. 2015; Bourne et al. 2016). Understanding the microbiome nature of marine animals is very important and for that generally small subunit (SSU) ribosomal RNA (rRNA) genes are targeted to gain information regarding the nature of association, physiology, and ecology (Ezenwa et al. 2012; McFall-Ngai et al.

2013). Consistent environmental changes and anthropogenic activities in marine environments have an impact role (Halpern et al. 2008; Doney et al. 2012) and changing microbial communities with respect to these activities are also under scrutiny.

Relationship between Hawaiian bobtail squid *Euprymna scolopes* (Mollusca) and bioluminescent bacteria *Vibrio fischeri* have been observed and it provides greater insight into their symbiotic nature that relates to biochemical interaction and fundamental signaling that occurred during symbiosis (McFall-Ngai 2014). *E. scolopes–V. fischeri* symbiotic relationship provides simplicity for the study of host–microbial interactions that could be very helpful in developing animal husbandry and genomic tools which later can be utilised for such microbiome–squid interaction. Advanced molecular biology tools such as transcriptomics and proteomics have been used to study energy transfer mechanisms that occurred between host and microorganism (Kleiner et al. 2012).

3.2 Organizational Adaptation Played by Symbiosis

As a critical factor driving evolution, symbiosis is becoming more widely acknowledged. The mitochondria and chloroplast organelles seen in contemporary eukaryotic cells serve as one of the greatest examples of how bacterial cells evolved into intracellular symbionts and provided eukaryotic cells with new capabilities for respiration and photosynthesis (Margulis 1970). Over a period of two billion years, these symbioses helped eukaryotic species grow and spread (Margulis and Sagan 1997). Lichen is a particularly remarkable example of extracellular symbioses culminating in biological innovation. In the mutualistic symbiosis between the cyanobacteria and the fungi, the cyanobacteria provide the fungi with suitable nutrition and the fungi protect the cyanobacteria within their branching network. Cyanobacterial cells are found in lichen within the structural framework that is provided by the hyphae of fungi. These organisms work as a holobiont, which is a composite of a host and one or more symbionts (Margulis and Fester 1991). The holobiont notion goes beyond mutualistic relationships.

The ability to produce a new functional characteristic or behaviour more quickly than through normal evolutionary processes is a major benefit of using symbioses to drive biological innovation (Margulis and Fester 1991). The idea of the hologenome, which is the combined genetic make-up of the host and any symbionts that might serve as a different level selection throughout evolution, was inspired by this proposal (Schulz et al. 2022). The terminology “holobiont” most generally relates to hosts that have microbial symbionts. Because holobionts “do not fit the criteria for being creatures, evolutionary individuals, or units of selection,” this idea is sometimes seen as contentious (Skillings 2016). Despite the debate around the concept, the term’s popularity suggests that people are becoming more aware of how important symbiosis is for promoting biological and ecological innovation on Earth (Sudakaran et al. 2017).

Sustained relationships among organisms are the result of various carefully tuned and tried-and-true procedures by the partners. Therefore, knowing the evolution of symbiotic relationships can help us better understand how particular adaptations came to be. Symbiosis's significance in significant evolutionary novelties, such as new behaviours, morphologies, metabolic pathways, and taxa, is referred to as "symbiogenesis." Most frequently, microbial endosymbiosis events are described as symbiogenesis (Margulis 1970). The preservation of the ancestral genomes in eukaryotes' mitochondria and chloroplasts, which are distinct from the eukaryotic cell's nuclear genome, is the most noteworthy indication of this type of occurrence. Researchers have found evidence of cospeciation patterns between two or more partners, which explains how one lineage would change in response to another (Ehrlich and Raven 1964). The Hawaiian bobtail squid (*Euprymna scolopes*) and the bacterium *Vibrio fischeri* are one of the most researched examples of a simple host–symbiont connection (also known as *Aliivibrio fischeri*). To establish an effective and mutualistic relationship, both partners have coevolved particular signalling and recognition features (McFall-Ngai et al. 2012). More recently, molecular studies have revealed patterns of phylosymbiosis, or linkages between a host and a larger symbiont population (such as the community of microorganisms linked with a host). According to O'Brien et al. (2019) and Wallin (1927), Similarities in the host lineage and community composition of the linked microbes serve as typical indicators of these linkages. There have been reports of phylosymbioses in the aquatic domain. There have been reports of phylosymbioses in the aquatic domain between microbes and sponges, corals, and reef fish (Chiarello et al. 2018).

3.3 Mechanistic Approach Adapted by Symbionts

Symbiotic species can co-evolve through a number of different processes. These pathways may also affect how organisms adapt to stress over a longer period of time in the marine environment. Unquestionably, one of the main processes resulting in genetic variety and new features within a species is genetic mutation. For instance, the sponge *Astroclera willeyana* has a gene in its genome that affects the expression of bacteria-derived cells that create spherulite. It is possible that the transfer of this gene caused the sponge's biocalcification procedure (Apprill 2020). *Nematostella vectensis*, a sessile organism that lives in salt marshes, contains bacterial genes involved in the shikimic acid cycle that may provide UV protection to the starlet sea anemone (Starcevic et al. 2008). Adaptive evolution and genetic differentiation are brought about by horizontal gene transfer, or the exchange of genetic materials between non-mating species. *Nematostella vectensis*, a sessile salt marsh resident, may be protected from ultraviolet radiation attributable to bacterial genes found in its genome that are involved in the shikimic acid cycle (Starcevic et al. 2008). Genomes typically serve as proof of the inherited genes. *Nematostella vectensis*, a sessile salt marsh resident, may be protected from ultraviolet radiation attributable to bacterial genes found in its genome that are involved in the shikimic acid cycle (Starcevic et al. 2008). Genomes typically serve as proof of the inherited genes. In crucial terms, few gene expressions have been carried out to determine whether the horizontally acquired genes are transcribed.

Another factor that encourages co-evolution between a host and symbiont is genome erosion, which reduces genetic information inside a genome. Most often, it is seen in genes that were used in a free-living organism's lifestyle but are no longer necessary when that organism becomes a symbiont for another creature. The organism lowers the energy requirements for cell maintenance and reproduction by minimizing its genome. The fact that many obligatory microbial symbionts have genomes that are significantly smaller than those of bacteria in facultative symbiotic partnerships serves as an example. For instance, the genomes of the internal, vital bacterial symbionts of *Calyptogena okutanii* hydrothermal vent clams are only 1.02 Mb in size. The genome of the bobtail squid symbiont *V. fischeri*, which has a free-living life stage, is more than 4 times larger (4.28 Mb) (Duperron 2016). In keeping with this pattern, many species' symbiotic gene requirements are extremely brief and only involve a small number of genes necessary for beginning and facilitating the symbiosis (Hoffmeister and Martin 2003). Generally, plasmid is the site for symbiosis genes that are small circular DNA molecules different from the DNA of a cell. They are therefore genetic components that are highly mobile and available for sharing and trading.

3.4 Symbiosis

3.4.1 Algae and Coral Reefs

The relationship amongst tropical and subtropical corals and the endosymbiotic algae that live inside of them is one of the best-known examples of a symbiosis that responds to stressful situations. Reefs' structural foundation is created by the calcium carbonate skeletons secreted by scleractinian or stony corals. According to LaJeunesse et al. (2018), stony corals contain tiny dinoflagellates from the Symbiodiniaceae family, which was recently expanded from the single genus Symbiodinium. Ninety percent of carbon is fixed is transferred to the host by these dinoflagellates that carry out photosynthesis within the host-acquired membranes known as symbiosomes in the host gastrodermal cells. The symbionts obtain stable lighting conditions, protection from predators, and access to micronutrients—which are occasionally scarce on oligotrophic coral reefs. Between 140 and 200 million years ago, during the Jurassic period, when calcifying corals began to adaptively develop and reefs started to spread, this symbiosis is assumed to have begun (LaJeunesse et al. 2018).

3.4.2 Coral and Bacteria

In addition to harbouring microalgae, corals also interact with a wide range of microorganisms, including other protists, endolithic algae, bacteria, archaea, fungus, and viruses (Kwong et al. 2019). For the coral holobiont's nutrient cycle and regeneration, bacteria are regarded to be one of the most important microorganisms (Rohwer et al. 2001). There is proof that coral-associated bacteria can synthesize antibiotics and other secondary metabolites, which may aid the coral's defence against illnesses (Ritchie 2006). With members spanning up to 69 recognised and candidate phyla, the bacterial communities of stony corals are among the most phylogenetically diverse of any animal (Huggett and Apprill 2019; Pollock et al. 2018). This tremendous phylogenetic variety is partly explained by the fact that the bacterial community's makeup varies across the different mucus, tissue, and skeletal components of adult corals as well as between the major coral life phases (Sweet et al. 2011; Zhang et al. 2015). For instance, the Caribbean coral porites have "Candidatus Amoebophilus" in the tissues and Tumebacillus in the mucus (Apprill et al. 2016). Across coral species, habitats, and stress-related circumstances, the durability of these kinds of particular coral–bacteria relationships appears to differ significantly (Morrow et al. 2018; Zaneveld et al. 2016).

3.4.3 Hawaiian Squid and *Vibrio fischeri*

The interaction between the bioluminescent bacterium *Vibrio fischeri* and the Hawaiian bobtail squid *Euprymna scolopes* has been studied as a model system for the colonisation of animal epithelia by symbiotic bacteria. Only the analysis of a bidirectional link makes it possible to achieve the fine resolution provided by the squid-vibrio light-organ system. How this association influences the partners' biology, including their ecology, evolutionary biology, and the underlying molecular mechanisms of symbiotic dynamics, has been extensively characterised. The mechanisms that encourage the initial colonisation of light-organs and, more recently, the maturity and long-term maintenance of the connection, have both been the subject of extensive research (Nyholm and McFall-Ngai 2021). Due to the exceptional specificity of the squid–vibrio interaction, only *V. fischeri* colonises the specialised light organ that develops from the embryonic hind gut-ink sac complex of the primitive host. Each generation of the host is colonised by environmental cells of *V. fischeri*, which account for less than 0.1% of the bacterioplankton within host environments, to establish the symbiosis. Numerous host- and symbiont-mediated system ensures specificity, and the light organ does not become colonised when *V. fischeri* cells are not present in the environment. The squid-vibrio symbiosis has been characterized by the ability to experimentally distinguish the colonisation process in the first few hours to days after the host hatches and the capacity to genetically modify *V. fischeri*, both of which have illuminated the host effects that specifically result from symbiont genetic manipulation (McAnulty and Nyholm 2017).

3.4.3.1 Endozoicomonas

Relating to coral in the coral holobiont, Endozoicomonas bacteria predominate. It is suggested that their relative abundance and Symbiodiniaceae abundance are directly correlated, with their relative abundance typically decreasing with heat-induced coral bleaching. Endozoicomonas is a hypothesised core coral bacterial community that is strongly linked to coral bleaching (Bourne et al. 2016; Neave et al. 2016). According to genome analysis, Endozoicomonas participates in the testosterone degradation, probiotic mechanism, DMSP degradation, and the Embden-Meyerhof-Parnas (EMP) glycolytic pathway(Tandon et al. 2020). Endozoicomonas populations in corals have been shown in numerous studies to decline during coral bleaching and then increase once the corals have recovered (Ziegler et al. 2017). As a result, it is anticipated that Endozoicomonas abundance is directly correlated with Symbiodiniaceae density (Bayer et al. 2013; Neave et al. 2017).

3.4.3.2 Osedax

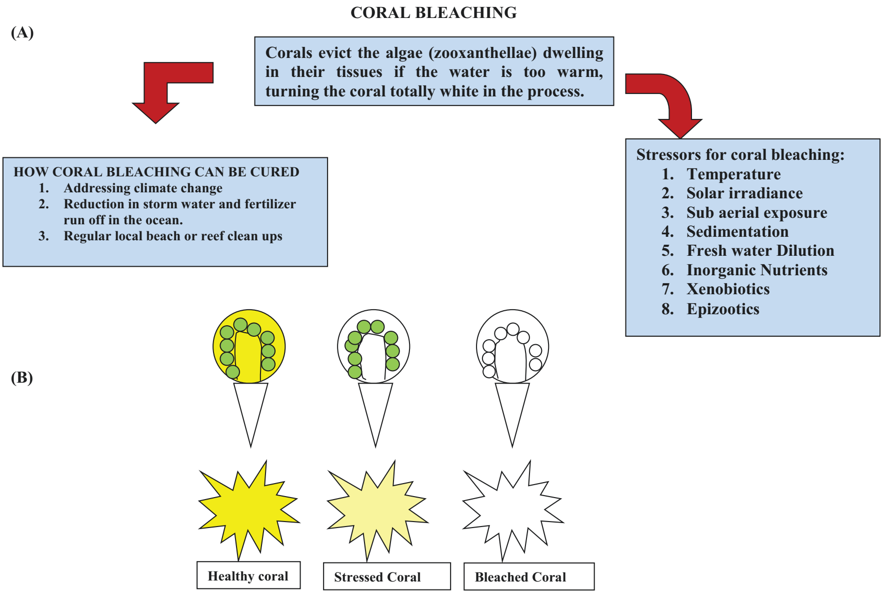
A genus of polychaete worms that dwell and eat on bones is called *Osedax*. Even the massive bones of baleen whales can vanish, sometimes in as little as 10 years, due to these tiny organisms. The worms' lower body is twisted and root-like; it pierces the bone and dissolves it using an acid secretion, releasing proteins and lipids. These nutrients are taken up by symbiotic bacteria that live inside the worms and appear to be crucial in some way for nutrient transmission to the worms. The worms themselves lack a mouth and a gut. At the front, feathery appendages draw oxygen from the water. Many more species that eat a range of marine animal bones, such as those from fish and turtles, have been found since the genus' original description (Rouse et al. 2004; Dipper 2022). The members of this particular Osedax-associated subgroup of proteobacteria are heterotrophic, as are their symbionts, which are also proteobacteria. This nutrient–exchange partnership is distinctive in the realm of symbiosis because they use the lipid-rich hydrocarbons within the bone marrow as a carbon source for their host (Elisha and Wood-Charlson 2013).

3.5 The Function of Bacteria in Coral Stress and Stress Response

Coral reefs are significant ecosystems for the economy because they are very prolific and diversified. Unfortunately, as a result of rising sea surface temperatures (SST) brought on by global warming, coral reefs are degrading, dying, and their distribution is declining globally (Hughes et al. 2017). Mass coral bleaching events have been more frequent and intense recently (Skirving et al. 2019; Sun et al. 2022). In addition, the health of corals can be impacted by global warming directly or indirectly, making them more susceptible to infectious diseases (Vega Thurber et al. 2014). The association between corals and complex microorganisms, such as Symbiodiniaceae, bacteria, archaea, fungi, endolithic algae, and viruses, is known as coral symbiosis (Rosenberg et al. 2007).

Coral reef ecosystems are currently at risk of experiencing significant losses due to the increasing frequency and severity of bleaching events. This underscores how important it is to understand how corals and the microorganisms that live in their symbiotic relationship interact. The creation and resilience of coral reef ecosystems as well as the cycling of carbon, nitrogen, and sulphur are all significantly influenced by bacterial populations, which are known to be extraordinarily abundant and diverse coral symbionts (Lesser 2011; Raina et al. 2009; Wegley et al. 2007; Thompson et al. 2015; Rosenberg et al. 2007). High temperatures put a strain on corals and the bacteria that live in them (Maynard et al. 2015). When corals are subjected to heat stress, the microbiome of the corals may shift in community structure. As a result, opportunistic microorganisms or bacteria linked to disease may be present (Patel et al. 2020; van Oppen and Blackall 2019); as well as an increase in the expression of genes related to coral pathogen virulence factors (van Oppen and Blackall 2019). Coral health is under danger due to these changes. Figure 3.1 depicts the coral bleaching cause and corrective measures.

This may also result in coral hosts losing their capacity to control bacterial symbioses in vivo. Corals are also very active in the early stages of whitening, according to the metatranscriptome studies (Sun et al. 2020) discovered that some cyanobacteria, along with other algae (Bacillariophyta, Chlorophyta), have the ability to substitute for photosynthesis, keeping corals' major source of energy during the early stages of bleaching until they restore symbiotic zooxanthellae from the seawater (Fine and Loya 2002). This demonstrates that shifts in the coral's microbial population occur dynamically from the beginning of the thermal stress phase to the end of



Corals evict the algae (zooxanthellae) dwelling in their tissues if the water is too warm, turning the coral totally white in the process.

HOW CORAL BLEACHING CAN BE CURED

1. Addressing climate change
2. Reduction in storm water and fertilizer run off in the ocean.
3. Regular local beach or reef clean ups

Stressors for coral bleaching:

1. Temperature
2. Solar irradiance
3. Sub aerial exposure
4. Sedimentation
5. Fresh water Dilution
6. Inorganic Nutrients
7. Xenobiotics
8. Epizootics

Fig. 3.1 The vulnerability of different corals to bleaching varies. All coral species exhibit consistent patterns of susceptibility, with a general tendency towards increased susceptibility in more complex, branching forms and reduced susceptibility in huge species, particularly those with fleshy polyps. If corals are often exposed to hotter temperatures or more sunlight, they may also develop a stronger tolerance to the stresses caused by bleaching. (**a**) Coral bleaching corrective measures and causes, (**b**) diagrammatic illustration of Coral bleaching the bleaching process. Examining variations in coral symbiotic bacteria can lead to a better understanding of the resilience and adaptation of coral symbionts in response to environmental changes and thermal stress.

3.6 Gain Grounding Monitoring of Microbial Symbiosis

Our understanding of the potential role of symbiosis and adaptability in tolerating stress could be improved by studying symbioses in the ocean, which present a tremendous opportunity for learning. There are around two million species of marine organisms that have been found, but few studies on their symbiotic interactions have been conducted, according to Mora et al. (2011). These animals most definitely interact with microbes, and it will be necessary to conduct survey-based genetic and microscopic examinations to determine how much they regularly or persistently host microbial symbionts. Understanding macroorganism interactions also requires observations, surveys, and familiarity with these species' typical habits, such as feeding, mating, and cleaning. The least studied regions are the twilight zone and the deeper ocean basins, which still contain a huge number of unidentified marine species. Although net tows and trawls can be used to collect samples of these creatures, there is more recent technology that will speed up research in this region.

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Part II

Communities of Special Interest

Cyanobacteria in Ocean

4

Sonam Dwivedi and Iffat Zareen Ahmad

Abstract

An important turning point in the Earth's surface's geochemical history may be seen with the appearance of cyanobacteria, which were widespread towards the end of the Pre-Cambrian. Marine cyanobacteria are not only an important source of atmospheric oxygen, but they are also prolific manufacturers of secondary metabolites, frequently in spite of having incredibly small genomes. These organisms create a wide variety of complex secondary metabolites, including pigments, fluorescent dyes, and physiologically active substances of particular interest to the pharmaceutical sector. A diverse phylum of nitrogen-fixing, photo-oxygenic bacteria with the ability to colonize a variety of settings is known as cyanobacteria. Aside from their primary function as diazotrophs, they also produce a large number of bioactive compounds, frequently as secondary metabolites, with a variety of biological and ecological activities that should be further studied. Of all the species that have been found, cyanobacteria are able to coexist in marine habitats in symbiotic partnerships with creatures like sponges, invertebrates. It has been shown that these symbioses significantly alter the physiology of cyanobacteria and cause the synthesis of bioactive chemicals that are typically not produced. In fact, an exchange of infochemicals causes metabolic alterations in cyanobacteria involved in symbiotic relationships and activates pathways that have been shut. Studies on drug discovery have shown that these compounds have intriguing biotechnological possibilities. This chapter reviews the importance of cyanobacteria in ocean and their economic importance, and future prospects.

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4.1 Introduction

Despite usually being referred to as blue-green algae, cyanobacteria are photosynthetic prokaryotes and, as evidenced by fossil records are thought to be among the planet's first living species (Demoulin et al. 2019). Due to their oxygenic photosynthesis, they contribute to the transition of the earth's atmosphere from one that is rich in carbon dioxide to one that is rich in oxygen (Allen et al. 2019). Cyanobacteria have evolved throughout the course of their prolonged evolutionary history to become several species with a range of morphologies and niche environments (Mutalipassi et al. 2021). Due to the high taxonomic diversity within the phylum, cyanobacteria can be found in a wide range (Gaysina et al. 2019). They can also be found in unnatural habitats for phototrophs like the subsurface of calcareous rocks (Khomutovska et al. 2020) and lava caves (Kosznik-Kwaśnicka et al. 2022), Arctic Ocean, Antarctic valleys and thermophilic lakes (Sohm et al. 2020). Through evolutionary history, cyanobacteria have evolved unique ways of interacting with other organisms and, through unique genetic pathways, produce various secondary metabolites (Pawlik-Skowrońska et al. 2019). Over the past 10 years, there has been an increase in interest from the pharmaceutical and public health sectors in cyanobacterial research, which has resulted in an expansion of the uses of cyanobacterial metabolites to include pigments, the production of food and fuel, and other biotechnological applications. Microcystins and cylindrospermopsin have historically been studied for their role in disease or for their therapeutic properties, such as UV protective, antibacterial, and anticancer. These are also able to induce gastrointestinal illness, liver disease, and kidney damage (Diez-Quijada et al. 2021; Kubickova et al. 2019).

In mesotrophic and oligotrophic oceans from temperate to tropic regions, prokaryotes and microbial eukaryotes phytoplanktons play an important role in primary production, biomass, and photosynthesis. While in temperate mesotrophic water, photosynthetic microbial eukaryotes contribute significantly to primary production, they can also play a large role in subtropical ocean ecosystems. They are widely distributed throughout the surface of oceans, and their significant biodiversity helps the marine ecosystems stay stable, durable, and efficient. In oligotrophic marine regimes, unicellular cyanobacteria are acknowledged as important photosynthetic prokaryotic participants in the microbial food webs and biogeochemical cycles. Despite being crucial parts of neritic and coastal food webs, especially those in areas that support significant fisheries, photosynthetic prokaryotes are frequently left out of routine management plans for coastal waters. It is well recognized that a fundamental aspect of the oceans is to understand how photosynthetic prokaryotes contribute to the trophic cascade of energy and matter in more productive environments (Trombetta et al. 2020; Sánchez-Baracaldo et al. 2022).

Temperature is considered to be the main factor restricting the geographic dispersal of a particular cyanobacterial flora that is specific to tropical marine habitats. Compared to the open ocean, the littoral zones have the greatest diversity of cyanobacteria. Here, they build symbiotic partnerships, particularly with sponges and ascidians, or exist as endoliths in carbonate substrates. They are also a source of a variety of bioactive compounds, some of which are essential as herbivore deterrents, and their diversity, which is still mostly unexplored, especially in the less accessible infralittoral. Although cyanobacteria are important photosynthetic contributions to benthic and open ocean primary production, it seems that their main role in tropical marine ecosystems is that of nitrogen fixers (Rajaneesh et al. 2020). In the frequently oligotrophic tropical oceans, non-heterocystous cyanobacteria play a key role by serving as a significant nitrogen source for the marine and global nitrogen cycles (Manning et al. 2021) and their different role as shown in Fig. 4.1 and Table 4.1.

Zeaxanthin and chlorophyll b are two pigments that point to the existence of *Synechococcus* and *Prochlorococcus* in the area; however, *Trichodesmium* blooms have been observed on occasion and have been associated with significant levels of nitrogen fixation (Moore et al. 2019). In many regions of the world's oceans, molecular-level investigations of phytoplankton population structure are currently frequent, but a thorough evaluation of the cyanobacterial in these important waters has not been carried out. High-throughput molecular analysis of both prokaryotic and eukaryotic phytoplankton, flow cytometry, and physicochemical data will be used to close this gap. It emphasized the unique biological and physiological traits

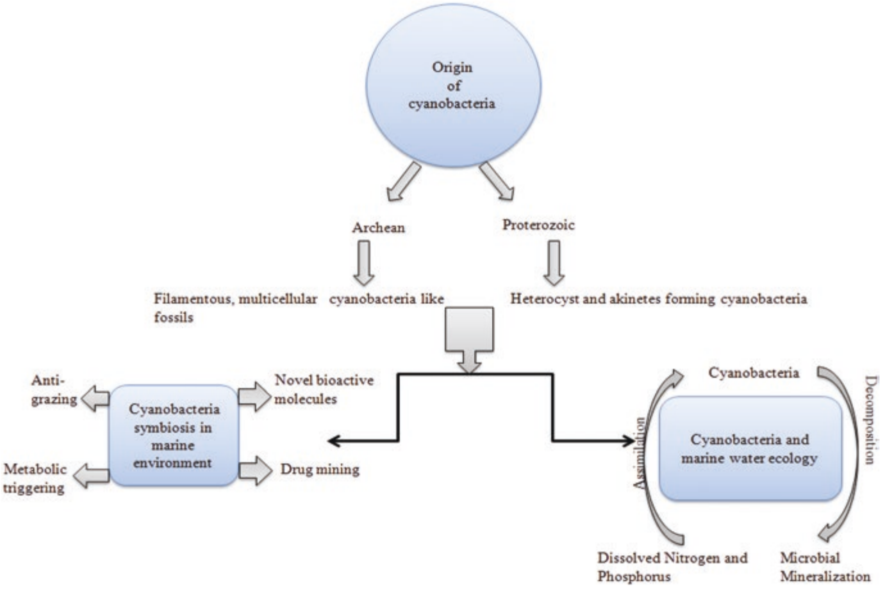

Fig. 4.1 Different roles of cyanobacteria in marine environments

Table 4.1 Significant role of cyanobacteria in marine environment

	Parameter	Species	Role	References
1	Eutrophication	*Microcystis*, *Aphanizomenon* (nitrogen-fixing cyanobacterial blooms)	Increase primary production, organic matter export to depth, and associated oxygen consumption by adding bioavailable nitrogen to the already over fertilized system	Beversdorf et al. (2013) and Munkes et al. (2021)
2	Diazotrophs	*Crocosphaera*, *Richelia,* UCYN-A and *Trichodesmium*	For the structure and operation of ocean ecosystems, cyanobacterial diazotrophs' nitrogen contributions are essential	Detoni et al. (2022)
3	Symbiosis	*Nostoc, UCYN-A*	Provide good understanding and knowledge for novel co-cultivation techniques, enhancing the environmentally sustainable production of desired compounds	Mutalipassi et al. (2021) and Harding et al. (2018)
4	Carbon mineralization	*Sprullina, Oxyrrhis marina,* Synechococcus sp., *Blennothrix ganeshii*	To interpret paleontological evidence, method for biological carbon absorption and storage, understanding of biologically induced carbonate mineralization (CCS)	Kamennaya et al. (2012) and Beltrán-Magos et al. (2013)
5	Alkane production	*Synechococcus* and *Prochlorococcus*	Populations of bacteria that break down hydrocarbons can quickly increase in number in response to localized releases of crude oil through natural seepage and human activity. Cyanobacterial alkane production is likely sufficient to support these populations	Lea-Smith et al. (2015)

of cyanobacteria that govern the type of interactions they have with particular ecosystem elements and the function of marine cyanobacteria in ecosystems as a whole. The distribution and biological variety of cyanobacteria in marine ecosystems and symbiotic relationships are now well understood thanks to these fresh discoveries.

4.2 Origin of Marine Cyanobacteria

Our planet's geochemistry has undergone a dramatic transformation due to cyanobacteria (Sánchez-Baracaldo et al. 2022). The existence of periods of major global environmental change at the start and end of the Proterozoic era is supported by a number of geochemical evidence. The Great Oxidation Event, which is known as the initial step in the oxygenation of the Earth's surface, occurred in the early Paleoproterozoic, according to geochemical data (Javaux and Lepot 2018). Around

800 to 500 Mya, a second, considerably steeper spike in oxygen levels known as the Neoproterozoic Oxygenation Event took place (Williams et al. 2019). Since the origin of planktonic cyanobacteria dramatically altered the nitrogen and carbon cycles towards the end of the Pre-Cambrian, understanding their evolution is an essential feature. However, it is still unknown what evolutionary processes gave rise to open-ocean planktonic cyanobacteria and how these processes link to geochemical evidence from the Pre-Cambrian. So far, it appears that nutrient availability and ocean geochemistry played a role in the Neoproterozoic delay in planktonic cyanobacteria's extensive colonization of open-ocean environments and apparent diversification. Marine phytoplankton today contributes to almost half of the Earth's total primary production. Only a few cyanobacterial species, including *Crocosphaera, Trichodesmium, Prochlorococcus,* and *Synechococcus,* dominated the open ocean. Since they influence primary production and the export of organic carbon to the deep ocean by converting nitrogen gas (N2) into ammonium (NH4+), which is then needed to make amino acids and proteins, N-fixing cyanobacteria are particularly significant among these species. *Prochlorococcus and Synechococcus,* two marine cyanobacteria, quantitatively dominate the majority of phytoplankton communities in today seas and play a significant role in primary productivity. Although certain planktonic cyanobacteria, such as *Crocosphaera, Prochlorococcus*, and *Synechococcus*, are unicellular and free-living cells, other cyanobacteria have developed symbiotic associations with prymnesiophyte algae (Flombaum et al. 2013). To investigate the time of significant evolutionary events that resulted in the late appearance of cyanobacteria, two distinct databases of protein and nucleotide sequences, as well as five different types of substitution models, were used (Zhou et al. 2022b). This study also demonstrates the evolution of marine cyanobacteria from benthic freshwater and marine ancestors (Boden et al. 2021).

4.3 Role of Cyanobacteria in Baltic Sea

Central northern Europe's Baltic Sea is a shallow, brackish, and partially confined body of water. Around 84 million people live in the densely populated drainage basin. The environment is put under stress by their imprint. The eutrophication issue is one that is extremely serious (Murray et al. 2019). The Baltic Sea receives anthropogenic fertilizers from rivers and air-sea fluxes (Ning et al. 2018). Numerous international environmental management plans have been implemented since the first Helsinki Convention in 1974, with varied degrees of effectiveness. On the one hand, multinational efforts have been effective in significantly reducing nutrient loads in the Baltic Sea, one of the most studied and managed oceans in the world (Pihlainen et al. 2020). However, despite all of the resource-intensive management initiatives, the ecosystem's condition has not yet greatly improved. Sedimentary processes are thought to be one of the main causes. Blooms of the nitrogen-fixing cyanobacteria, which are thought to increase in frequency with rising temperatures, are another issue. Cyanobacteria's capacity to use dinitrogen, an almost infinite source of nitrogen in the air, and transform it into accessible nitrogen ties their dynamics closely to the Baltic Sea's eutrophication issue by adding nutrients to an already overfarmed ecosystem (Olofsson et al. 2021). The fixed nitrogen is well considered to provide a substantial contribution to the total nutrient budget. Quantitative estimates place the phytoplankton community's share of the total new nitrogen supply between 20 and 50% (Piontek et al. 2019). How the overall amount of fresh nitrogen input will fluctuate in the future is yet unknown. The effects of environmental management-induced changes in nutrient loads are less clear, even though increasing temperatures are likely to result in more blooms. One argument is that reducing the loads will not have a significant impact on the nutrient budget because cyanobacteria will make up for the loss by fixing more atmospheric nitrogen. According to the opposing viewpoint, decreased loads would result in decreased primary productivity since nitrogen fixation is capped and can't entirely make up for decreased nutrient loads. Some of the cyanobacteria studies that have been evaluated make the assumption that the Baltic Sea's already severe oxygen shortage will get worse due to global warming. Lower oxygen concentrations result from a decrease in the solubility of oxygen in seawater due to warming. Furthermore, because they are better suited to oligotrophy and gain from the increased light levels that occur along with increasing stratification in response to increased air-sea heat fluxes, warming circumstances may favour cyanobacteria (Carstensen et al. 2020). Increased primary production and subsequent export of organic matter to depth are both aided by increased nitrogen fixation, which also aids in overcoming oligotrophy (Stigebrandt and Andersson 2020). The propensity of some species to release poisons is another reason to comprehend the dynamics of cyanobacteria in the Baltic Sea. There is evidence that cyanobacterial toxins can produce more as nitrogen levels rise. In addition, it has been observed in cultures that toxicity peaks during the time when the growth of the relevant cyanobacteria is at its best. This is an issue as the toxins may cause large-scale extinctions of fish, animals, and filter-feeding creatures. *Nodularia spumigena,* one of the most important cyanobacteria species in the Baltic, generates the toxin *Nodularin. Dolichospermum* sp. and *Aphanizomenon flos-aquae,* which can also create poisons, provide a further thread (Munkes et al. 2021). Intense cyanobacterial blooms can cause a harmful loss of water clarity in addition to being toxic. This can shadow benthic macrophytes in shallow coastal locations, effectively decreasing their development and survival, which has a severe impact on invertebrates and fish that depend on macrophytes for food and refuge. Despite cyanobacteria's significance for the Baltic Sea environment, little is known about the processes that lead to cyanobacterial bloom production. Many biotic and abiotic variables that support the growth of cyanobacteria have been proposed and are frequently the subject of heated debate (Munkes et al. 2021).

4.4 Role of Cyanobacteria in Proterozoic Oceans

For geobiologists, figuring out how biology fits into the evolution of planets continues to be a major difficulty. The lack of well-preserved rocks from the Archean to Proterozoic Era is impeding efforts to solve this riddle for Earth. A poor fossil record was also left by the microscopic life that pre-dominated the Earth's biota for the most of its history. This record mainly consists of membrane-derived hydrocarbon molecules, uncommon microbial fossils, and lithified microbial mats (Hamilton et al. 2016). The formation of oxygen or other oxidizing radicals, however, significantly altered Earth's surface and atmosphere throughout the Proterozoic Era, pushing it away from the more reducing conditions that prevailed during the Archean, as shown by the sulphur isotope record and other geochemical proxies (He et al. 2021). We can reconstruct Earth's redox evolution using information from ancient rocks as well as current biota-catalyzed biogeochemical processes. The evolution of oxygenic photosynthesis in prehistoric cyanobacteria was one of the most important microbiological breakthroughs in Earth's history (Demoulin et al. 2019; Sánchez-Baracaldo and Cardona 2020). Currently, oxygenic photosynthesis is the primary source of O_2 in the atmosphere. Therefore, the study of microbial metabolisms and evolution offers a crucial link between the knowledge from the geologic record and the current biota. Many publications combine current knowledge of these ancient microbial mediators in planetary redox history with insights from the geological record and cyanobacteria currently present in early Earth analogue environments. Research lends credence to the idea that during the Proterozoic Era, anoxygenic photosynthesis, including the work of metabolically adaptable cyanobacteria, was crucial in postponing the oxygenation of Earth's surface ocean (He et al. 2021).

One of the most intriguing problems in contemporary geoscience is figuring out how the chemistry of the Earth's surface has changed over time. According to the prevalent theory, the early Earth had far less oxygen than it does now, slightly decreasing circumstances and traces levels of CH4 in the atmosphere during the Archean Eon (Evans et al. 2019). The sun shows on Earth's surface at this time as well, but it may have been brighter than it is now due to the larger hydrogen-to-helium ratio in its core (Owen et al. 2020). The great oxidation event (GOE) is also known as the first large rise in atmospheric oxygen. Even though there is a significant lot of controversy regarding atmospheric oxygen concentrations during the Proterozoic era, ozone levels after the sudden increase of GOE were lower than the current atmospheric level (Olejarz et al. 2021). According to current estimations, oxygen levels in the atmosphere did not reach their current level for 2 Gyr after the GOE. The virtually total domination of prokaryotes was finally overthrown when oxygen reached its current levels, entering in a new period marked by an abundance of multicellular life (Hamilton et al. 2016). The elimination of mass-independent sulphur isotope fractionation in mineral sulphides and sulphates provides the strongest evidence yet for the increase in oxygen (Luo et al. 2016). The mass-independent fractionations of gas-phase sulphur compounds produced by volcanoes during the Archean epoch were brought about by short-wavelength photochemical reactions. Since photochemical processes required very low oxygen levels for them to persist in the rock record, the disappearance of this signal in rocks dating indicates that oxygen was prevalent in the atmosphere (Catling and Zahnle 2020). The emergence of rusty red soils, the rise of ringed iron formations, and the disappearance of sedimentary detrital grains made of easily oxidized minerals like pyrite and uraninite all occur at this time (Hamilton et al. 2016). Cyanobacteria, the only life forms capable of oxygenic photosynthesis, are likely the first substantial biotic supply of oxygen on early Earth. However, the biological production of oxygen by oxygenic bacteria engaged in nitrite-driven anaerobic methane oxidation may also have contributed to the GOE (Welte et al. 2016). Regarding the relative chronology of the genesis of intra-aerobic denitrification and oxygenic photosynthesis, there is still some doubt (Rizzo et al. 2019).

The deep waters most likely remained anoxic until 0.5 Gya, when atmospheric oxygen levels increased to levels close to those of today. According to the prevailing theory, the deep ocean was oxygen-poor, iron-rich, and sulphidic during this epoch, especially in constrained basins and along productive margins. Due to oxygenic photosynthesis and geological processes, sulphidic environments would have been unstable as atmospheric oxygen levels rose (Camacho et al. 2017). Rocks from the Proterozoic period, however, provide evidence of persistently low oxygen levels (Miletto et al. 2021). Mn is a very good redox analogue because it may be oxidized by high-potential oxidants like O_2 or photosystem II.

4.5 Role of Cyanobacterial in Alkane Production in the Ocean

Hydrocarbons are a common presence in the vast oceans, where both natural seepage and human activities contribute to the release of crude oil. It is estimated that annually the ocean ecosystem is affected by anywhere between 0.4 and 4.0 million tons of crude oil. This can have a significant impact on marine life and the overall health of our oceans (Love et al. 2021). Even in the least contaminated marine surface waters, alkanes like pentadecane and heptadecane, albeit their sources are still unknown (Lea-Smith et al. 2015). Particulate matter >0.7 m in diameter is related with a minor percentage of alkanes, from 1 to 60 fg/mL (Chen et al. 2021). In marine systems, populations of hydrocarbon-degrading bacteria, also known as hydrocarbonoclastic bacteria, are present and play a significant part in the turnover of these chemicals (Xu et al. 2018; Chernikova et al. 2020). These bacteria include many species that cannot utilize alternative carbon sources. Obligate hydrocarbon-degrading bacteria must use an alternative hydrocarbon source because they are present in waterways that do not have large quantities of crude oil pollution (Yakimov et al. 2022). Cyanobacteria can create C15 to C19 hydrocarbons through two different methods. The widespread distribution of cyanobacteria in the marine environment raises the possibility that the waters are home to a sizable and widely scattered geographic hydrocarbon production (Parveen and Yazdani 2022).

4.6 Role of Cyanobacteria in Tropical and Subtropical Marine Environments

These cyanobacteria aid the host organism in symbiotic partnerships in marine environments by fixing nitrogen or releasing dissolved organic carbon, which is crucial in oligotrophic waters (Mills et al. 2020). There are 150 genera and over 2000 species of cyanobacteria. The two smallest known cyanobacteria in aquatic environments, termed as picophytoplankton (3 m in size), are *Prochlorococcus* and *Synechococcus* (Jakubowska and Szeląg-Wasielewska 2015). These tiniest creatures make up a large portion of phytoplankton in freshwater and marine environments, including those with nutrient-rich to depleted habitats. They also play a key role in primary productivity and overall phytoplankton biomass. *Synechococcus* was the first of them to undergo extensive research. They are rod- to coccoid-shaped organisms that can range in size from 0.8 to 1.5 m and split into equal halves through binary fission in one plane. They are the predominant phycobilisome-containing cyanobacteria in freshwater and marine aquatic ecosystems, and they are often more prevalent in oligotrophic than nutrient-rich areas (Reinl et al. 2021). *Synechococcus* is divided into two categories based on the composition of its phycobilisomes, one of which contains phycoerythrin and the other phycocyanin. Although the latter is only found in freshwater and estuarine habitats, the former group is present in all types of aquatic systems (Grébert et al. 2022). The discovery of *Prochlorococcus* was a turning point in the study of biological oceanography. They stand in for the little plant in the aquatic ecology. This genus has tiny (0.60.8 m) diameter members that can survive in oligotrophic environments. They are the most prevalent photosynthetic organism on the earth and have the coccoid shape. They are non-motile, free-living cells. In oligotrophic waters, *Prochlorococcus* can contribute 13% to 48% of the net primary and 21% to 43% of the photosynthetic biomass (Rii et al. 2016). It is well known that *Trichodesmium* frequently makes up a significant portion of the nitrogen-fixing cyanobacterium in tropical, oligotrophic environments and contributes significantly to the biogeochemical cycle, in addition to *Prochlorococcus* and *Synechococcus* (Bergman et al. 2013). This filamentous cyanobacterium is thought to be responsible for around half of the ocean's estimated nitrogen fixation. Due to the limited number of shipboard sightings, nothing is currently known about its global spread. However, remote sensing methods can offer a remedy to close that gap in worldwide *Trichodesmium* mapping (Blondeau-Patissier et al. 2014; Groom et al. 2019). By creating a group-specific algorithm based on the data acquired by calculating the bio-optical properties of specific genera using remote sensing photos, the large-scale biomass distribution of a given group may be analyzed. For the same reason, algorithms are used to find *Trichodesmium* from space. The first global maps of the occurrence of *Trichodesmium* blooms from satellite oceans colour data and an explanation of their regional and temporal distribution throughout the world's oceans (Groom et al. 2019). The southwest and northeast monsoons have an impact on the tropical regions of India, which distinguishes these waters from those in other areas. Due to monsoonal activities, the freshwater discharge during monsoon and tidal activity during non-monsoon seasons primarily determine the annual variance in hydrodynamics in coastal waters (Naik et al. 2020). The Arabian Sea is located in the northwest corner of the Indian Ocean, and because of its semi-enclosed nature, it has a peculiar hydrography, climate, and biogeochemical processes (Rixen et al. 2020). The physical characteristics of the Bay of Bengal also exhibit a great deal of variation. Studies on cyanobacteria in these waters are currently receiving attention, particularly those on *Synechococcus* and *Prochlorococcus*. These studies demonstrate how these cyanobacterial growths, community structure, and dispersion are impacted by temperature and salinity change, water column stability, and freshwater discharge (Xia et al. 2017; Affe et al. 2018). Numerous studies on cyanobacteria, their economic significance, biofuel, fertilizer, medication development, etc., have been conducted on a global scale. These issues, which primarily concern the freshwater ecology, are the subject of ongoing research in the tropical and subtropical waters off the coast of India. More specialized research on marine habitats is now needed, particularly on bloom forms, symbiotic interactions between the creatures that generate blooms, and their ecology. Apparently, cyanobacterial blooms can cause the oxygen levels in the water to plummet, which can have serious consequences for the health of fish and other aquatic creatures. Additionally, these blooms can release harmful chemicals that can be dangerous for humans who come into contact with the water. The nitrogen-fixing cyanobacteria seem to thrive in the presence of other bacteria and archaea. These microorganisms work together to create a healthier environment for themselves and other living things. The nitrogen-fixing cyanobacteria benefit greatly from the presence of bacteria and archaea (Bhadury and Singh 2020). Since *Prochlorococcus* lacks chlorophyll a but does have marker pigments like divinyl chlorophyll a and divinyl chlorophyll b, it differs biochemically from other cyanobacteria (Barrera-Rojas et al. 2018). In the ocean, *Prochlorococcus* fills a specific biological niche (Aldunate et al. 2020). Divinyl chlorophyll b/divinyl chlorophyll a ratios are lower in *Prochlorococcus* strains that have acclimated to high light (HL) than they are in bacteria that have adapted to low light (LL) (Ulloa et al. 2021). Both HL- and LL-adapted *Prochlorococcus b*acteria have been found in the Arabian Sea, according to studies. The molecular analysis of these populations is not well understood, yet. Recent research on single-cell genomics has revealed the existence of a few more ecotypes and a number of coexisting subpopulations in the wild *Prochlorococcus*. According to recent findings, physical factors including convective mixing, density stratifications, and upwelling have a significant impact on where cyanobacteria are found in the Indian Ocean. For instance, the abrupt disappearance of Prochlorococcus in the northern Indian Ocean following the winter mixing is due to the shifts in the oxygen and light regimes, which have a significant impact on their survival. However, the exact mechanism behind such an effect is still not known. In general, phycoerythrobilin (PEB)-rich populations predominate in mesotrophic or coastal waters, whereas phycourobilin (PUB)-rich populations are exclusively seen in oceanic waters. PUB:PEB ratios exhibit an upward trend with water column depth. *Synechococcus* strain CSIRNIO1, a member of phylogenetic group II, was found to contain phycoerythrin I, a pigment that effectively uses green light. Temperature was primarily responsible for controlling the vertical spread of *Synechococcus* in the Arabian Sea (Rajaneesh et al. 2017). *Synechococcus* abundance was low despite the high nutritional condition at a depth of 75,100 m. This shows that the effects of temperature and irradiance dominate the availability of nutrients for their growth. The number and spread of *Synechococcus* in the Arabian Sea are significantly influenced by physical factors such as convective mixing and advection (Bemal et al. 2018). The Arabian Sea's little-understood grazing pressure may also contribute to the low *Synechococcus* abundance. Due to a lack of sophisticated tools, *Synechococcus* has hitherto been overlooked in estuary and coastal water research. In addition, it is believed that small-sized cells are unimportant in settings abundant in nutrients

(Xia et al. 2017; Wei et al. 2019). However, *Synechococcus,* particularly during hot weather, produces more than 50% of the total phytoplankton biomass in estuarine and coastal waters (Paerl et al. 2020). In the Zuari estuary, Goa, it was shown that the spatiotemporal variation of *Synechococcus* was strongly correlated with hydrographic changes (Śliwińska-Wilczewska et al. 2018). Red and blue light are significantly reduced in turbid waters and green light transmits the most. One of the variables affecting the dispersion of *Synechococcus* in marine, coastal, and estuarine environments is the difference in light quality. It illustrates the significance of the blue-green light for the accessory pigments in *Synechococcus.* Therefore, it is crucial from an ecological perspective to include Synechococcus in studies of phytoplankton. Picophytoplankton cyanobacteria were found to predominate in Goa's coastal bay waters, according to a daily observation study. This finding emphasizes the significance of high-frequency sampling in dynamic coastal regions so that transient responses can be recorded and used as tracers of environmental forcing brought on by tides and freshwater influx (Albrecht et al. 2017; Zheng et al. 2019). *Synechococcus* harbouring phycoerythrin and phycocyanin displayed different ecological niches in these waters. Eutrophic waters had high levels of *Synechococcus* phycocyanin, whereas clear waters had high levels of *Synechococcus* phycoerythrin. These results suggest that the distribution pattern of *Synechococcus* can be used as a trophic status indicator for coastal water bodies. Since 1942, *Trichodesmium* blooms have been documented (Kumar et al. 2017; Rousset et al. 2018). Additional research found that these blooms are primarily restricted to the spring intermonsoon season. *Trichodesmium* appears to have grown more rapidly during this season. There have been blooms in the Bay of Bengal on multiple times, according to earlier researchers. Due to the creation of tropical cyclones and the seasonal reversal of winds and currents affected by monsoon, the Bay of Bengal, which is the north east part of the Indian Ocean, is characterized by riverine flow clays. It has been suggested that the Bay of Bengal has stratified conditions for the majority of the year, which favours *Trichodesmium* predominance. Because of these characteristics, the Bay of Bengal is a unique oceanic region, and knowledge of the spatiotemporal fluctuation in the distribution of *Trichodesmium* in this region will reveal new details about the long-term viability of this significant nitrogen-fixing organism. *Trichodesmium* in the Bay of Bengal is dispersed mostly by ocean surface currents. A small stream that travels from the Malacca Strait to southern Sri Lanka is known as the North Equatorial Current (Martin and Shaji 2015). It is most noticeable from March to April. This characteristic of dispersal is further strengthened by observations made elsewhere, including the encouragement of high *Trichodesmium* abundance in coastal locations. The cyanobacteria group contains a particular carotenoid marker pigment called zeaxanthin, which is why in theory it can be used as a tool to comprehend their presence or absence in the samples, according to this method, which is also known as "chemotaxonomy." Given that cyanobacteria are challenging to find using conventional methods and have thus eluded scientists for a considerable amount of time, this was a significant accomplishment in the field of biological oceanography. Recent research has also pointed to cyanobacteria as a significant source of the marine atmosphere's crucial trace gases for the climate (Zhou et al. 2022a). One such instance is halocarbons, which are significant due to their capacity to cause greenhouse warming and deplete ozone in the stratosphere.

Trichodesmium blooms have been closely associated with their presence in the water column throughout the Arabian Sea's intermonsoon summer (Follett et al. 2018). *Trichodesmium* and *Richelia intracellularis* make up more than 60% of the marine diazotrophs that fix nitrogen in the pelagic zone of the world's seas (Wu et al. 2018; Tuo et al. 2021). *R. intracellularis* has demonstrated symbiotic relationships with numerous diatom species (Mutalipassi et al. 2021). These results reveal the extensive endosymbiotic consortia of *R. intracellularis* in the world's waters and call for a more in-depth analysis to determine its regional contributions to primary and new production (Pierella Karlusich et al. 2021).

4.7 Ecological Role of Cyanobacteria in Marine Environment

Trichodesmium and other cyanobacteria's associated epibionts have been studied for their potential in biotechnology and the removal of pollutants like hydrocarbons. They together have a significant impact on the cycle of carbon, phosphorous, and nitrogen in subtropical surface oceans. The ecological significance of these associations, their current and potential contributions to the world's nutrient cycles, and their potential applications in biotechnology and the remediation of blooms and pollutants can all be better understood with increased efforts to identify these epibiont communities and their metabolic potential. The possibility of using heterotrophic bacteria, which have the capacity to break down cyanobacterial toxins, to reduce cyanobacterial blooms in drinking water reservoirs has been explored. A Sphingopyxis strain that removed microcystins from water using sand filters was identified. Cyanobacterial blooms may be controlled by using bacteria that have growth-inhibiting activities against cyanobacteria. However, these steps can only be implemented after thorough examinations of the traits of these bacteria and their interactions with cyanobacteria have established their safety. All oceans contain cyanobacteria, which can take on a variety of morphological forms depending on the time of year and the environment. They are a component of the freshwater and marine plankton and benthos. Cyanobacteria create physiologically active compounds and a sizeable amount of the oxygen that enters the atmosphere as they are at the base of the majority of food chains. The majority of cyanobacterial species are widespread and global, meaning they can be found in a variety of ecological niches. Particularly fascinating are marine cyanobacteria. Oceanic phytoplankton does not include a large number of them, while inland seas with enhanced eutrophication may have up to 20 co-occurring species. Phytoplanktons of the order Oscillatoriales are very abundant. Our understanding of the structure and operation of marine ecosystems was fundamentally altered by the late nineteenth discovery of unicellular cyanobacteria within the marine microbiota. Coccoid cyanobacteria of the genus *Synechococcus Nägeli, Synechocystis Sauvageau*, and *Prochlorococcus* are the principal members of this ecological group. The immense expanses of the world's seas are dominated by photoautotrophic picoplankton, which also plays a significant role at the start of marine food webs as a potential carbon source. Sargasso

Sea's output is made up of the bigger *Synechococcus,* whose dominance can result in the creation of relatively large granules that make the efficient export of carbon to higher trophic levels possible. The filamentous nitrogen-fixing Trichodesmium is widely distributed in the Pacific Ocean. In the summers of 1985 and 1986, chroococcoid cyanobacteria were abundant in the surface waters of the southern section of the ocean between Australia and Antarctica. Temperature, which had an exponential relationship with cell number, was the primary factor of their abundance. Occasionally, increased salinity could act as a hindrance to the development and spread of cyanobacteria. However, certain native forms show resistance to variations in this parameter; as a result, Cyanobacteria can be classified according to how sensitive they are to, or how well they can adapt to, increased salinity. High geographic variability in phytoplankton abundance and biomass, as well as its associations with temperature and nutrient availability, were found in water samples from the northern Ionian Sea.

4.8 Economic Importance of Marine Cyanobacteria

Possibly the most significant class of microbes on Earth is the cyanobacteria. They were among the first people to settle the desolate areas of various maritime areas. In the waters of the planet, cyanobacteria perform crucial ecological tasks by making significant contributions to the carbon and nitrogen budgets on a worldwide scale. The use of marine cyanobacteria in the field of biotechnology has received a lot of interest lately. They have been the subject of numerous recent investigations due to their wide range of industrial applications, including biofuels, coloured pigments, food additives, and biofertilizers. In addition, they are applied in nanobiotechnology and used in the manufacturing of bioplastics, hydrogen production, water treatment, cosmetics, forestry, and animal feed. The most in-demand biofuels include bioethanol, biodiesel, hydrogen, and gas. Also mentioned were the comparative yields of the biofuels made from cyanobacteria, microalgae, and other natural sources. The global cyanobacteria can be found in practically any environmental niche. The only prokaryotic multicellular creatures with functionally specialized cells are these bacteria. Cyanobacteria are quite biologically diverse and can adapt to a wide range of environments, even harsh ones, in marine ecosystems. Cyanobacteria are one of the most crucial elements of food chains and, in some situations, directly influence the formation of a community due to the peculiarities of their metabolism. They also play a significant role in the balance of nutrients in marine ecosystems, particularly carbon and nitrogen. However, the widespread emergence of cyanobacteria, particularly toxin-producing species, can be detrimental to the marine organism population. Toxic blooms that result in the destruction of natural ecosystems can seriously harm both public health and economic activity. It is important to consider the potential effects of cyanobacterial overgrowth in semi-artificial environments, such as marine aquariums and aquaculture. In recent years, it has become increasingly clear that cyanobacteria play a vital role as collaborators in symbiotic relationships with a range of uni- and multicellular eukaryotic organisms. The biological characteristics of cyanobacteria are of particular significance for comprehending the evolution of life on Earth and for reestablishing contemporary ecosystems following natural and man-made calamities. An effective indicator for assessing the state of the ecosystem is cyanobacteria. Thus, knowledge of the diversity of cyanobacteria must unquestionably be taken into account when researching marine ecosystems and the processes that shape and transform them.

Thylakoids are one of the distinguishing features of cyanobacteria. One of the earliest signs of life on Earth was the production of stromatolite reefs, which are still discernible as fossilized forms in the oldest known rocks. One of the oldest fossil records of any group of animals, this cyanobacterial fossil record may be as old as 3500 million years (myr). The formation of multiple shallow reefs, which served as cyanobacteria's habitat, occurred over the subsequent 3000 myr. Even while cyanobacteria have been mostly replaced by eukaryotic algae on modern coral reefs, particularly the dinoflagellate *Symbiodinium* sp. and coralline red and green algae, they nevertheless play a significant role in the ecology of reefs today. The benthos and plankton compartments of coral reef ecosystems now contain cyanobacteria. Although symbiosis was once dismissed as an ad hoc evolutionary phenomenon, there is now abundant evidence that these obligate or facultative relationships between microorganisms and their multicellular hosts were crucial in the development of phenotypic diversity and complex phenotypes that could colonize new environments. Comparative examinations of complete genome sequences and the ability to recreate evolution at the molecular level have both shown that the integration of genes from many origins has taken place on a very big scale. Although lateral gene transfer is unquestionably significant in prokaryotes, symbiotic association—that is, a sustained close relationship with another species—is frequently required in multicellular eukaryotes to recruit foreign genes and subsequently acquire novel metabolic capacities. Symbiosis connects living things from all spheres of existence and has drastically altered their genomes and structural makeup. By promoting gene transfer from one genome to another and causing the loss of genes from one genome that is present in both symbiotic partners, symbiosis has an impact on genome evolution. Symbioses, particularly those containing cyanobacteria, are a highly promising potential source of novel chemical entities useful for the creation of functional components and the identification of new drugs, for all of the aforementioned reasons. Understanding the environmental variables that control cyanobacteria's growth, dispersion, and interspecific interactions in marine habitats may improve our understanding of how to promote the development of bioactive compounds for drug discovery. In this chapter, the significance of cyanobacteria's symbiotic relationship with a wide variety of marine creatures has been evaluated. These molecules have interesting biotechnological activities. The food, cosmeceutical, nutraceutical, and pharmaceutical industries can all use them.

4.9 Concluding Remarks and Future Perspectives

The evolutionary processes that resulted in the most recent evolution of marine planktonic cyanobacteria are clarified by this work. The formation of planktonic forms was influenced by the loss of filamentous forms, a reduction in cell diameter, and a switch in habitat choice from freshwater to marine. Most modern cyanobacterial groupings can be located in the Mid-Proterozoic. Benthic, terrestrial, and/or coastal marine habitats were probably home to early cyanobacteria. In contrast to the enormous oceans, cyanobacteria were first restricted to terrestrial and coastal habitats, which help to explain why the oxidation of the Earth's surface during the Pre-Cambrian Period was delayed. When planktonic phytoplankton started to dominate during the end of the Pre-Cambrian and the beginning of the Phanerozoic, primary productivity would have significantly increased. Due to monsoonal activities, the tropical Indian region is considerably distinct in terms of hydrographic conditions from the subtropical, temperate, and polar regions. These areas can shed light on several crucial functions that cyanobacteria play in the aquatic ecology. *Prochlorococcus* and *Synechococcus* make up the majority of the oligotrophic *Prochlorococcus* and *Synechococcus* in the waters of the Indian Ocean, with intermittent *Trichodesmium* blooms. An acute interest in the need for more research on *Synechococcus* and *Prochlorococcus* role in the microbial food web in transferring energy to a higher organism to maintain fisheries has been sparked by some of the intriguing data on their distribution and seasonality. To ensure the security of the environment and even their mode of symbiotic connections with other creatures, more study of the cyanobacterial blooms in Indian waters is also necessary. Furthermore, because cyanobacteria's significance was only recently realized and because the Indian Ocean has seen relatively little exploration, our knowledge of their involvement in marine biogeochemistry is currently quite limited. In coral reef ecosystems, cyanobacteria can be found everywhere: on the reef itself, inside and out, as symbionts of sponges, and covering soft bottoms as microbial mats in the water column. In addition, they have contributed to the reef's formation and erosion. They are significant primary producers, provide an organic food supply for benthic and planktonic heterotrophic species, and add nitrogen to the ecosystem.

It is difficult to comprehend how photosynthesis contributes to the increase in oxygen on Earth. Recent research into settings with both sulphide and oxygen present in the sun has discovered cyanobacteria with a wide range of metabolic capabilities that can do both oxygenic and anoxygenic photosynthesis. Our finest models for examining the ecological and environmental limits on primary productivity and oxygen production in Earth's past are these living creatures and ecosystems. It is necessary to have a better understanding of the physiology, ecology, metabolic diversity, and evolutionary history of cyanobacteria in these prehistoric analogue habitats. Omics methods, which are well within our technical capabilities, have the potential to shed light on the identity and evolutionary history of the genes and regulatory systems that regulate flows of carbon, nitrogen, sulphur, and oxygen. They may also offer new insights into the organic, isotopic, and geochemical biosignatures of cyanobacterial life that can help us interpret the rock record. Because their freshwater counterparts have historically received the majority of the attention in regard to metabolite production, marine cyanobacteria provide a largely untapped resource in terms of genetic variety and industrial potential. They are prolific makers of numerous complex secondary metabolites, many of which have potential uses in bioengineering, biofuels, and medicine. Due to their tiny genomes and minimal resource requirements, they make excellent candidates for genetic and metabolic engineering. When combined with their ability to convert sunlight into energy, these organisms have the potential to operate as low-cost, adaptive cellular factories capable of producing high-value commodities and biofuels with little environmental impact. Future creative endeavours will depend more and more on cyanobacteria and, in particular, marine life, from aerospace programmes to idea initiatives in sustainable building. Innovative architectural designs that incorporate algae have the potential to increase waste recycling, boost temperature control, and reduce the carbon footprint of commercial constructions. Despite the fact that there is still much to discover about marine cyanobacterial metabolites, important advancements are being achieved as a result of recent advances in molecular technologies. Maximizing the biotechnological potential of marine cyanobacteria will benefit from collaborations spanning the domains of ecology, genetics, chemistry, health research, and engineering. New technologies will be created as a result, such as ways to maximize the production of biofuel by using copious saline water resources and non-arable land, as well as ways to increase the spectrum of cyanobacterial metabolites' usage beyond their current applications.

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Marine Algae and Their Importance

5

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Abstract

Simple, non-flowering marine algae are a diverse group that range in size from unicellular (2 μm to 30 m) to multicellular forms (kelps up to 70 m). They are crucial for the formation of habitats and as a food source in the marine environment. Alga is the oldest member of the plant world, with origins going back several million years. They support life in the marine ecosystem by creating food webs, producing oxygen, and acting as the largest primary producer in the marine environment. They also act as habitats for many creatures. Algae are a major primary producer in the marine ecosystem and contribute more than 90% of the world's photosynthesis. They are made up of many kinds of big macroalgae and tiny algae. Photosynthetic organisms called marine algae (also known as seaweeds) inhabit the seas and oceans. They are acknowledged as having a number of advantages and serving as a source of several significant bioactive chemicals. We discuss facts about marine algae, including their taxonomy, distribution, and significance, in this chapter. Because it doesn't need land, irrigation infrastructure, additional nutrients, or fertilizers, marine algae have an advantage over terrestrial-based crops developed for biofuels. Farms that cultivate macroalgae for human and animal consumption are widespread around the world, whereas biofuel-focused farms are still in the experimental phase. For scientists conduct-

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ing studies in this field, the information presented in this review provides a scientific basis on marine algae.

Keywords

Anaerobic digestion · Brown algae · Dietary fibers · Marine algae · Red algae

5.1 Introduction

Marine algae are photosynthetic plant-like organisms that are found in aquatic environments, mainly in the sea; but some species are also found in rivers, lakes, and even wastewater. Algae have the ability to adapt to a wide range of environmental conditions such as temperature, salinity, pH, moisture, and different light intensities, which describes their distribution worldwide (Anbuchezhian et al. 2015). Algae are a very large and diverse group of more than 30,000 species which range from unicellular to multicellular types. They have different characteristics in which even their color, size, habitat, and nutrition are quite different (Pooja 2014). Algae are considered the primary producers of the aquatic environment due to the key role they play in the productivity of many nutrients and the marine food chain at large (Anbuchezhian et al. 2015; Pooja 2014; Hasan and Rina 2009). They are also considered the fastest-growing species that are largely productive, chemically unique, and biologically active, which gives a broad scope for their characteristics. They use different mechanisms to fix the atmospheric carbon dioxide and effectively utilize the nutrients that could be converted into biomass (Hasan and Rina 2009). Algae, like higher plants, exhibit inherent cellular mechanisms for using solar energy (light) for the photosynthesis process. Algae are classified into two major types: macroalgae (seaweed) and microalgae (Pooja 2014; Hasan and Rina 2009).

Macroalgae are large-size algae that are visible to the naked eye and are multicellular photoautotrophic organisms which in addition to being primary producers they also play a crucial role in the structuring and preservation of the marine ecosystem. Macroalgae differ in various aspects such as size (some species can grow up to tens of meters), morphology, ecophysiology, and longevity. They play a huge role in the marine ecosystem in that they also hold fast to the sediment and prevent coastal erosion; while other species have gas-filled-like structures to help in buoyancy. Based on their pigmentation, macroalgae are classified into three classes which are green algae (Chlorophyta), brown algae (Phaeophyta), and red algae (Rhodophyta) (Anbuchezhian et al. 2015; El Gamal 2010). Their growth rate could be more than 30 times faster than terrestrial plants and the oil content in macroalgae is also 30 times more than conventional crops. The oil derived from macroalgae is of better quality and the algal source is sulfur free and entirely biodegradable. Macroalgae are a prospective source of fuel for the production of biodiesel, bioethanol, biomethane, and biohydrogen due to their water content that is rich in lipids, protein, and carbohydrates (Godvin Sharmila et al. 2021). The percentage of lipids in macroalgae is very low, hence, macroalgae are more considered for natural sugars and the carbohydrates which they contain in large quantities for the production of biogas or alcohol-based fuel. The algae chemical composition can vary depending on the season of harvest, site of collection, environmental conditions, growth habitat, and time (Godvin Sharmila et al. 2021). In recent years, macroalgae have received more attention owing to their various health-promoting properties that can decrease the risks of many ill-health. In most Asian countries, hundreds of marine macroalgae are mainly used for human nutrition. Furthermore, macroalgae have also found use in water treatment and in agriculture as natural fertilizers, therefore improving the quality of the soil and agricultural products and limiting the use of chemical fertilizers. Due to their capacity to reduce carbon dioxide emissions, these aquatic organisms are further exploited as sustainable feedstock for biofuel production. The use of macroalgae as a source of renewable energy has been explored for years with constant improvement being done in this area (Anbuchezhian et al. 2015; Pooja 2014; Hasan and Rina 2009; Warner et al. 2015).

Unlike macroalgae, microalgae are microscopic organisms that are also found in freshwater and marine environment. They are highly diverse organisms with the ability to generate a wide range of useful chemicals and metabolites. Microalgae are unicellular microorganisms, which can exist individually, in chains or in groups. Their size ranges from a few micrometers to a few hundred micrometers depending on the type of species. Microalgae have a rapid growth rate compared to their terrestrial counterparts (Bajhaiya et al. 2017). A typical microalgae species can be evaluated by its ability to transform solar energy into biomass production and subsequently the formation of metabolites. Microalgae are photosynthetic autotrophic organisms, which are capable of producing complex compounds by using the available simple substances in their environment (Bajhaiya et al. 2017). Due to their ability to utilize carbon dioxide and solar energy to generate products without needing organic carbon, photosynthetic microalgae are attractive and a potential alternative to microbial factories that use bacteria and fungi. It is estimated that there are about 200,000 to 800,000 species of microalgae, with about 50,000 species identified. These aquatic microorganisms have developed a defense mechanism to survive in harsh and unfavorable environments because they are found in ever-changing climate conditions that unfortunately lead them to produce a wide range of chemical compounds with novel properties and various biological activities. The production of these chemical compounds varies from species to species, and no single species produce the same compound as they are depending on factors such as life cycle, environmental conditions, seasons, etc. By producing natural bioactive compounds, microalgae are a good natural substitute for the chemical synthesis of certain bioactive compounds that are of commercial interest (Mobin et al. 2019). In addition, these chemical compounds are physiologically active substances and offer high-value bioproducts for commercial use. Microalgae are classified based on their various characteristics that include their pigmentation, morphology, photosynthetic membranes, and storage arrangements. Microalgae species are divided into four groups, namely diatoms (Bacillariophyceae), green algae (Chlorophyceae), blue-green algae (Cyanophyceae), and golden algae (Chrysophyceae) (Rajkumar et al. 2014).

In this chapter, we go over some information concerning marine algae, such as their taxonomy, distribution, and importance. Marine algae have an advantage over terrestrial-based crops created for biofuels because it doesn't require land, irrigation infrastructure, additional nutrients, or fertilizers. Worldwide, there are many farms that grow macroalgae for human and animal consumption, but biofuel-focused farms are still in the experimental stage. The data offered in this review offers a scientific foundation on marine algae for researchers working in this area.

5.2 Properties of Marine Algae

Studies have demonstrated the positive effects that bioactive substances taken from marine algae have on human health. However, the commercialization of these high-value compounds has been restricted because of the poor bioactive chemical extraction yield, regulatory approval standards, and the high production costs. To guarantee the safety, effectiveness, and quality of the substances derived from marine algae, it is also necessary to address potential side effects, allergic reactions, heavy metal contamination, and toxins. Here are a few of the effects that bioactive substances derived from marine algae have.

We understand that most marine algae's primary structure is polysaccharide in nature though in conjunction with other chemical compounds in their diverse percentages as per the source of the algae.

Most of the compositional monosaccharide configuration, molecular weight (MW), backbone, as well as structure–function correlation of polysaccharide-based three main species of algae (red, brown and green algae) are summarized in Table 5.1.

5.3 Microalgae Harvesting Methods

Microalgae harvesting is crucial for the extraction of useful algal biomass for further processing and for end-product applications. Harvesting is the process of removing water from the microalgae culture, hence, the aim of harvesting is to generate slurry with around 2–7% algal suspension (total solid matter). There are four main methods of microalgae harvesting: centrifugation, floatation, filtration, and sedimentation (gravity settling), which have been extensively explored for both pilot and large-scale harvesting of algal biomass (Cassini et al. 2017). A pre-treatment method such as flocculation may be necessary for improving the harvesting yield. To increase the effectiveness of the harvesting method, the combination of two or more of these methods is often utilized. Selection of a harvesting method largely depends on several factors such as density and size of the microalgae and the desired final products (Fu et al. 2017; Balasubramaniam et al. 2021). An effective harvesting is a key to a good economical yield of the overall process. There is typically no best method when it comes to harvesting, as each method has its own

Table 5.1 The monosaccharide composition, molecular weight, backbone, polysaccharide type, and structure-function relationship of polysaccharide-derived from three main species of algae (red, brown, and green algae)

Species	Polysaccharide type	Molecular weight (Da)	Monosaccharide	Backbone	Biological activities	Ref
Red algae						
Mastocarpus stellatus	Carrageenan	1248 k	Gal:Glc:Xyl:Man = 87.8:5.4:4.4:2.4	β-1,3-Gal and α-1,4-Gal	Anticoagulant	Youssouf et al. (2017) and Gómez-Ordóñez et al. (2014)
Chondrus armatus	Carrageenan	88 k	Gal	β-1,3-Gal and α-1,4-Gal	Antiviral	Kalitnik et al. (2013)
Nemalion helminthoides	Sulfated mannan	43.8 k	Man:Xyl:Sulphate = 1:0.01:0.64	α-1,3-Man	Immunomodulatory	Pérez-Recalde et al. (2014)
Ahnfeltiopsis flabelliformis	Sulfated galactan	–	Gal:3,6-AnGal:Glc:Xyl:SO_3Na = 34.9:15.0:2.0:2.1:18.7	β-1,3-Gal and α-1,4-Gal	Anticoagulant	Kravchenko et al. (2014)
Porphyra haitanensis	porphyran	277 k	Gal	β-1,3-Gal	Antitumor	Wang and Zhang (2014)
Gracilaria fisheri	Sulfated galactan	–	Gal	β-1,3-Gal and α-1,4-Gal	Antioxidant	Imjongjairak et al. (2016)
Cryptonemia seminervis	Sulfated galactan	51.6 k	Gal, trace in Glc, Ara	β-1,3-Gal and α-1,4-Gal	Anti-metapneumovirus	Mendes et al. (2014)

(continued)

Table 5.1 (continued)

Species	Polysaccharide type	Molecular weight (Da)	Monosaccharide	Backbone	Biological activities	Ref
Gelidium crinale	Sulfated galactan	300–600 k	Gal	α-1,3-Gal and α-1,4-Gal	Antiinflammatory	Assreuy et al. (2012)
Brown algae						
Alaria marginata	Galactofucan	–	Fuc:Gal:Xyl = 47.5:47.3:5.2	→3)-α-l-Fuc-(2,4-SO_3^-)-(1→	Anticancer	Usoltseva et al. (2016)
Hizikia fusiforme	–	–	Fuc:Gal:Xyl:Glc = 1.00:0.50:0.24:0.21	–	Immunomodulatory	Jeong et al. (2015)
Cystoseira sedoides	Fucoidan	642 k	Fuc and Uronic acid	α-1,3 or α-1,4-Fuc	Antiinflammatory	Hadj Ammar et al. (2015)
Coccophora langsdorfii	Fucoidan	–	Fuc	α-1,3 and α-1,4-Fuc	Anticancer	Imbs et al. (2016)
Eisenia bicyclis	Laminaran	19–27 k	Glc	β-1,3 and β-1,6-Glc	Anticancer	Wozniak et al. (2015)
Scytothamnus australis	Sulfated fucan	–	Fuc:Xyl:Glc = 40.8:1.5:1	α-1,3-Fuc	Anti-HSV1	Wozniak et al. (2015)
Sargassum fusiforme	Laminaran	27.6 k	Glc:Gal = 1.13:0.38	β-1,3-Glc, β-1,6-Glc	–	Jin et al. (2014)
Laminaria japonica	Laminaran	–	Man:Ara:Glc:Gal:Fuc = 3.27:8.61:4.23:12.12:46.93	–	Antioxidant	Cheng et al. (2011)

Species	Polysaccharide type	Molecular weight (Da)	Monosaccharide	Backbone	Biological activities	Ref
Green algae						
Enteromorpha linza	Rhamnan sulphate	108.4 k	Rha:Xyl:Man:Glc:Gal = 3.6:1.0:0.31:0.28:0.19	1,4-Rha	Antioxidant	Wang et al. (2013)
Codium divaricatum	Sulphated galactan	37.9 k	Gal:Glc = 97.8:2.16	1,3-β-Gal	Anti-coagulant	Li et al. (2015)
Capsosiphon fulvescens	Ulvan	–	Rha:Xyl:Man = 45.0:44.1:10.2	4)-β-Xyl-(1→4)-α-Rha-(1→	Anticoagulant	Synytsya et al. (2015)
Ulva armoricana	Ulvan	140–500 k	Rha:Gal:Glc:Xyl = 40.0:6.7:26.2:4.4	–	Antiviral	Hardouin et al. (2016)
Ulva pertusa	Ulvan	28.2 k	–	–	Antiradiation	Shi et al. (2013)
Monostroma angicava	Rhamnan sulphate	88.1 k	Rha	α-1,2-Rha, α-1,3-Rha	Anticoagulant	Li et al. (2017)
Gayralia oxysperma	Rhamnan sulphate	109 k	Rha:Xyl:Glc = 76.0:17.3:4.4	α-1,3-Rha	Antitumor	Ropellato et al. (2015)

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Gal galactose, *Glc* glucose, *Xyl* xylose, *Man* mannose, *3,6-AnGal* 3,6-anhydro-ᴅ-galactose, *Ara* arabinose, *Fuc* fucose, *Rha* rhamnose

Table 5.2 Comparison of microalgal harvesting methods

Method	Advantages	Disadvantages
Flocculation	Simple and low cost High yield of biomass harvesting	Potential contamination of harvested algal biomass by chemical coagulants Synthetic coagulants negatively influence biochemical compounds (proteins, lipids, carbohydrates)
Floatation	Simple to operate	High maintenance cost Small air bubbles are energy intensive Requires flocculants
Filtration	Reliable and can handle even the delicate cells	High maintenance cost Slow process High operational costs Membrane fouling or clogging
Centrifugation	Easy, rapid, and efficient Can handle most algal types	High energy input High maintenance cost Internal damage of cells
Sedimentation	Simple, cost-effective Low energy input	Often slow and produce low yield Suited for dense algal cultures

advantages and disadvantages (Table 5.2). For macroalgae, manual harvesting works well or often times the use of mechanical techniques is used.

5.3.1 Flocculation

Flocculation is considered to be an inexpensive harvesting method in which the addition of flocculants causes the microalgae cells to agglomerate by forming larger particles known as flocs. Chemical and bio-flocculants are the two types of flocculants available. The flocculants could either be cationic polymers or multivalent cations (Cassini et al. 2017). Iron and aluminum salts are chemical flocculants that are of low cost, readily available, and used widely in industries. And the biopolymer bio-flocculant being used is chitosan. Several factors such as the type of microalgae, cell concentration, type of flocculant, flocculant dosage, pH, charge density, growth phase, salinity, the presence of algal organic matter, etc., could affect the flocculation process, thus the selection of an appropriate flocculant is imperative (Balasubramaniam et al. 2021). Various mechanisms such as bridging, surface charge neutralization, electrostatic patching neutralization or sweeping may be used to explain the flocculation process. Flocculation is often used with the combination of a filter compressor (Branyikova et al. 2018).

5.3.2 Floatation

Floatation is the separation process where microalgal cells float on the surface of water using air or gas bubbles to the solid particles. Harvesting involves the pumping of air bubbles from underneath the sedimentation tank to aid gravitational separation where the generated bubbles will attach and carry the solid particles to the collection area. Sometimes a chemical coagulant is added to allow the floatation process in increasing the harvested biomass, or an adjustment of pH is carried out to enhance the separation (Mustafa et al. 2021). The four main types of floatation techniques used in the harvesting of algal biomass are dissolved-air, electro-floatation, dispersed air, and ozone floatation (Mustafa et al. 2021). The dissolved air floatation method uses tiny air bubbles with a size range of 10–100 μm, which forces or pushes the solid microalgae cells to rise to the surface where they may be collected using the skimming method. This makes the dissolved air method time and energy-consuming, while the dispersed air floatation method uses air bubbles with a diameter of 700–1500 μm created from strong mechanical agitators. Although this method uses less energy, it has substantial maintenance costs. The floatation technique is economically feasible due to the operational costs, simple to operate and a high yield of biomass harvesting (Balasubramaniam et al. 2021).

5.3.3 Filtration

Filtration is the dewatering process used after flocculation to enhance the harvesting efficiency. The process separates the alga biomass from the liquid culture medium by passing through specific filters such as porous membranes, screens or microstrainers. Porous membranes are typically used for microalgae with low density and also performed on a small scale. The pressure difference throughout the filter is crucial to separate the solid particles and the liquid which can either be by gravity or vacuum. The few available filtration methods are conventional filtration, microfiltration, and ultrafiltration. The conventional process is convenient for harvesting large microalgae (Balasubramaniam et al. 2021). Membrane filtration could be used to meet the requirements of both dewatering and thickening of the microalgae harvesting. However, this procedure for algal biomass harvesting always suffers from clogging or membrane fouling, especially with high biomass concentrations that could be time-consuming, increase operational costs, and reduce the effectiveness of the process. The drawbacks of the filtration process of microalgae harvesting include high maintenance cost, slow operation, and high operation (Mustafa et al. 2021).

5.3.4 Centrifugation

Centrifugation of the microalgae is the separation of the algal biomass from the culture media using centrifugal force to separate the solids according to their cell size and density difference. This method is preferred over other harvesting techniques because it is reliable and rapid for all microalgae species, not time-consuming and can harvest almost all microalgae without the use of any flocculants or chemicals. Due to the ability of this process to harvest large amount of the microalgae biomass, it is commonly used for saturated fatty acids, pharmaceuticals, and other high-value products (Balasubramaniam et al. 2021). As a result, the effectiveness of the entire process depends on the rotating speed used to separate solids that are suspended in a liquid, which enables it to harvest the majority of the algal biomass from the culture media. However, this process is also time-consuming and costly due to the high amount of energy input required. Furthermore, there is a chance that the mechanical spinning of the microalgal cells could be destroyed under the high shear and gravitational forces. One of the benefits of using the centrifugation methods is that it is effective and does not use chemicals that can contaminate the end-product. Centrifugation is often done at a laboratory or pilot scale (Mustafa et al. 2021).

5.3.5 Sedimentation

Sedimentation is the initial step of separating the microalgae from water. Harvesting of microalgae using this method yields a wet and large amount of sludge, which results from the slow settling and poor velocity. Factors that could influence the success of this method are the weight of the microalgae, sedimentation velocity, light intensity, temperature, time, and size. Sedimentation method works effectively in the combination of the flocculation process, which enhances the microalgal removal and settling. The method is also cost-effective, simple and requires low energy input (Mustafa et al. 2021). However, the process is slow and produces a low yield of microalgal harvest, especially when the microalgae culture did not go through the process of flocculation.

5.4 Bioactive Compounds from Marine Algae and Their Biological Activity

Marine algae produce various bioactive compounds that have been used in a wide range of applications ranging from nutritional supplements to bioactive substances for health benefits. The content and key compositions of the bioactive compounds in marine algae vary from species to species, and no species produces the same compounds. These bioactive compounds include and are not limited to polysaccharides, vitamins, minerals, proteins, and lipids. Research has shown that these bioactive compounds have numerous health-promoting benefits such as antimicrobial, anti-inflammatory, anticancer, and antibiotic effects. Figure 5.1 gives a summary of the bioactive compounds and their properties. Both macroalgae and microalgae contain more of the bioactive compounds, although this section will focus more on microalgae (Hakim and Patel 2020).

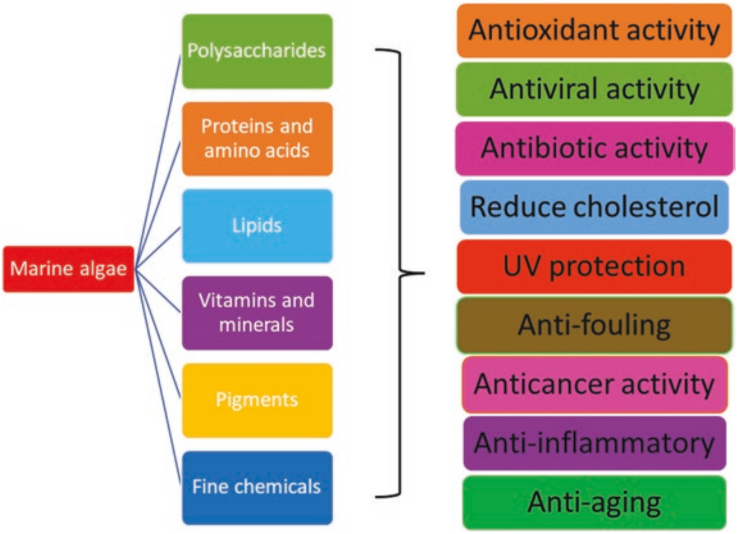


Fig. 5.1 Summary of bioactive compounds and some of their properties

5.4.1 Polysaccharides

Polysaccharides are long chains of carbohydrates (cellulose, starch, or glycogen) made up of smaller carbohydrates that are commonly used in the human body for cellular structure and energy. Fructose, glucose, xylose, and galactose make up the majority of polysaccharides, whereas sulfate, protein, and uronic acid are only present in trace levels. Marine algae produce a wide range of polysaccharides with varying value and largely depends on their degree of purity, availability, and use (Mahata et al. 2022). With the right conditions, around 15–55% of the dry biomass weight of marine algae can be extracted as polysaccharides. Among the available polysaccharides, sulfate polysaccharides are the most significant algal polysaccharides from the biological activity perspective and a significant source of bioactive natural compounds with properties such as antithrombotic, antitumor, antimicrobial, antimutagenic, anti-inflammatory, immunomodulatory, and antiviral effects. The polysaccharides are also promising therapeutics for atherosclerosis due to their properties, abundant availability, cost-effective, and minimal toxicity (Øverland et al. 2019).

5.4.2 Proteins and Amino Acids

Marine algae can have a protein concentration that ranges from 6 to 52% of their dry weight. However, the protein content contained in microalgae are up to 71% of their dry weight, although these amounts are strongly impacted by the environmental conditions, including temperature, salinity, light, pH, mineral content, CO_2 supply, population density, growth phase physiological status, the season, and the species (Hakim and Patel 2020; Øverland et al. 2019). Because of this high protein content, microalgae are promising to be used as a protein source in food industries. Essential amino acids determine the quality of food proteins. Although glutamine and aspartic acid are the two most abundant amino acids in microalgae, the algae can also accumulate large concentrations of other amino acids such as alanine, arginine, valine, leucine, lysine, threonine, isoleucine, and glycine. The properties of flavor development are observed in glutamine and asparagine. Because microalgae can produce practically all amino acid compounds, they are preferred to other sources of protein in protein-rich diets (Hakim and Patel 2020; Mahata et al. 2022). In addition, genetically modified microalgae are capable of producing a variety of proteins effectively.

5.4.3 Lipids

Marine macroalgal species contain a very low lipid content; however, most microalgae are rich in oil content. Because microalgae have a high lipid content, they could substitute fish oil in aquaculture and for human consumption. Microalgal lipids are abundant in essential long-chain polyunsaturated fatty acids (PUFAs), such as omega-3 and omega-6 oil, which are needed because humans and many other animals cannot produce them on their own. They are also beneficial for functional components of the cell membranes. The bioactive chemicals of prostaglandins and thromboxane, which are required for the maintenance of cholesterol and triglycerides in the body as well as for protection against some diseases including dermatitis and osteoarthritis, are produced by PUFAs, which benefit both human and animal health (Mobin et al. 2019). Omega fatty acids have drawn a lot of interest because of their role in growth, nutrition and therapeutic and pharmaceutical use. For human consumption, marine fish like salmon and cod are currently the main source of these bioactive compounds. However, the quantity and composition of algal lipids differ depending on the species, region, season, temperature, salinity, amount of light, or a combination of these factors. Phospholipids, which are frequently used in the cosmetics industry as wetting agents, emulsifiers, solubilizers, and liposomes, are another component of microalgae that is abundant (Mahata et al. 2022). They also have medicinal usefulness due to their anti-inflammatory and antithrombotic properties. About 10% to 50% of the total lipids in marine microalgae can be phospholipids.

5.4.4 Vitamins and Minerals

Micronutrients such as vitamins and minerals are crucial for the human body metabolism, just like macronutrients like protein, fat, and carbohydrates. They all co-exist in biochemical pathways where they eventually take part in a number of biological processes, including boosting immunity, promoting growth and development and repairing cell damage. Moreover, a lack of vitamins can cause a number of illnesses, such as rickets, beriberi, and scurvy. Vitamins can be found in abundance in marine microalgae (Mahata et al. 2022). These aquatic microorganisms are capable of producing all the vitamins that are produced by higher plants. Spirulina sp., for example, has large amounts of vitamins A and B complex, which have a direct effect on how the brain and cells operate, as well as how well they prevent illness. Compared to soybeans and cereals, microalgae accumulate more vitamins, but this varies depending on algal species, season, alga growth stage, and environmental parameters. In addition, the growth and maintenance of the body depend on a variety of minerals, including calcium, phosphate, potassium, magnesium, sodium, iron, zinc, and copper. Although an inadequate potassium diet might result in convulsions, a calcium and phosphate deficit results in bone abnormalities (Mobin et al. 2019). Magnesium, iron, copper, and zinc all play important roles in maintaining healthy bones, eyesight, and energy levels. These elements, which could be used in the diet of humans or animals, are fortunately present in marine microalgae. Macroalgae also possess a high mineral content, which has traditionally been given to farm animals as a mineral supplement (Mobin et al. 2019; Øverland et al. 2019).

5.4.5 Pigments

The presence of pigments within microalgae cells gives them their characteristic colors. The two main types of pigments are water-soluble and fat-soluble. Carotenoids and chlorophylls are fat-soluble pigments, whereas phycobilin is a water-soluble pigment. These high-value compounds made from microalgae have potential uses in medicine as antioxidants, immunological boosters, neuroprotectants, and vitamin precursors. Algal pigments have been proposed as a potential anti-aging ingredient for skin care products (Mahata et al. 2022). Marine microalgae and cyanobacteria have the ability to generate up to 8% of the fluorescent proteins called phycobiliproteins. Based on their long-wavelength absorption maxima, the phycobiliproteins can be divided into four main classes: allophycocyanin (650–660 nm), phycocyanin (610–625 nm), phycoerythrin (490–570 nm), and phycoerythrocyanin (560–600 nm). Due to their widespread usage in immunity assays, phycocyanin and phycoerythrin are the two most well-known phycobiliproteins. Carotenoids are naturally occurring, lipophilic (fat-soluble) pigments created by starving microalgae. In addition, marine Spirulina species, *Porphyridium* species, and *Chlorella protothecoides* are abundant in natural pigments that may be used as food additives, antioxidants, food identifiers, and therapeutic substances (Mahata et al. 2022).

5.4.6 Marine Algae-Based Fine Chemicals

5.4.6.1 Carotenoids

Carotenoids are a significant group of pigments that are fat-soluble and are potent antioxidants that are essential for oxygen photosynthesis. Carotenoids cannot distribute the energy they acquire from the sun directly to the photosynthetic pathway; hence, they can only pass it from one chlorophyll molecule to the other. Carotenoids typically make up 0.1 to 2% of the dry weight of the majority of algae. Only a few of the 400 known carotenoids, such as β-carotene and astaxanthin are of great commercial significance. Other carotenoids are of less significance (lutein, zeaxanthin, lycopene, and bixin). The physico-chemical characteristics of the aquatic environment, including water temperature, salinity, light, and nutrient availability, might affect the carotenoid composition of microalgae (Surget et al. 2017). The majority of environmental factors change seasonally, which might eventually promote or impede the formation of carotenoids.

5.4.6.2 Antioxidants

Antioxidants are abundant in microalgal biomass, which has potential uses in human and aquatic diet, cosmetics, and medicine. Applications of antioxidants in health has sparked interest due to the free radicals and oxidation used in many physiological functions. Due to the harsh environmental conditions that microalgae have adapted to, they experience a significant amount of oxygen and radical stresses. As a result, their bodies have developed various and effective defence mechanisms to prevent the accumulation of free radicals and reactive oxygen species. This process shields microalgae from cell-damaging activities (Surget et al. 2017). Astaxanthin is one of the strong antioxidants.

5.4.6.3 Phenols

Phenols are a significant class of natural products produced by microalgae as secondary metabolites. These substances have biological and antioxidant properties and they are crucial for algal cell defense against biotic and abiotic stress. The basic functions of algae, such as photosynthesis, cell division, and reproduction, are not directly affected by these substances. Purified phenolic compounds have properties that include antioxidant, anti-radical, UV protection, metal chelation, and antifouling. However, the antioxidant properties are the primary bioactivity linked to phenolic chemicals (Surget et al. 2017). Some algal phenolic compounds have reportedly been linked to anti-inflammatory activity, including rutin, hesperidin, morin, caffeic acid, catechol, catechin and epigallocatechin gallate, a phenolic compound that may be able to combat free radicals.

5.4.6.4 Minerals

The high mineral concentration of microalgae is well known. Spirulina, a microalga, has ash in excess of 6.7% of its dry weight. The general mineral content is greatly influenced by the ambient growing conditions, including season, temperature, physiological status, geographic variances, etc. (Øverland et al. 2019).

5.5 The Importance of Marine Algae

5.5.1 Edible and Poisonous Algae

For long years, people have grown algae and consumed it as food. Dried microalgae not only have a lot of provitamin A but also have other elements such as proteins, minerals, vitamins, and antioxidants. The micronutrients in minerals are iodine, iron, zinc, copper, selenium, molybdenum, fluoride, manganese, boron, nickel, and cobalt. The macronutrients in minerals are sodium, calcium, magnesium, potassium, chlorine, sulfur, and phosphorus (Thomas 2002). Thousands of tons of consumable algae and algal-derived products are produced annually around the world for use as dietary supplements, food additives, functional foods, and pharmaceuticals (Suter 2011). As a sort of brown algae, edible seaweed is a vegetable of the sea that both marine life and people consume in a variety of ways. Asian cuisines, notably those of Japan and Korea, have traditionally harvested and consumed edible seaweed that is low in calories and high in nutrients (Veluchamy and Palaniswamy 2020).

Seaweed is primarily recognized as a source of iodine because it has a proportion of iodine that exceeds the minimal dietary needs. Brown algae have the highest iodine content, with dry kelp containing between 1500 and 8000 ppm and dry rockweed (Fucus) containing between 500 and 1000 ppm (Cole and Sheath 1990). In dried seaweeds, red and green algae (Fig. 5.2) typically have lesser levels (between 100 and 300 ppm), yet they still have large concentrations compared to all other terrestrial plants. Very little amounts of seaweed could meet the current 150 μg/day recommendation for daily adult needs. Even green and red algae, such as the purple nori used in Japanese cuisine, give 100–300 μg of iodine per gram. Just 1 g of dried brown algae provides between 500 and 8000 μg (Hoek et al. 1995).

According to studies, the thyroid gland is the principal tissue that uses iodine in the human body, and it adjusts quickly to greater iodine consumption (it is a component of thyroid hormones). Due to the extremely low iodine content of the soil, plants, and animals that are used as common food sources, large percentages of the world's population receive insufficient amounts of iodine. To ensure that proper quantities are reached, iodine is frequently added to table salt in many nations. A few emerging nations, nevertheless, are still catching up and experiencing the negative effects of inadequate iodine intake. China is home to the most people who have a history of consuming little iodine, followed by India (Kandale et al. 2011).

Seaweed is one of the richest plant sources of calcium, second only to iodine, although compared to dietary needs, its calcium concentration is far inferior to that of iodine. Usually between 4 and 7% of the dry matter of seaweeds is calcium. One gram of dried seaweed has 70 mg of calcium (7%), which is less than the 1000 mg recommended daily intake. Even yet, this is more than a serving of the majority of items without a milk base (Kandale et al. 2011).

Along the oceanic coasts of the world, sea lettuce, a type of edible green seaweed, grows. It has long been a staple diet for people as well as water creatures like manatees and sea slugs. Spirulina, a marine alga, contains a remarkable amount of

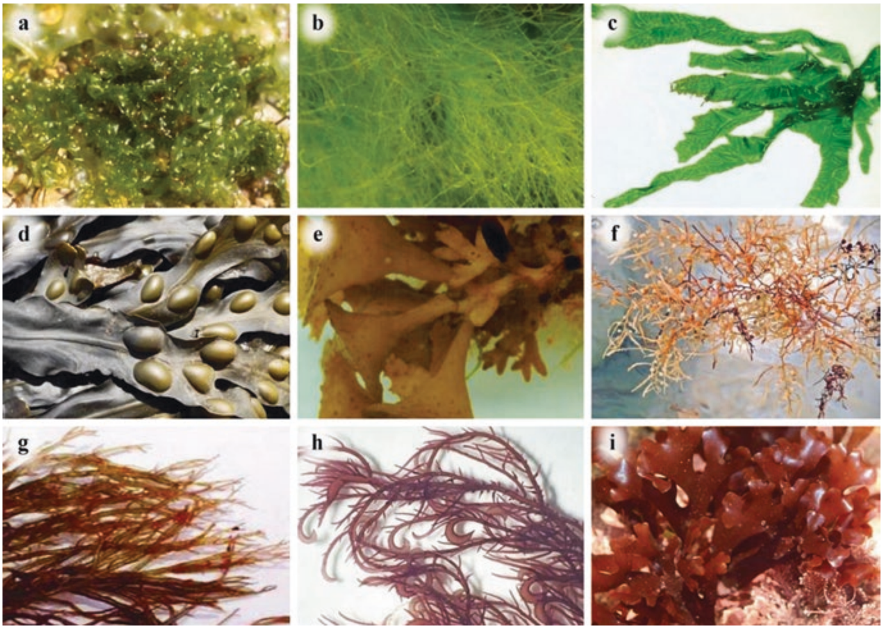


Fig. 5.2 Different seaweeds images such as green, brown, and red. (**a**) Ulva reticulata Forsskål; (**b**) Chaetomorpha linum (O.F.Müller) Kützing; (**c**) Ulva lactuca f. fasciata (Delile) Hering; (**d**) Fucus vesiculosus Linnaeus; (**e**) Turbinaria turbinata (Linnaeus) Kuntze; (**f**) Sargassum natans (Linnaeus) Gaillon; (**g**) Gracilaria edulis (SGGmelin) PCSilva; (**h**) Hypnea musciformis (Wulfen) J.V.Lamouroux; (**i**) Chondrus crispus Stackhouse. (Reproduced with copyright permission from Pradhan et al. (2022), Elsevier 2022)

protein, of which 90% is easily digestible. Spirulina is a microalga that may offer a promising source of protein for people who are undernourished or suffer from protein insufficiency (Suter 2011).

High quantities of unsaturated fatty acids are found in the oils from certain algae. For instance, the triglyceride pool of Parietochloris incisa has a very high amount of arachidonic acid, up to 47% of the total. Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are long-chain, necessary omega-3 fatty acids that can be found in some types of algae that are popular among vegetarians and vegans (Bigogno et al. 2002). Oleic and alpha-linoleic acids are found in significantly larger concentrations in green algae, whose fatty acid composition is most similar to that of higher plants. Red algae are rich in EPA, which is primarily found in animals, particularly fish (Kandale et al. 2011).

Alginate, agar, and carrageenan, gelatinous compounds together known as hydrocolloids or phycocolloids, are gelatinous substances that are extracted from seaweeds through harvesting or cultivation. As food additives, hydrocolloids have come to economic prominence. The food sector takes advantage of their emulsifying, water-retention, and gelling abilities, among other physical qualities. Agar is a food additive that is used in molded foods, confectionary, meat and poultry items, desserts, and beverages. Carrageenan is a preservative that is used in dairy products, baked goods, salad dressings and sauces, dietetic foods, and meat and fish products (Kandale et al. 2011). Chemical dyes and coloring agents can also be replaced with the natural pigments produced by algae (Bigogno et al. 2002).

Edible algae are a rich source of dietary fiber, minerals, and proteins (Kuda et al. 2002). Between 32% and 50% of the dry matter in seaweed is made up of fiber. The soluble fiber fraction makes up 51–56% of the total fibers in red (agars, carrageenans, and xylans) and green (ulvans) algae and 67–87% in brown algae (laminaria, fucus, and others). In general, soluble fibers are thought to reduce cholesterol and have hypoglycemic properties (Kandale et al. 2011).

Marine algae are another popular source of antioxidants (Nagai and Yukimoto 2003). Eisenia bicyclis (arame) (Cahyana et al. 1992) and fucoxantinein Hijikia fusiformis (hijiki) (Yan et al. 1999) contain phylopheophytin, one of several active antioxidant substances from brown algae. The amount of protein in seaweed varies. Brown algae have a low dry matter content (5–11%), whereas some species of red algae have a dry matter content (30–40%) that is comparable to legumes. Green algae, which are still rarely harvested, have up to 20% of their dry matter in protein, which is a significant amount. A microalga called spirulina is well recognized for having a very high concentration, or 70% dry matter (Kandale et al. 2011).

These algae are typically boiled, steam-treated, dried, and stored while in process. According to Jiménez-Escrig et al., a brown alga called Fucus had 98% less ability to scavenge radicals after being dried at 50 °C for 48 h. In addition, these dried goods are reconstituted with 20 to 40 times their original volume of water before consumption. Agars are used in the food business and for lab media culture. They are made from red seaweeds like Gracilaria (Jiménez-Escrig et al. 2001).

But certain algae can actually be dangerous to people. For instance, eating tropical fish that have consumed alga like Gambierdiscus or Ostreopsis can have devastating effects on humans and induce the ciguatera sickness. Fish-eating algae with the names Heterosigma and Dictyocha (classes of Raphidophyceae and Dictyochophyceae, respectively) are also suspected. Arsenic poisoning can occur if some seaweeds are consumed since they contain significant amounts of the toxic metal. Brown algae called hizoka contain enough arsenic to be used as rat poison (Britannica E 2022).

5.5.2 Anticancer Activity of Marine Algae

Recently, researchers have been more interested in bioactive substances with anticancer characteristics that were derived from marine algae. According to studies, -carotene, an antioxidant also obtained from marine algae, is extremely beneficial in the early phases of cancer treatment. Another biomolecule that is obtained from marine algae and has anti-inflammatory and antioxidant characteristics is phycocyanin. In both developed and developing nations, cancer is recognized as one of the top causes of mortality. The growth in the average global life expectancy has made it a significant health issue and a burden for the majority of public healthcare systems everywhere (Yao et al. 2022). GLOBOCAN estimates that there are 18.1 million new instances of cancer worldwide, affecting people of all sexes and ages, while 9.6 million people died from cancer in 2018 (Bray et al. 2018). By 2030, it is anticipated that there will be 13.1 million cancer-related deaths worldwide (Rashid et al. 2019). Oncology has made considerable strides in recent years, developing cancer treatments like surgery, radiation, chemotherapy, molecule-targeted therapeutics, and cell-based therapies (Miller et al. 2016; Halim et al. 2019; Wang et al. 2022). However, there are still issues that make it difficult to use and render these cancer therapies ineffective. For instance, chemotherapy, the most popular cancer treatment, also harms or kills healthy cells, causing significant side effects in the patients. To improve the quality of life for cancer patients, it is imperative to find novel anti-cancer chemicals that target cancer cells while having less of an impact on normal cells. Discovering molecules that can aid in the prevention and treatment of cancer has increasingly relied on natural products (Özyalçin and Sanlier 2020; Tang et al. 2020).

Over a hundred Marine algal polysaccharides (MAPs) have been examined through in vitro and in vivo animal studies over the course of the last ten years, indicating the excellent anti-cancer effects of MAPs in a wide spectrum of cancer cell lines. Numerous MAPs effectively stop the growth of tumor cells and harm cancer cells without having any negative side effects, addressing the main problems with traditional chemotherapy. According to recent research, MAPs primarily combat cancer by preventing cancer cells from proliferating, causing cancer cells to undergo apoptosis and cell cycle arrest, preventing tumor tissues from forming new blood vessels or metastasizing, scavenging reactive oxygen species (ROS), triggering an immune response, and controlling the gut microbiota (Yao et al. 2022).

5.5.2.1 MAPs Inhibit the Proliferation of Cancer Cells

Through direct cytotoxicity, MAPs prevent and destroy cancer cells and reduce colony development in cancer cells. The red alga *Gracilariopsis lemaneiformis* (*Gp. lemaneiformis*) has been shown to have antitumor properties, and its polysaccharides of ganoderma lucidum (PGL) has been shown to have potent anticancer properties. PGL is known as a neutral polysaccharide with a linear structure made up of repeating units of the disaccharide agarobiose and is composed of 3,6-anhydro-L-galactose and D-galactose. In a study, Khang and colleagues looked at the ability of the human gastric cancer cell line MKN45, the lung cancer cell line A549, and the cervical carcinoma cell line HeLa to proliferate after the addition of PGL. The CCK-8 assay findings for cell viability showed that PGL had the most pronounced anticancer effect on A549 lung cancer cells. In addition, trypan blue staining's results on cell proliferation matched up with the results of the cell viability test. Furthermore, they discovered that PGL hindered cell proliferation, decreased cell viability, and changed cell shape, and that these effects were time- and concentration-dependent. In an effort to better understand the molecular mechanism of the PGL-induced antitumor phenotype, they calculated the gene expression values by Cufflinks and the distribution, and 758 differentially expressed genes were observed (Kang et al. 2016a).

Nikolova et al. carried out research to demonstrate a cell-specific effect of a recently isolated extracellular polysaccharide from the red microalga Porphyridium sordidum in an effort to shed some light on the effectiveness of polysaccharides on normal and cancer cells. The xylose:glucose and galactose:manose:rhamnose in the red microalga Porphyridium sordidum had a molar ratio of 1:0.52:0.44:0.31. As we look more closely at the isolated polysaccharide's anti-proliferative effects. MDA-MB231 high metastatic and MCF-7 low metastatic cancer cell lines, together with one normal cell line (MCF10A), were examined. Polysaccharide concentrations of 10, 25, 50, 75, or 100 g/mL were applied to the cells during 24 and 48 h. The 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) test assay was used to examine the effects of polysaccharide on cell proliferation. The results revealed that polysaccharides could not significantly alter cell viability 24 h after incubation, but that 48 h later, cell survival appeared to be dose- and cell-type-dependent. Furthermore, the combination of 200 V/cm electroporation and the application of 75 g/mL polysaccharide caused changes in cell morphology and a 40% reduction in the viability of MDA-MB231 cells, whereas control cells (MCF10A) maintained their normal morphology and vitality (Nikolova et al. 2019).

In a study against colorectal cancer, Choi and colleagues examined the sulfated glucuronorhamnoxylan polysaccharide (abbreviated SPS-CF) that was isolated from the green alga Capsosiphon fulvescens. At 500 μg/mL, the SPS-CF treatment caused a dose-dependent reduction of the proliferation of HT-29 human colon cancer cells by up to 40%. Figure 5.3e demonstrates that, in comparison to untreated control cells, the cell viability was dose-dependently reduced to 93, 90, 80, and 64% at doses of 0, 50, 100, 200, and 500 μg/mL, respectively. Although the treatment of 200 and 400 mg/kg SPS-CF intraperitoneally had no effect on the change in mice body weight (Fig. 5.3b), it considerably slowed the growth of colon tumors when compared to control mice (mice treated with phosphate-buffered saline) (Fig. 5.3a). Following 14 days of treatment, the average tumor volumes were 2822 mm^3 for the SPS-CF 200 mg/kg/day group, 2691 mm^3 for the SPS—CF 400 mg/kg/day group, and 3561 mm^3 for the control group (Fig. 5.3c). The 400 mg/kg dose resulted in a roughly 20% reduction in tumor weight when compared to the control group (Fig. 5.3d) (Choi et al. 2019).

Three human cancer cell lines (Fig. 5.4), including HepG2 (hepatocellular carcinoma), MCF7 (human breast cancer), and Hela, were tested for the cytotoxic effects of an ulvan isolated from the green seaweed Ulva lactuca (cervical cancer) in a study by Thanh et.al. The figure shows the ulvan's cytotoxic effects against the HepG2, MCF7, and Hela cancer cell lines at different concentrations (0.8, 4, 20, and 100 μg/mL). According to the study, the percentage of cell viability decreased as ulvan concentration grew, reaching zero at a concentration of 100 μg/mL (Thanh et al. 2016).

At a concentration of 200 μg/mL, fucoidan derivatives from the brown alga Saccharina cichorioides were discovered to exhibit inhibitory activity on the development and colony-forming capacity of HT-29 human colorectal cancer cells (Anastyuk et al. 2017), while HT29 and HCT-116 human colorectal cancer cells

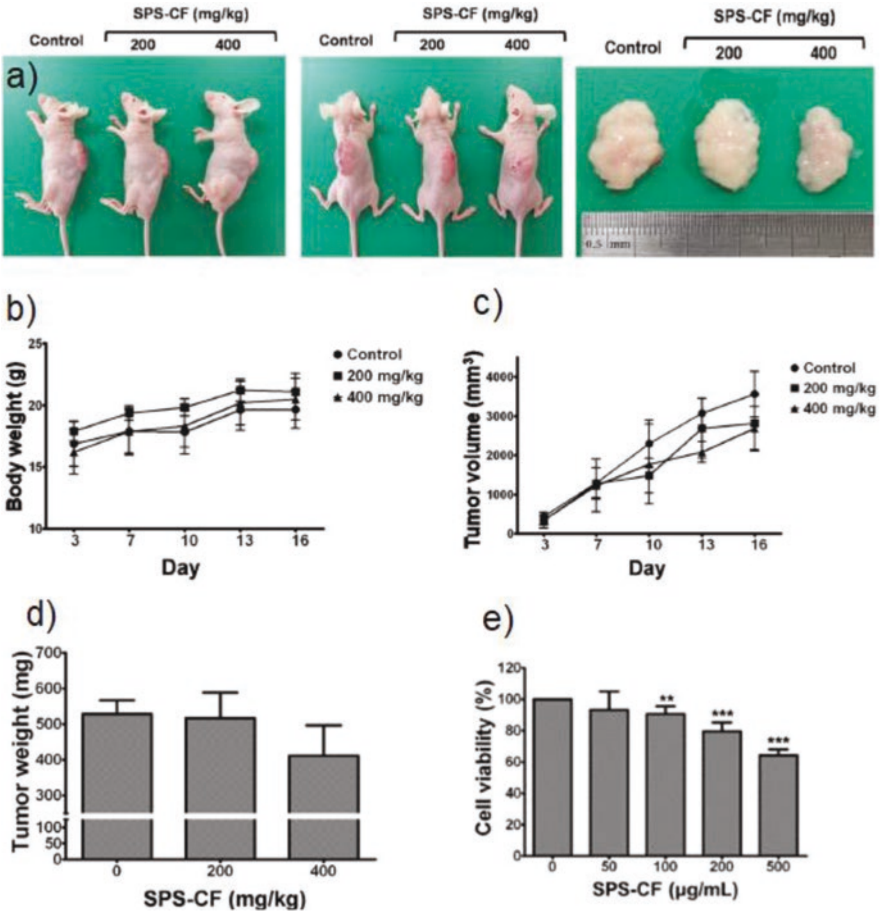


Fig. 5.3 In vivo efficacy of SPS-CF against human colon cancer xenografts. (**a**) Tumor size in the tissues of SPS-CF treated mice maintained smaller tumor sizes compared with the control group. (**b**) The average body weight of each group was expressed as the means ± SD (*n* = 4 per group). (**c**) The average tumor volume of each group was measured using a caliper and expressed as the means ± SD (*n* = 4 per group). (**d**) The average tumor weight of each group was expressed as the means ± SD (*n* = 4 per group). (**e**) Effects of SPS-CF on the cell viability and cell cycle in HT-29 cells—Inhibitory effects of SPS-CF on the growth of HT-29 cells were determined by MTT assay. (Reproduced with copyright permission from Choi et al. (2019), Elsevier 2019)

showed signs of being inhibited by polysaccharides from Fucus evanescens. At a concentration of 200 g/mL, the polysaccharides F1 and F3 inhibited the colony growth of HCT116 cells by 28 and 32%, respectively, although they only had a small impact on the colony growth of HT-29 cells (16 and 27%, respectively) (Hmelkov et al. 2018).

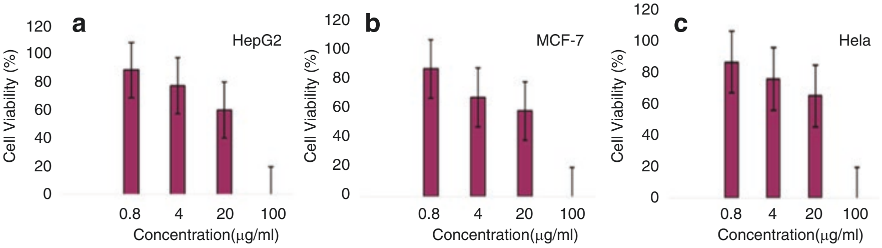


Fig. 5.4 Cytotoxic effect of the ulvan at various concentrations against (a) HepG2, (**b**) MCF7, and (**c**) Hela cancer cell lines. The data shown are the mean ± SD of triplicate assays, and the experiment was repeated three times. (Reproduced with copyright permission from Thanh et al. (2016), Elsevier 2016)

5.5.2.2 MAPs Induce Apoptosis in Cancer Cells

Organisms eliminate unneeded or diseased cells from their bodies through a physiological process known as apoptosis, often known as programmed cell death. It takes part in a variety of pathogenic conditions, such as cancer. Cell shrinkage, membrane blebbing, nuclear condensation, and the creation of an apoptotic body are all characteristics of apoptosis. When cell division and apoptosis are out of balance, tumors develop. Exploring new medications that can cause cancer cells to die by inducing apoptosis is desirable because apoptosis is thought to be a controlled and regulated process (Kandeel et al. 2018). MAPs are powerful anti-cancer agents with a strong therapeutic potential against a range of malignancies, as they can control cancer cell apoptosis and promote cancer cell death, according to extensive research conducted over the past few decades. In a concentration-dependent manner (0 mg/L, 20 mg/L, 80 mg/L, and 320 mg/L), a study found that polysaccharides extracted from the brown alga Laminaria japonica (LJP) strongly triggered apoptosis (mostly late apoptosis) in HONE1 human nasopharyngeal cancer cells. Where after LJP treated for 72 h, HONE1 apoptosis was dominated by late apoptosis, and HONE1 apoptosis in different concentrations of LJP was listed as following: 0 mg/L (8.81 ± 1.25) % (Fig. 5.5a), 20 mg/L (18.58 ± 2.43) % (Fig. 5.5b), 80 mg/L (32.24 ± 2.49)% (Fig. 5.5c), 320 mg/L (49.51 ± 1.89%)% (Fig. 5.5d) (Zeng et al. 2017).

In Caco-2 human epithelial colorectal adenocarcinoma cells, FHs 74 Int normal human small intestine cells, HepG2 human hepatocellular carcinoma cells, and Fa2N-4 human hepatocytes, degraded -carrageenan from Kappaphycus alvarezii promoted the morphological hallmarks of apoptosis. In cells treated with -carrageenan, the morphological signs of apoptosis, such as cell detachment, membrane blebbing, chromatin condensation, nuclear disintegration, and the production of apoptotic bodies, were seen (Zainal Ariffin et al. 2014). In addition, MCF-7 human breast adenocarcinoma cells and HCT-15 human colon adenocarcinoma cells were both induced to undergo apoptotic morphological alterations and cell-mediated death by fucoidan isolated from the brown alga Sargassum polycystum. When cells were left untreated with F2, the nuclei in the control cells fluoresced uniformly

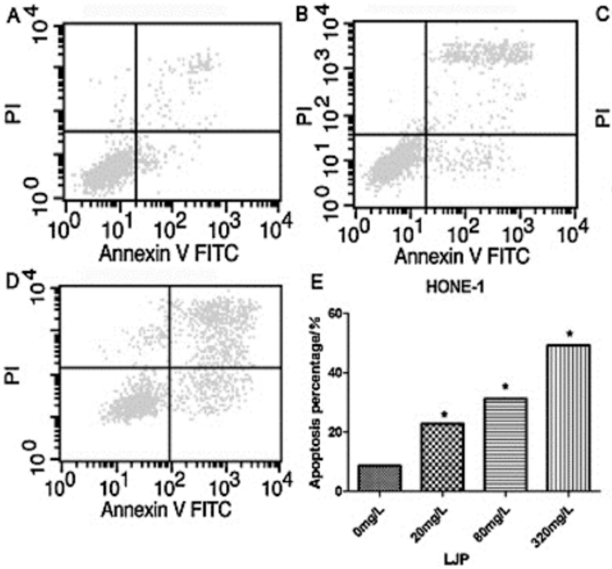


Fig. 5.5 LJP significantly induced apoptosis of HONE1 cells. Flow cytometry was used to detect the apoptosis of NPC cells. HONE1 apoptosis in different concentrations of LJP was detected, and HONE1 apoptosis in different concentrations of LJP was listed as following: (**a**) 0 mg/L (8.81 ± 1.25) %, (**b**) 20 mg/L (18.58 ± 2.43) %, (**c**) 80 mg/L (32.24 ± 2.49) %, (**d**) 320 mg/L (49.51 ± 1.89%) %. (**e**) The differences of apoptosis rate between the LJP group and the control group were statistically significant ($P < 0.01$), and so was the differences between groups in different concentration ($P < 0.01$). (Reproduced with copyright permission from Zeng et al. (2017), Elsevier 2017)

green, indicating that the cells were healthy and their nuclei were intact (Fig. 5.6i and iia). The number of apoptotic cells, however, significantly increases in the MCF-7 and HCT-15 cells treated with F2 at IC_{50} and 100 μg/mL doses (Fig. 5.6i and iib and c). This demonstrates nuclear condensation and fragmentation. These findings made it abundantly evident that cells containing DNA and nuclei displayed uniform bright green, while cells exhibiting early apoptotic yellow staining and late apoptotic reddish or orange staining were identified (Palanisamy et al. 2018).

In a different experiment, polysaccharides from the red alga Porphyra haitanensis (PHP) caused apoptosis in SGC-7901 human gastric cancer cells, with a definite apoptosis peak in the concentration range of 10–500 μg/mL, showing a clear apoptosis peak in Fig. 5.7. The rate of apoptosis increased as PHP concentration increased. The ratio of apoptosis to PHP concentration was strongly associated. The apoptosis rate was 19.65% when PHP concentration was 500 g/mL, which was substantially higher than the control group (Chen and Xue 2019). Although Yu et al. investigated the anticancer activity of Auricularia polytricha polysaccharides (APPs) against A549 human lung cancer cells and its underlying mechanisms, they discovered that cells treated with lower concentrations of APPs moderately accumulated in the G0/G1 phase of the cell cycle when compared to the control group (43.25% for control; 50.35% for 25 μg/mL APPs) (Yu et al. 2014). The cleaved poly

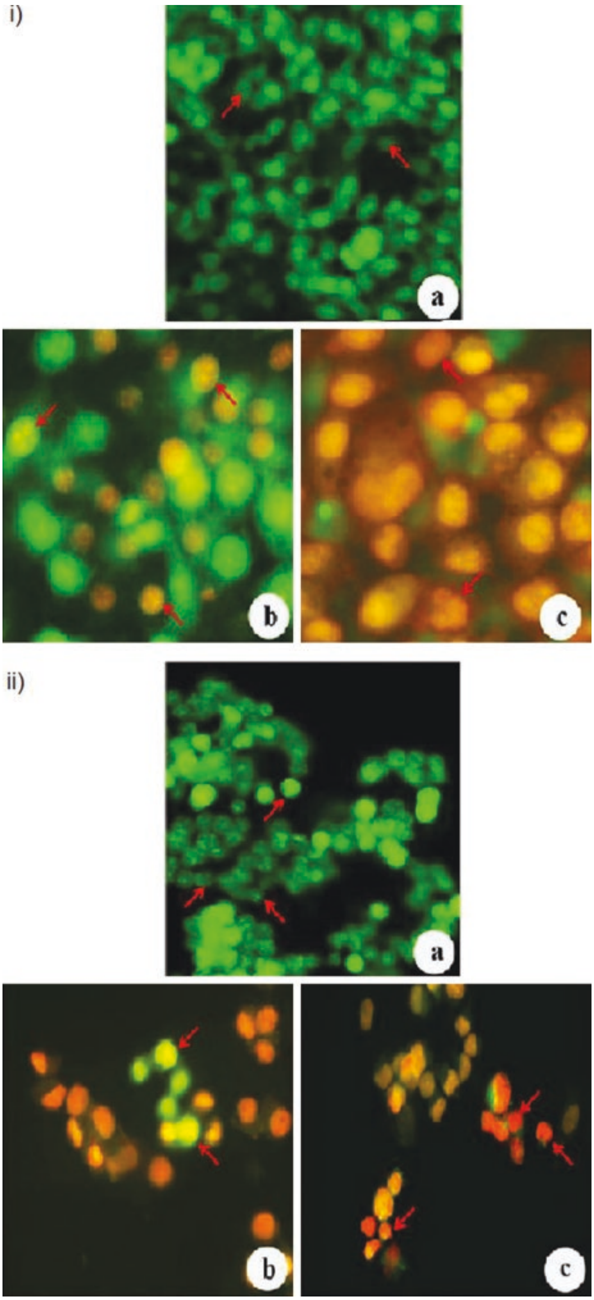


Fig. 5.6 Effect of fraction 2 on apoptosis morphological changes of breast and colon cancer cell lines under fluorescence microscope after 24 h of incubation. (*i*) Breast cancer (MCF-7) cell lines; (**a**) control, (**b**) IC50 concentration (20 μg/mL), (**c**) maximum concentration (100 μg/mL). (*ii*) Colon cancer (HCT-15) cell lines; (**a**) control, (**b**) IC50 concentration (50 μg/mL), (**c**) maximum concentration (100 μg/mL). (Reproduced with copyright permission from Palanisamy et al. (2018), Elsevier 2018)

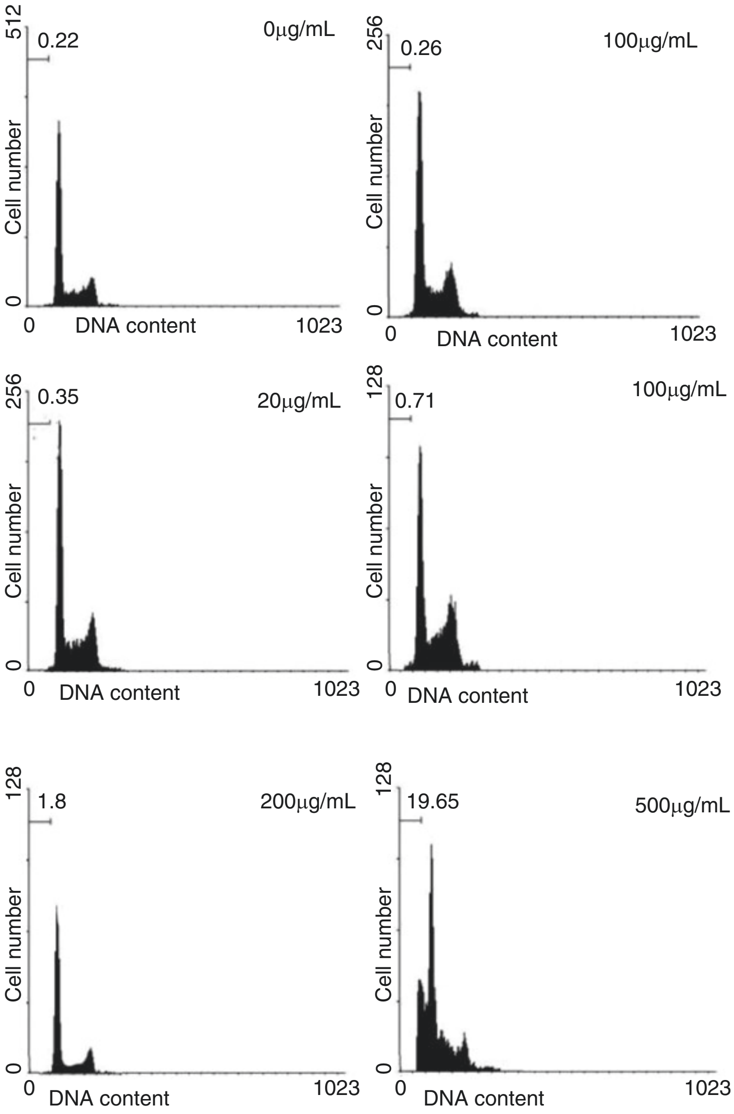


Fig. 5.7 Apoptosis rate of SGC-7901 cells treated by PHP with different concentrations for 48 h. (Reproduced with copyright permission from Chen and Xue (2019), Elsevier 2019)

(ADPribose) polymerase (PARP) level was dramatically increased by the sulfated glucuronorhamnoxylan polysaccharide isolated from the green alga *Capsosiphon fulvescens*, and DNA fragmentation was induced. These effects were seen in HT-29 human colon cancer cells in a study by Choi et al. (2019).

5.5.3 Antioxidants Property of Marine Algae

Antioxidants are defined as the quantity of free radicals eliminated or neutralized by antioxidant compounds. Numerous studies have demonstrated their versatility as food additives. They shield living things from reactive oxygen species (ROS), which can damage lipids, DNA, and proteins and cause a number of chronic diseases, including diabetes, atherosclerosis, rheumatoid arthritis, cancer, and Alzheimer's (Sato et al. 2019). The majority of algal colorants are well-known for having antioxidant effects. As a result, in recent years, interest in these natural antioxidants as sources of antioxidants has increased (Marinho et al. 2019).

Carotenoid is one of the algae pigments whose antioxidant properties have received the most attention. Numerous health advantages of carotenoids, including the preservation of cognitive function, have been demonstrated (Johnson 2012; Devore et al. 2013); for skin protection (Stahl and Sies 2012); in older adults' resulting in mineral density increase and decrease in their rheumatoid arthritis symptoms (Rodriguez-Amaya 2019) and as a provitamin source (Pérez-Gálvez et al. 2020). The removal of electrons from carotenoids or the formation of carotenoid-radical adducts are two methods by which antioxidants scavenge free radicals (FRs). This characteristic appears to have therapeutic potential for a number of disease states, including the treatment of cardiovascular disease, macular degeneration, breast, prostate, gastrointestinal system, and lung malignancies (Pérez-Gálvez et al. 2020).

Fucoxanthin is one of the most common xanthophyll carotenoid pigments. Fucoxanthin can protect tissue when there is a lack of oxygen because all other carotenoids are unable to quench active radical species (D'Orazio et al. 2012; Xia et al. 2013; Tavares et al. 2020). Fucoxanthin, which has been shown to have a variety of health-promoting benefits including anti-obesity, anti-cancer, and UV protection capabilities, is primarily found in brown algae (Holdt and Kraan 2011). Several other studies have also demonstrated fucoxanthin's potent capacity to scavenge free radicals (Marinho et al. 2019; Peng et al. 2011; Koduvayur Habeebullah et al. 2018).

The most significant provitamin A source among the carotenoids is beta-carotene, which is followed by alpha-carotene and beta-cryptoxanthin. Strong antioxidant properties of astaxanthin allow it to prevent oxidation of low-density lipoprotein caused by azo-compound (Wang et al. 2012). Codium fragile, a digestible seaweed that is a mainstay of the Japanese diet, is rich in siphonaxanthin [19-(trans-Δ2-dodecenoate)] (10). Siphonaxanthin is a dietary ingredient that reduces the viability of human leukemia HL-60 cells 2014 (Sugawara et al. 2014); (Batista et al. 2019). In addition, it is anti-angiogenic. Their research revealed that siphonaxanthin is a significant bioactive carotenoid with a lot of potential for use in the medical or food industries.

Another family of color compounds with anti-inflammatory, antimutagenic, and antioxidant characteristics is the chlorophyll pigment family (Tumolo and Lanfer-Marquez 2012; Chen and Roca 2018; Nwoba et al. 2019). Studies on chlorophyll claim that it transforms into derivatives such pheophorbides and pheophytins, which are absorbed at a rate akin to some carotenoids both during digestion and after absorption to the intestines (Pérez-Gálvez et al. 2020; Chen and Roca 2018, 2019).

Research is continuing to gain a better understanding of the metabolic processes, the mechanisms of oxidation, and the absorption of chlorophyll (Pérez-Gálvez et al. 2020).

Antioxidant activity can also be impacted by chlorophyll's shape and arrangement. According to studies, chl a is a more potent antioxidant than chl b (Fernandes et al. 2017; Saldarriaga et al. 2020). In addition, by substituting the central magnesium ion with copper, metallo-chlorophyll or copper chlorophyllins are produced, which have a better antioxidant capacity and provide a stable food coloring ingredient (Viera et al. 2019). According to the research by Cho et al. (Cho et al. 2011), pheophorbide gives Ulva prolifera's chlorophyll outstanding antioxidant potential, including DPPH and hydroxyl radical scavenging action. On the other hand, Phormidium autumnale has 200 times the antioxidant power of alpha-tocopherol (Hsu et al. 2013).

The results of recent efforts to incorporate algae into novel food formulations have been positive. According to a study by Batista et al. (Batista et al. 2019), adding microalgae such as *Arthrospira platensis*, *Chlorella vulgaris*, *Tetraselmis suecica*, and *Phaeodactylum tricornutum* to wheat crackers significantly increased antioxidant activity. Chlorophyll has the power to halt degenerative diseases in their tracks, according to reports (Dashwood 2021). However, the antioxidant properties of chlorophylls are fragile and easily broken down into chemicals (Indrasti et al. 2018). The microencapsulation technology has been used to preserve chlorophyll. According to studies, the antioxidant quality was both conserved and boosted by the microencapsulated Spirulina in pasta and the kale chlorophyll in whey protein (Zen et al. 2020; Zhang et al. 2020).

According to Pan-utai and Iamtham (2019), phycobiliprotein pigments have a variety of health-promoting qualities and may be used as food coloring or additives. Numerous investigations on the antioxidant activity of phycobiliproteins (PBPs) have been done recently (Pleonsil et al. 2013; Thangam et al. 2013; Sonani et al. 2015). These studies showed how well PBPs can bind and lessen ferrous ions. Phycoerythrin's antioxidant function has a stronger reducing power and a lower ability to chelate than phycocyanin or allophycocyanin. On the other hand, the chelating and lowering properties of phycocyanin and allophycocyanin are comparable. For the phycobiliproteins, it is discovered that the antioxidant activity of phycoerythrin > phycocyanin > allophycocyanin is dose-dependent (Sonani et al. 2015). They have also shown that Caenorhabditis elegans can age more slowly due to phycoerythrin's antioxidant properties. However, preservation methods may have an impact on the antioxidant activity of PBPs (Tello-Ireland et al. 2011).

Cyanosarcina sp., *Phormidium* sp., *Scytonema* sp., and *Leptolyngbya* sp. provided phycobiliproteins that were extremely thermostable and had significant antioxidant activity (Pumas et al. 2011). A gastrointestinal digestion of the phycoerythrin from Bangia fusco-purpurea can produce certain peptides that have considerable antioxidant activity, according to a study by Wu et al. (2015). On the basis of this, PE can be applied to the creation of functional foods. In addition, research into the phycoerythrin from *Grateloupia filicina*'s possible health advantages revealed that PE shielded astrocytes from hydrogen peroxide's oxidative damage (Jung et al.

2016). In addition, cytoprotective against damage caused by hydrogen peroxide was the phycoerythrin found in dulse (*Palmaria* sp.) (Sato et al. 2019).

Numerous health benefits of phycocyanin, including anticancer, antioxidant, anti-inflammatory, and hepatoprotective qualities, have been documented in addition to its nutritional value and abundance of important amino acids (Bertolin et al. 2011; Park et al. 2018; Osman et al. 2019). According to Abdel-Daim et al. (2015), the multiple health advantages associated to phycocyanin are the outcome of this antioxidant property. Spirulina platensis-derived phycocyanin showed dose-dependent antioxidant activity (Wu et al. 2016). In addition, they noticed that phycocyanin is light-sensitive at normal temperatures and that its thermal stability is compromised at temperatures higher than 45°C. But, it has been claimed that sodium chloride can successfully stabilize phycocyanin and maintain its effectiveness. Along with its antioxidant abilities, phycocyanin is also known to have anti-lipid peroxidation characteristics. Strong anti-lipid peroxidation activity was shown by the extract of Geitlerinema sp. (Renugadevi et al. 2018).

A powerful antioxidant, allophycocyanin (APC) is a naturally occurring pigment (Bertolin et al. 2011). Contrary to phycoerythrin, the elimination of ROS is accomplished by the responses of antioxidant proteins expressed by the antioxidant genes rather than through direct oxidation-reduction reactions (Kim et al. 2018). Allophycocyanin has excellent therapeutic potential, according to a study. Human erythrocytes' oxidative stress could be successfully reversed by APC and selenium-containing APC, and an oxidant's ability to promote lipid oxidation was also prevented (Zhang et al. 2011). Furthermore, they demonstrated that how Se-APC can stop the production of intracellular reactive oxygen species. The ability of APC to reduce free radical production has been noted by researchers to have potential applications in the treatment of illnesses. Allophycocyanin from Palmaria palmata has stronger antioxidant activity than PE and exhibited cytoprotective effects on human neuroblastoma SH-SY5Y cells (Sato et al. 2019).

The amount of polyphenols in various kinds of algae varies. Compared to most other algae, brown algae have more content of polyphenols. Algal polyphenols are anti-diabetic, anti-Alzheimer's, anti-allergy, anti-aging, and lower the risk of cardiovascular and cancer disorders, according to studies (Rodrigo et al. 2011; Stagos et al. 2012; Cha et al. 2016; Peñalver et al. 2020). Algal polyphenols are sufficiently powerful that they could replace synthetic antioxidants in the food sector (Hermund et al. 2018). Due to its effects on various enzymes as catalase, glutathione peroxidase, superoxide dismutase, and free radical elimination, polyphenols have a substantial antioxidant capacity (Gómez-Guzmán et al. 2018; Sanchez et al. 2019).

Phlorotannins, polyphenols derived from brown algae, have numerous health advantages, including lowering the risk of cancer, metabolic, and neurological diseases (Gómez-Guzmán et al. 2018). Like other tannins, they are present in soluble form in the cytoplasm or the intercellular spaces of cell organelles (Generalić Mekinić et al. 2019). Numerous phenolics also function as a type of chemical defense against bacteria, grazers, and fouling organisms. As a result, polyphenols have significant pharmacological and nutraceutical value and may be included in functional meals to help with weight management or digestion (Holdt and Kraan

2011; Generalić Mekinić et al. 2019; Le Lann et al. 2016). Phlorotannin from *Sirophysalis trinodis*, also known as *Cystoseira trinodis*, was isolated from *Sargassum muticum* and demonstrated antioxidant and antiproliferative action in breast cancer cells (Namvar et al. 2013; Sathya et al. 2017). Phlorotannin is said to have ten times the antioxidant activity of any known biological molecule (Lomartire et al. 2021). They could potentially be included in skin care products and other cosmetic applications (Bedoux et al. 2014). A study on the health advantages of algae species revealed that frequent consumption of food high in phlorotannins decreased the risk of hypercholesterolemia and cardiovascular disease (Namvar et al. 2012). With so many beneficial components present in algae, it is difficult not to include algae in your regular diet. For greatest health benefits, Machu et al.'s (Machu et al. 2015) recommendation is to consume algae directly as food rather than as a supplement.

Strong antioxidant capabilities are present in algal anthocyanins, and they are comparable to those of alpha-tocopherol, quercetin, and catechin (Alappat and Alappat 2020). According to a study by Kongpichitchoke et al. (2015), algal anthocyanin plays a crucial part in the electron transfer route by providing unpaired electrons from free radicals with electron donations. The substance had a variety of qualities, including those that were anticancer, anti-diabetic, anti-inflammatory, anti-obesity, and lowered fasting sugar (Alappat and Alappat 2020; Pojer et al. 2013; Sarikaphuti et al. 2013; Bontempo et al. 2015; Lee et al. 2017; Lin et al. 2017; Strugała et al. 2019).

5.5.4 Anti-Obesity Properties of Marine Algae

Metabolic disorders like dyslipidemia, hyperglycemia, and hypertension are associated with obesity. The burden of obesity is primarily derived from its associated chronic disorders, including type II diabetes mellitus, cancer, lung diseases, and cardiovascular diseases (Adams et al. 2006; Finucane et al. 2011). The etiology of the relationship between obesity and chronic diseases is still being studied. It is interesting to note that chronic low-grade inflammation has been connected to the development of obesity and disorders that are related to it (Greenberg and Obin 2006; Osborn and Olefsky 2012).

The effects of the Sabah brown seaweed Sargassum polycystum on body weight and blood plasma levels in rats given a high-fat diet supplemented with various doses of the seaweed powder were examined by Awang and colleagues. To supplement their high-fat diets, the low dose group (LDG), medium dosage group (MDG), and high dosage group (HDG), respectively, received supplements of 2.5, 5.0, and 10.0% seaweed powder. When compared to the control group, they discovered that the HDG (10.0% seaweed treatment diet) had the greatest effect in preventing weight gain, followed by the MDG (5.0% seaweed treatment diet) and LDG (2.5% seaweed treatment diet) (Awang et al. 2014). Another study by Kang et al. found that *Plocamium telfairiae* extract (PTE) had the best inhibitory effect on lipogenesis in adipocytes among the red algae extracts examined and was therefore chosen as a possible anti-obesity treatment. Over the course of 14 weeks, the body weight was assessed once each week (Fig. 5.8). In comparison to mice fed a normal diet, mice fed a high-fat intake had a higher body weight at 14 weeks. However, compared to the mice who received the high-fat dose alone, the body weights of the mice treated with PTE (100 mg/kg) also experienced a considerable drop (Kang et al. 2016b).

Li et al. evaluated the impact of diet-induced obesity in C57BL/6J mice on the development of siphonaxanthin-rich green algae (Codium cylindricum). A low-fat diet (LF; 7% fat, w/w), a high-fat diet (HF; 35% fat, w/w), or a high-fat diet supplemented with 1% or 5% green algal powder (1GA or 5GA) were given to the mice over the course of 78 days. The findings demonstrated that the 5GA group's body weight and perirenal white adipose tissue (WAT) were significantly lower than those of the HF group. In addition, the green algae's dietary fiber's inhibitory effect on fat absorption was cited as a contributing factor in the lowering of WAT (Li et al. 2018).

Ben Abdallah Kolsi and colleagues studied the effects of Cymodocea nodosa sulfated polysaccharide (CNSP) on lipase activity in vitro and in vivo to high-fat diet (HFD)-rats on body weight, lipid profile, and liver-kidney functions. In comparison to untreated HDF-rats, the treatment of CNSP causes obese rats' body weight to drop and their intestinal and serum lipase activity to be inhibited. In HFD-rats, this decrease in lipase activity causes an increase in high-density lipoprotein cholesterol (HDL-C) levels and a decrease in total cholesterol (T-Ch), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C) (Kolsi et al. 2015)

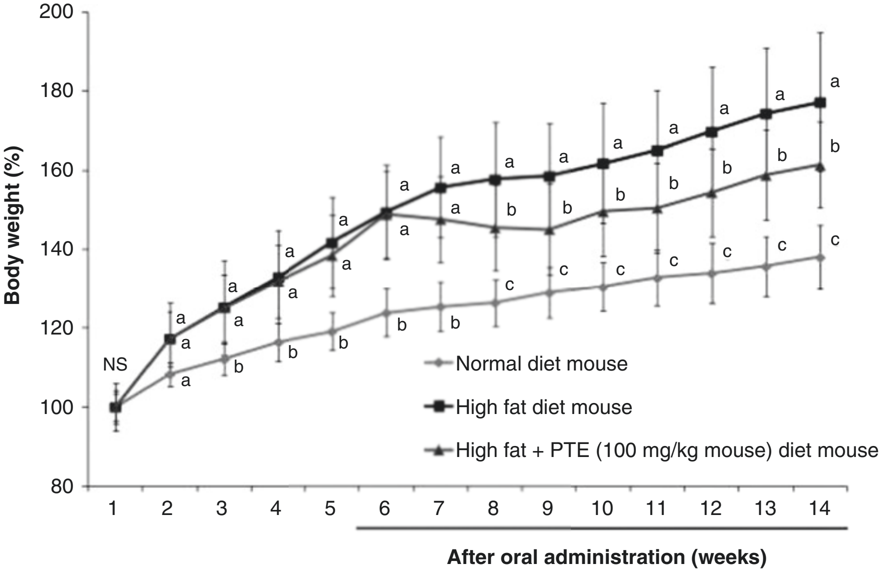


Fig. 5.8 Body weight gain of mice fed experimental diets for 14 weeks. The results are expressed as mean ± standard error ($n = 10$). (Reproduced with copyright permission from Kang et al. (2016b), Elsevier 2016)

5.5.5 Anti-Diabetic Properties of Marine Algae Anti-Obesity Properties of Marine Algae

The aberrant metabolism of glucose that occurs in diabetes mellitus is partly brought on by peripheral tissue resistance to the action of insulin. Polyuria, polydipsia, and polyphagia are the identifying signs. The most serious chronic disease that is becoming more and more prevalent in an aging and fat world is diabetes mellitus. Hyperglycemia is one of the multiple disorders that makes up diabetes mellitus. Diabetes mellitus is primarily divided into type 1 diabetes, which is insulin-dependent, and type 2 diabetes, which is not insulin-dependent (type 2 diabetes). Type 2 diabetes, which is the most common type of diabetes, is in particular a growing global health concern (Zimmet et al. 2001). The onset of type 2 diabetes and its accompanying consequences, such as micro- and macro-vascular illnesses, are significantly influenced by hyperglycemia (Baron 1998). Therefore, avoiding or curing diabetic complications and increasing the quality of life for diabetic patients depend on good blood glucose level management (DeFronzo 1999).

Currently, treatment options for type 2 diabetes include insulin and a number of oral anti-diabetic medications, including sulfonylureas, metformin, rosiglitazone, -glucosidase inhibitors, and thiazolidinediones. However, these treatments have either a meager efficacy or major adverse effects based on the mechanism of action, such as hypoglycemia, flatulence, weight gain, and worsening of gastrointestinal issues. As a result, interest in complementary therapies and the therapeutic use of natural diabetes medications, particularly those made from herbs, has grown recently (Chang et al. 2006; Jung et al. 2007). This is due to the fact that plant sources are typically thought to be less hazardous and to have less adverse effects than manufactured ones (Lee and Jeon 2013).

Chinese traditional medicine has used marine algae as a treatment for diabetes among alternative medicines because they include a number of biologically relevant compounds (Brown et al. 2014). Table 5.3 lists numerous Marine algal compounds with specific anti-diabetic characteristics, such as phloroglucinol (23), eckol (24), dieckol, phloroeckol, and phlorofucofuroeckol A. (Lee and Jeon 2013). The anti-diabetic activities of other algal chemicals like bromophenol (Zhao et al. 2018) and fucosterol (Unnikrishnan et al. 2015) are also well documented. Several isolated carotenoids from algae, including astaxanthin (7) and fucoxanthin (1), have anti-diabetic properties. For instance, the brown alga fucoxanthin (1), which is widely distributed, has significant potential as a functional food that fights diabetes and obesity (Miyashita and Hosokawa 2017). Similar results have been seen with astaxanthin (7), a reddish carotenoid pigment that is frequently present in *Haematococcus pluvialis* (Chlorophyta), which has a notable anti-diabetic impact.

Astaxanthin (7) oral treatment was shown by Wang et al. (2012) to greatly reduce postprandial hyperglycemia and postprandial area under the curve (AUC). There is anti-diabetic action in the Ecklonia species extract. These mainly contained phlorotannins and shown anti-diabetic action with an IC_{50} value of 10.7 μM. With IC_{50} values for the phlorofucofuroeckol A and dieckol at 1.37 μM and 1.61 μM, respectively, in comparison to the reference medication acarbose (IC_{50} = 51.65 μM)

Table 5.3 Marine algal compounds with specific anti-diabetic characteristics

Active compounds	Species	Anti-diabetic action	Ref.
Fucoxanthin (**1**)	*Eisenia bicyclis*	PTP 1B inhibition, Aldose reductase	Jang et al. (2018)
Eckol (**24**), dieckol, and 7-phloroeckol		Inhibition α-amylase and α-glucosidase	Abdelsalam et al. (2019)
Dieckol	*Ecklonia cava*	α-Glucosidase inhibitor	Lee et al. (2009a)
	Ecklonia cava	Postprandial hyperglycemia-lowering effect	Lee et al. (2012a)
	Ecklonia cava	Glucose uptake effect in skeletal muscle	Guan (2011)
	Ecklonia cava	PTP 1B inhibition	Moon et al. (2011)
	Ecklonia cava	Protective effect against diabetes complication	Lee et al. (2010)
Fucodiphloroethol G	*Ecklonia cava*	α-Glucosidase inhibitor	Lee et al. (2009a)
6,6′-Bieckol	*Ecklonia cava*	α-Glucosidase inhibitor	Lee et al. (2009a)
7-Phloroeckol	*Ecklonia cava*	α-Glucosidase inhibitor	Lee et al. (2009a)
	Ecklonia cava	PTP 1B inhibition	Moon et al. (2011)
Phloroglucinol	Ecklonia stolonifera	α-Glucosidase inhibitor	Moon et al. (2011)
	Eisenia bicyclis	PTP 1B inhibition	Moon et al. (2011)
Dioxinodehydroeckol	Ecklonia stolonifera	α-Glucosidase inhibitor	Moon et al. (2011)
	Eisenia bicyclis	PTP 1B inhibition	Moon et al. (2011)
Diphlorethohydroxycarmalol	*Ishige okamurae*	α-Glucosidase inhibitor	Heo et al. (2009)
	Ishige okamurae	Postprandial hyperglycemia-lowering effect	Heo et al. (2009)
	Ishige okamurae	Protective effect against diabetes complication	Heo et al. (2010)
Eckol	*Ecklonia stolonifera*	α-Glucosidase inhibitor	Moon et al. (2011)
	Eisenia bicyclis	PTP 1B inhibition	Moon et al. (2011)
Octaphlorethol A	*Ishige foliacea*	Glucose uptake effect in skeletal muscle	Lee et al. (2012b)

(continued)

Table 5.3 (continued)

Active compounds	Species	Anti-diabetic action	Ref.
Polyphenolic-rich extract	Ascophyllum nodosum	α-Glucosidase inhibitor	Nwosu et al. (2011)
Phlorotannin-rich extract	Ascophyllum nodosum	Postprandial hyperglycemia-lowering effect	Roy et al. (2011)
	Fucus vesiculosus	Postprandial hyperglycemia-lowering effect	Roy et al. (2011)
Polyphenolic-rich extract	Ecklonia cava	Glucose uptake effect in skeletal muscle	Kang et al. (2010)
Dieckol-rich extract	Ecklonia cava	Improvement of insulin sensitivity	Lee et al. (2012c)
Polyphenolic-rich extract	Ishige okamurae	Improvement of insulin sensitivity	Min et al. (2011)
Phlorofucofuroeckol A	*Ecklonia stolonifera*, *Ecklonia cava*	α-amylase inhibitor PTP 1B inhibition ACE inhibitor α-glucosidase inhibitor	Machu et al. (2015) and de Jesus Raposo et al. (2013)
Diphlorethohydroxycarmalol	*Ishige okamurae*	α-glucosidase inhibitor (IC50 = 0.16 ± 0.01 mM) α-amylase inhibitor (IC50 = 0.53 ± 0.018 mM)	Motshakeri et al. (2014)
Extract of *Sargassum hysterix*	*Sargassum hysterix*	α-amylase inhibitor (IC_{50} = 0.58 ± 0.01 mg/mL) α-glucosidase inhibitor (IC_{50} = 0.59 ± 0.02 mM)	Gotama and Husni (2018)
Pheophytin-A	*Eisenia bicyclis*, *Ecklonia stolonifera*	Aldose reductase Inhibition (fucosterol IC_{50} HLAR = 18.94, HRAR = 144)	Jung et al. (2013)
Bromophenols	*Symphyocladis latiuscula*	Phosphatase 1B (PTP1B), glucosidase, −amylase, and aldose reductase	Abdelsalam et al. (2019)

(Abdelsalam et al. 2019). They also showed that eckol (24) had higher alpha-glucosidase activity than dioxinodehydroecko (IC_{50} = 34.60 μM and phloroglucinol, IC_{50} = 141.18 μM) and lower IC_{50} (IC50 = 22.78 μM) than both of these compounds.

The brown alga Ishige okamurae has recently been demonstrated to contain numerous phlorotannins, including diphlorethohydroxycarmalol, octaphlorethol A, and phluroglucinol 6-6-bieckol, which may have anti-diabetic potential (Yang et al. 2019). The therapy of Sargassum polycystum extract displayed a hypoglycemic effect on streptozotocin-induced type 2 in diabetic rats, according to a study by Motshakeri et al. (2014). They discovered that this edible alga's ethanol and aqueous extract dramatically lowered the glycemic index of diabetic rats by 27% and 35%, respectively. The activity of the alpha-amylase and alpha-glucosidase enzymes has been reported to be inhibited by a number of compounds identified in Eisenia bicyclis, including eckol (24), dieckol, 7-phloroeckol, and fucosterol (Abdelsalam et al. 2019; Jung et al. 2013).

5.5.6 Antiviral Application of Marine Alga

The utilization of marine algae as source material for the synthesis/isolation of bioactive antiviral materials has been explored for centuries though their adoption within the last two decades has been intensified among researchers and industrialists (Tassakka et al. 2021).

The pharmacology of a number of marine natural products have antiviral action against the human immunodeficiency virus type 1 (HIV-1), dengue virus, SARS-CoV-2 virus (Abdelsalam et al. 2019), hepatitis B virus, influenza virus, and West Nile virus.

In an interesting study, red marine alga "*Halymenia durvillei* (Rhodophyta)" extracts as natural antiviral medication have been utilized effectively to inhibit SARS-CoV-2 virus (Tassakka et al. 2021). The authors extracted 37 compounds along with their identifications. As per their report, in contrast, cholest-5-En-3-Ol (3.Beta.)- had a high fitness score in molecular docking studies both in the monomer and dimer state compared to the N3 inhibitor and remdesivir affinity scores, suggesting the potential of compounds 1-2 tetradecandiol and E,E,Z-1,3,12-nonadecatriene-5,14-diol for therapeutic purposes. These natural substances may work well for the treatment of COVID-19 infection as they have competitive affinity scores against the 3CL-Mpro (Tassakka et al. 2021). In light of the encouraging findings that demonstrated the potential of *H. durvillei* as a substitute treatment in treating COVID-19 infection, they recommended that the ADME (absorption, distribution, metabolism, and excretion) and pharmacokinetic studies should also be utilized to evaluate the ability of the natural compounds as oral pharmaceuticals (Tassakka et al. 2021).

A group of researchers have proposed in their report the Four identified marine sulfated glycans have been found to have antiviral properties against enveloped (the herpesvirus human cytomegalovirus) and non-enveloped (adenovirus) DNA viruses (Zoepfl et al. 2021). These include a sulfated galactan from the red alga *Botryocladia occidentalis*, a sulfated fucan from the sea urchin *Lytechinus variegatus*, and a sulfand a sulfated fucan and a fucosylated chondroitin sulfate from *Isostichopus badionotus*, a sea cucumber. According to this study's authors, all four new glycans prevented viral entrance and attachment, most likely through interacting with virions. The antiviral profiles of the sulfated fucans, which both lack anticoagulant activity, imply that their activities are due to other physicochemical factors as well as their potential conformational shapes in solution and when interacting with virion proteins, rather than just their sulfation content or negative charge density. Exploring the connections between glycan structure and antiviral activity is made possible by the structural and chemical characteristics of these marine sulfated glycans (Zoepfl et al. 2021).

In another study, four marine red alga Rhodomela confervoides-based ureidobromophenols with great antioxidant properties have been identified where Compound 1 featured a bromophenol but also butyric acid units fixed to the same N-atom of ureido moiety and the other isolated ureidobromophenols have strong anti-DPPH as well as anti-ABTS properties (Li et al. 2021). The authors made note of the possibility that the seaweed functional food ingredients or dietary food supplements in the food business can benefit from the ureidobromophenols, isolated from marine algae, which have antioxidant properties.

The antiviral property of diterpenes extracted from marine algae "*Dictyota menstrualis*" against HIV-1 virus has been demonstrated by Pereira et al. (2004) in their work. The antiviral performance was credited to the solubility of the algae (*Dictyota menstrualis*) "CH_2Cl_2/MeOH" against the replication of the HIV-1virus as per their in vitro studies. Two diterpenes, (6R)-6-hydroxydichotoma-3,14-diene-1,17-dial (Da-1), and (6R)-6-acetoxi-dichotoma-3,14-diene-1,17-dial (AcDa-1), were shown to have antiretroviral action. The culture media of HIV-1-infected PM-1 cells was supplemented with Da-1 or AcDa-1 at various points after infection or during virus adsorption/penetration. The findings suggested that the chemicals had an impact on a preliminary stage of the viral replication cycle. Each diterpene was tested for its ability to prevent virus binding and entry into host cells, but no inhibitory impact was found (Pereira et al. 2004). The viral protease coding sequence was amplified from total cellular DNA to examine provirus DNA synthesis and integration into the host genome. Infected cells treated with the diterpenes did not contain any proviral DNA. The recombinant HIV-1 reverse transcriptase (RT) was evaluated in vitro in the presence of each diterpene to examine the impact of the diterpenes on the reverse transcription of the viral genomic RNA. The RNA-dependent DNA-polymerase activity of HIV-1 RT was dose-dependently suppressed by Da-1 and AcDa-1. Together, their findings show that both diterpenes impede HIV-1 RT and, as a result, virus replication (Pereira et al. 2004).

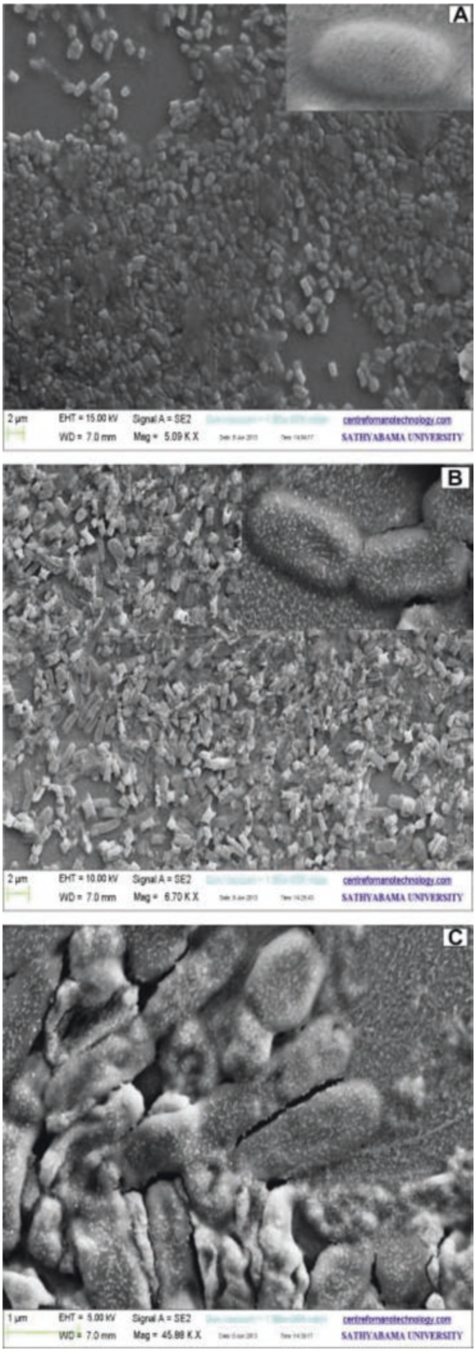
Seeing this is not an exhaustive discussion though inclusive, we have presented few instances based on available literature where it is clearly shown that the active compounds in marine algae are responsible for their antiviral activity though there is a great need for more exploitation in this area.

5.5.7 Antibacterial/Antifungal Application of Marine Algae

The antibacterial and antifungal characteristics of diverse marine algae have been exploited by humans for centuries without proper documentation; however, researchers/industrialists have beamed light on marine algae utilization as source material for the preparation of diverse antibacterial and well as antifungal agents within the recent decades (de Felício et al. 2015).

In this vein, the antibacterial efficacy of silver chloride nanoparticles synthesized from marine algae (*Sargassum plagiophyllum*) has been reported (Stalin Dhas et al. 2014). The destruction of *E. coli* bacterial cells as presented in Fig. 5.9 was reported by these group of researchers (Stalin Dhas et al. 2014).

Fig. 5.9 Morphological analysis of *E. coli* using FESEM before (**a**) and after (**b** and **c**) treatment with silver chloride nanoparticles (20 µg/mL). Inset: figure shows individual bacterial cells before and after treatment. (Reproduced with copyright permission from Stalin Dhas et al. (2014), Elsevier 2014)



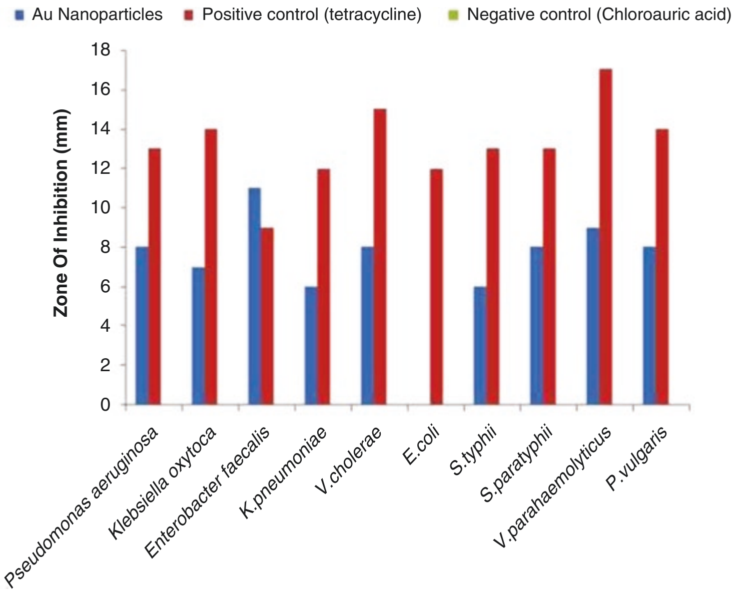


Fig. 5.10 Antibacterial activity of gold nanoparticles synthesized by the reduction of gold chloride with the *S. marginatum* biomass against some selected bacterial pathogens. (Reproduced with copyright permission from Arockiya Aarthi Rajathi et al. (2012), Elsevier 2012)

In another study, the antimicrobial activity of brown marine algae synthesized Au nanoparticles has been demonstrated (Rajathi et al. 2012), even as the authors showed as depicted in Fig. 5.10 that the nanoparticles demonstrated the highest antibacterial activity against *E. faecalis* (~11 mm) and the minimal zone of inhibition against *K. pneumoniae* was recorded, and it was higher than that of the positive control tetracycline (6 mm). *E. coli* did not exhibit any inhibition (0 mm) (Rajathi et al. 2012). They also noted that the green technique to nanoparticle synthesis produced highly efficient nanoparticles against gram-negative bacteria. Au nanoparticles' antibacterial properties complied with an antibacterial activity's method of action.

In another report, Ma et al. (2021) have demonstrated three 5-hydroxyepicyclonerodiol oxide (1) and 4-hydroxyepicyclonerodiol oxide (2), and one new naturally occurring halogenated trichothecane derivative, trichodermol chlorohydrin (3), sesquiterpenes from the marine-alga-epiphytic fungus Trichoderma hamatum Z36-antibacterial 7's activity (Ma et al. 2021). According to their research, *Amphidinium carterae*, *Chattonella marina*, and *Prorocentrum donghaiense* were used to test the effects of compounds 1 through 3 on different marine phytoplankton species. All of the isolates have the ability to stop *C. marina* from growing, as demonstrated in Table 5.4. When compared to 2 and 10-cycloneren-3,5,7-triol, 1 had a greater impact on *P. donghaiense* and less of an impact on *C. marina*. These variations could be related to cyclization of the side chain moiety and variation (C-4 or C-5) of the hydroxy group. The antibacterial activity of these isolates was further

Table 5.4 Antimicroalgal and antibacterial activities of 1–3

	IC_{50} (µg/mL)			Inhibitory zone diameter (mm) at 40 µg/disk			
Compd	*A. carterae*	*C. marina*	*P. donghaiense*	*V. anguillarum*	*V. harveyi*	*V. parahaemolyticus*	*V. splendidus*
1	na	55	35	10	8.5	7.7	7.7
2	na	24	na	9.0	7.7	8.0	6.7
3	97	26	35	0	0	7.0	0
$K_2Cr_2O_7$	1.4	1.1	1.1				
Chloramphenicol				30	40	35	40

Reproduced with copyright permission from Ma et al. (2021), Elsevier 2021
na no activity at 100 µg/mL

tested against *Vibrio anguillarum*, *Vibrio harveyi*, *Vibrio parahaemolyticus*, and *Vibrio splendidus* (Table 5.4). All four bacteria are capable of being inhibited by compounds 1 and 2; however, compound 3 only exhibits sporadic activity against *V. parahaemolyticus*. Despite the chlorine atom's presence in 3, it doesn't appear to be able to enhance the antibacterial activity. None of the isolates, taken collectively, perform better than positive controls.

At present, literature on the application of marine algae-derived bioactive compounds and their utilization as antibacterial or antifungal agents is vast; we will refer readers interested in further reading to the review article by Mayer et al. (2022).

5.5.8 Marine Algae Application in Cosmetics

During growth, algae generate a large number of secondary metabolites. Due to their specific characteristic, they have been recognized as a significant natural source of these bioactive chemicals with a wide range of biological activity in cosmetic compositions.

For the purpose of enhancing biological effects in skin, many cosmetics that incorporate algae or even algal extracts have been produced. However, the majority of these studies, which are usually contained in patents, do not specify the specific biocompounds or underlying mechanisms behind each cosmetic performance. In addition, it is unclear if the overall effect of algal extract in cosmetic products is a result of many chemicals working together or if each bioactive acts independently.

To make it easier to identify biocompounds for the creation of novel cosmetic products with commercial applications, not only the active components' characterisation but also the profiles of algal extracts might be examined.

To assess the true commercial potential of algae for the manufacturing of industrial cosmetics, stability, compatibility, and even toxicity, research must be taken into account.

Sandolera and colleagues looked into the marine GABA and its derivative GABA-alanine, both of which are made by the cultured red microalga *Rhodosorus marinus*, and found that its mechanism of action involves the suppression of TRPV1 expression in healthy human astrocytes in vitro. The treatment of "sensitive skin, atopia, dermatitis, and psoriasis" could therefore be accomplished with these chemicals (Scandolera et al. 2018).

Secondary metabolites generated from algae are renowned for their beneficial effects on the skin (Pangestuti and Kim 2011). The cosmetics industry has been funding the research and development of new products that contain ingredients or extracts from natural sources due to a global trend toward products that are viewed as healthful, environmentally sustainable, and obtained ethically.

Algae are naturally exposed to oxidative stress, and they develop a number of effective defense mechanisms against reactive oxygen species and free radicals. They also produce substances that can protect cosmetics from the damaging effects of UV radiation by acting similarly to the organic and inorganic filters currently on the market (Sousa et al. 2008). For instance, growing *C. vulgaris*, Nostoc, or

Spirulina platensis in the presence of UV light results in an increase in the production of both chlorophyll and carotenoids (Sharma and Sharma 2015). In addition, due to their antioxidant capabilities, these compounds may aid in preventing the oxidation of oil in formulations, particularly in emulsions with a significant quantity of oily phase (Sharma and Sharma 2015).

By encouraging the expression of heme oxygenase-l (HO-l), a molecule that stops the formation of heme on the skin by eliminating heme catabolites, fucus vesiculosus extract helps to diminish the appearance of dark circles on the skin area around the eye. In topical preparations, the extract's anti-inflammatory and antioxidant capabilities may help diminish fine lines and wrinkles while also helping to lessen the appearance of eye bags and eye puffiness. Moreover, utilizing cosmetics and sunscreens could delay or even prevent skin aging (Sun and Chavan 2017).

Inhibiting some inflammatory processes and accelerating the healing process, as well as preserving skin hydration, are all possible effects of particular secondary metabolites of specific microalgae (Kim et al. 2008).

Red microalgae extracts are also used in anti-aging creams, anti-irritant peelers, skin care, sun protection, hair care, emollient, revitalizing, and regeneration products (Sun and Chavan 2017; Borowitzka 2013; Sanghvi and Martin Lo 2010; Arad and Yaron 1992).

In cosmetic compositions, algae are primarily used as thickening, water-binding, and antioxidant agents. However, each species may have made more than one contribution, as shown in Table 5.5 (Hasan and Rina 2009).

These were listed in accordance with the category of cosmetic product to help people understand how algae affects cosmetics.

5.5.8.1 Sunscreen

Frequently used UV filters that shield the skin against a variety of harms, such as photoaging, sunburn, photodermatoses, and skin cancer (Nohynek et al. 2010; Peres et al. 2016; Pereira et al. 2015; Mercurio et al. 2015). Some types of algae produce compounds with specific chemical structures that not only absorb UV light but also prevent the production of melanin (Hagino and Saito 2010).

Chlorogloeopsis spp. extract helps keratinous tissue by protecting it from UVA and UVB damage (which causes the creation of free radicals), preventing photoaging, wrinkle formation, and skin sagging (O'Connor et al. 2011).

Isochrisis algae could block UV transmission with a profile similar to that of a formulation with solely organic and inorganic filters and an SPF of 15. In addition, nannochloropsis algae proved excellent at blocking UVA and UVB transmission. In addition, compared to a commercial formulation, the incorporation of cyanobacteria in sunscreen formulation produced higher UVB-UVA absorption (290 to 400 nm) and good visible spectrum absorption (400–650 nm) (Huner et al. 2004).

The cyanobacterium Nostoc sp. R76DM also makes amino acids that are similar to mycosporine and absorb UV light (MAAs). The antioxidant and reactive oxygen species (ROS) scavenging ability of MAAs palythine, asterina, porphyra, and palythene was dose-dependent in vitro (Rastogi et al. 2016). Although these MAAs were used in the development of no cosmetic product.

Table 5.5 Studies of the use of algae in cosmetics

Algae	Form of use in the formulation	Extraction Solvent	Percentage in formulation (%)	Galenic form	Cosmetic effect	Ref
Genus *Porphyra* and *Wakame* *Spirulina* sp. *Chlorella* sp.	Protein and peptides (dry powder)	Organic solvents	0.01–10	Skin lotion Milky lotion Skin cream Body soap Shampoo Rinse Bath agents	Gloss and moisture on the skin Smoothness, moisture and gloss on the hair	Hagino and Saito (2010)
Coccoid and *Filamentous*	Extract	–	0.1–2.5	Emulsion Lotion Powder product	Enhancing skin barrier and collagen formation Anti-aging effect	Einarsson et al. (2014)
Phaeodactylum tricornutum	Extract	Isopropanol or polar Solvent (Ethanol) with later use of Aqueous-Ethanolic and Heptane	0.1–10	Cream Emulsion Emulsion-Gel	Protecting the skin from the adverse effects of UV exposure Preventing and/or delaying the appearance of skin aging effects	Nizard et al. (2007)
Fucus vesiculosus	Extract	Polar solvents	0.1–10	Liquid Crystal Cream	Reduce the appearance of dark circles Stimulates collagen	Sun and Chavan (2017)
Chlamydocapsa sp.	Lipsomal Extract	–	3.0	Cream Hydrogel Hair mask	Prevent or delay skin aging Avoid loss of the barrier function Reduce transepidermal water loss (TEWL)	Stutz et al. (2012)
Genus *Spirulina*, *Donaliela*, *Hematococcus*, *Nannochloropsis*, *Tetraselmis*	Cell algae	–	1.0–20	Gel, Emulsion (Water/Oil and Oil/Water)	Sunscreen	Lotan (2012)
Cyanobacteria	Extract	Methanol and acetone	0.001–25	Cream	Sunscreen	Huner et al. (2004)

(continued)

Table 5.5 (continued)

Algae	Form of use in the formulation	Extraction Solvent	Percentage in formulation (%)	Galenic form	Cosmetic effect	Ref
Chlorogloeopsis spp.	Extract	Water	0.05–20	Sun Creams and lotions, Shampoo lipsticks	Photoaging, wrinkle, sagging skin, sunscreen, and prevent sun damage to the hair and nail	O'Connor et al. (2011)
Genus *Prototheca*, *auxenochlorella*, *Chlorella* or *Parachlorella*	Cell algae or extract	Water	1.0–90	Cream, soap, lotion, Shampoo, facial wash	Sun protection, hydration, anti-aging, exfoliant to skin or hair	Schiff-Deb and Sharma (2015)

5.5.8.2 Moisturizers

Cosmetics have the power to maintain or enhance the skin's natural protective barrier, maintaining a healthy appearance. They are recommended in atopic dermatitis situations as well as when a client develops dry skin conditions, such as when there has been a change in the filagrin gene, which produces the skin's natural moisturizing factor (NMF) (Ramos-e-Silva et al. 2013; Chaves et al. 2014).

Some proteins including their hydrolyzates from the *Porphyra* genus, *Spirulina* species, and *Chlorella* species have a strong affinity for skin and hair, retaining moisture and having the right viscosity (Hagino and Saito 2010). Products for skin and hair care, body soap or bath products, creams, shampoos, rinses, hair restorers, solutions for permanent waves, shampoos, and rinses all contain cosmetics containing algal peptides (Hagino and Saito 2010).

When compared to the control, skin gloss and moist feel actions in cosmetics containing Porphyra were higher. Cosmetics for hair care that contain spirulina give a moisturizing sensation, gloss, smooth combing, and good sensory qualities. (Hagino and Saito 2010).

As a non-toxic, non-irritating, and non-sensitizing substance that promotes antistatic and emollient actions in moisturizing creams, squalene, which is derived from the Thraustochytriales plants Schizochytrium, Aurantiochytrium, and Thraustochytrium, is used in cosmetics to stimulate ideal skin properties (Pora et al. 2018; Kaya et al. 2011).

5.5.8.3 Anti-Aging Products

Cosmetics used to counteract environmental and intrinsic (smoking, UV radiation, and environmental circumstances) factors that promote aging of the skin (natural physiological changes or genetically determined). For instance, they could lessen the effects of aging and boost the consumer's self-esteem, resulting in a higher standard of living (Mukherjee et al. 2011).

Phloroeckol and tetrameric phloroglucinol, two antioxidant substances chemically categorized as phlorotannins, were discovered in the brown macroalga *Macrocystis pyrifera* recently. The anti-diabetic and antioxidant properties of these phlorotannins may help to delay the aging process of the skin (Leyton et al. 2016).

To increase collagen stimulation, anti-aging treatments can contain extracts of *Monodus* sp., *Thalassiosira* sp., *Chaeloceros* sp., and *Chlorococcum* sp. (Zanella et al. 2018).

Chlorella vulgaris extracts increase the production of collagen in the skin, promoting tissue regeneration and the prevention of stretch marks. Arthrospira extracts could heal the indications of early skin aging and prevent the development of wrinkles (Kim et al. 2008; Spolaore et al. 2006). Numerous vital vitamins, minerals, and fatty acids, such as omega-3 and -6, which are well recognized to promote skin health and cell regeneration, are contained in brown macroalgae's makeup (Kim et al. 2008).

Free radicals may activate metalloproteinase (MMP), harming skin fibroblasts' collagen, cell membranes, and nuclei. It may be possible to prevent UV-induced skin issues such as stratum corneum thickening, rough texture, wrinkles, and flaccidity by using a green algae extract to decrease MMP activity and increase the amount of collagen and elastin in the fibroblast (Shih and Shih 2009).

In this regard, extracts of Coccoid and Filamentous, two blue-green algae found in saline hot water, also contain unidentified biocompounds that have the potential to stop skin photoaging. In addition, by promoting keratinocytes' terminal differentiation and the expression of crucial skin barrier genes, they may improve skin barrier performance (Einarsson et al. 2014). In addition, it promotes the synthesis of new collagen to speed up skin regeneration and stop the aging process (Einarsson et al. 2014).

Extracts from *Phaeodactylum tricornutum* may increase the activity of the proteasome in skin cells, notably keratinocytes, fibroblasts, or melanocytes. In additionto improving skin elasticity and firmness, this extract can shield the skin from the damaging effects of UV radiation. It might prevent wrinkles from forming or lessen the depth of existing wrinkles (Nizard et al. 2007).

In products for the skin and even hair protection, extracts of the snow algae *Chlamydocapsa* sp. are applied topically to function primarily in oxidative reactions, such as photoaging. In addition, it could lessen transepidermal water loss (TEWL), prevent the development of wrinkles brought on by exposure to UV rays, cold, or dryness, and shield against the loss of the barrier function brought on by environmental exposure (Stutz et al. 2012).

5.5.8.4 Whitening

Cosmetics that block the tyrosinase enzyme prevent excessive skin pigmentation and promote whitening (Babitha and Kim 2011). *Nannochloropsis oculata*'s pure extract, which contains the anti-tyrosinase zeaxanthin, was trademarked for use in creams (Babitha and Kim 2011).

5.5.8.5 Hair Care

For formulations to stop hair loss, extracts of *Monodus* sp., *Thalassiosira* sp., *Chaeloceros* sp., as well as *Chlorococcum* sp. are suggested because they could alter melanogenesis in human skin and hair, enhancing and promoting keratinocyte differentiation, melanocyte proliferation, and the growth of human hair and hair follicles (Zanella et al. 2018).

Skin and hair can be softened and made more flexible by cosmetic formulations for sun protection and anti-aging that comprise species of the microalgae *Chlorella* genus, which mostly consist of intact microalgal cells and contain oil derived by dry weight (Brooks and Franklin 2013).

Finally, taking into account the current market, alguronic acid appears as a new topical cosmetic product capable of enhancing skin appearance and health. It is made of a combination of polysaccharides extracted from biomass of the cyanobacteria *Chlorella protothecoides* (UTEX 31) and Parachlorella, which are grown in the dark under heterotrophic conditions (Im et al. 2012). Further research to support the product's effect is not yet available, though. We therefore propose additional research on bioactive chemicals to enhance the biological activities of algae, taking into account that the use of algae in cosmetics is a promising topic for the cosmetics sector.

5.5.9 Others

There exist several other applications of marine algae in niches such as antifouling, anti-coagulants (Li et al. 2017), and so on: the list is unending seeing that there is a current drive by researchers/industrialists toward the utilization of these materials for advanced uses.

5.5.10 Potential Uses of Marine Algae

5.5.10.1 Potential Use in Dermatology

Algae Against Acne Vulgaris

Many teens and young adults suffer with acne vulgaris, sometimes known as acne, which is a prevalent skin problem. It is distinguished by pimples, blackheads or whiteheads, greasy skin, and even scars. Years of acne can leave lifelong scars, deformity, and negative impacts on physiological development (Leyden 1995). Acne has a complicated and multifaceted etiology. Although acne is typically thought of as an inflammatory condition, it can also be caused by germs, hair follicle keratinization, and sebum release (Farrar and Ingham 2004). Acne is typically caused by *Staphylococcus epidermidis, S. aureus, Pseudomonas aeruginosa,* and *S. aureus (*Yamaguchi et al. 2009*).* Traditional treatments for acne vulgaris caused by bacterial overgrowth include clindamycin and erythromycin. Nevertheless, widespread use of antibiotics has resulted in bacterial resistance. In addition, antibiotics may irritate and induce skin allergies. Therefore, the bioactive substances produced from sea algae may be a secure, all-natural substitute. It has been observed that macroalgae extracts contain antibacterial and antifungal properties (Pérez et al. 2016). When extracts from different marine algae were tested for their ability to kill skin-related bacteria, some potent antibacterial chemicals were discovered. In addition, some macroalgal extracts have anti-inflammatory properties and have the capacity to control collagen and growth factor levels, which may help to treat acne and hasten skin restoration (Lee et al. 2009b).

5.5.10.2 Algae Protects Skin from UV Radiation Injury

In recent years, photoaging brought on by excessive sun exposure has become a major issue. Fig. 5.11 illustrates the method by which UV promotes the creation of reactive oxygen species (ROS) (Pallela et al. 2010); this is essential for maintaining homeostasis and cell signaling. However, the concentration of ROS will increase

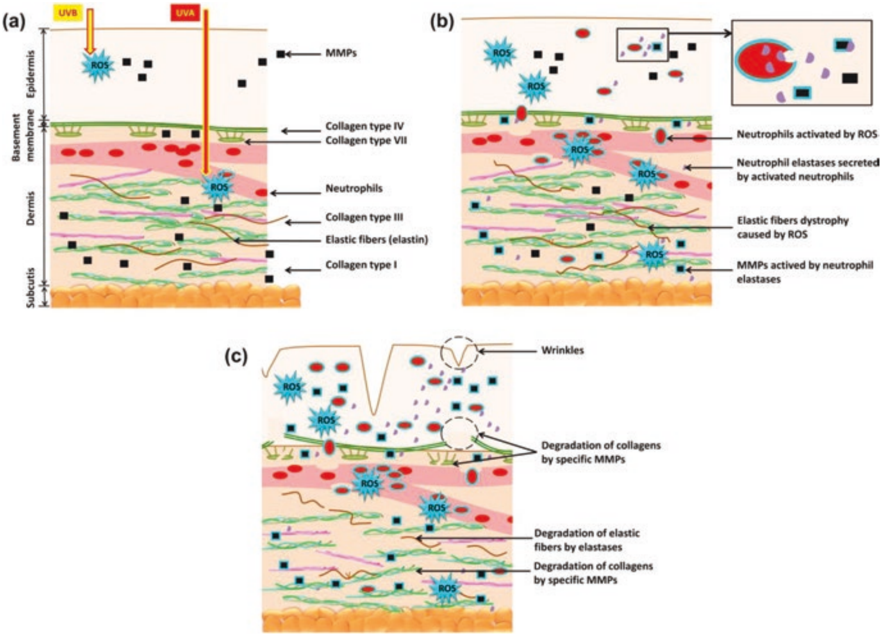


Fig. 5.11 The mechanism of photoaging: (**a**) The amount of ROS increased by UV exposure: UVB usually reach the epidermis, while UVA can penetrate the epidermis, and reach the dermis. When skin is exposed to UV radiation, the concentration of ROS will rise due to the skin antioxidant defenses. (**b**) The damage caused by ROS: When expose to UV radiation, ROS proliferates quickly, and neutrophils will be activated by high concentrations of ROS. Then ROS cause elastic fibers dystrophy and activated neutrophils secrete neutrophil elastases which activate matrix metalloproteinases (MMPs). (**c**) The damage caused by MMPs: The degradation of collagen is caused by MMPs, and the degradation of elastic fibers is caused by elastases, leading to collagen-support reduce and loss of skin elasticity, finally promote winkle formation and accelerate skin aging. (Reproduced with copyright permission from Wang et al. (2017), Elsevier 2017)

quickly when skin is subjected to unfavorable conditions, such as UV irradiation or high temperatures, and a high concentration of ROS may harm cell structure. Collagen and elastin in the dermis support the epidermis and help keep skin supple and elastic. High quantities of ROS will cause neutrophils to become activated when exposed to UV irradiation (Fig. 5.11a). Once elastic fiber dystrophy is brought on by ROS, active neutrophils produce neutrophil elastases, which in turn activate matrix metalloproteinases (MMPs) (Fig. 5.11b) (Chen et al. 2012). Elastic fiber breakdown is likewise a result of elastases, whereas collagen degradation is a result of MMPs. The skin begins to lose its elasticity and develop wrinkles when exposed to excessive UV light. MMP buildup is therefore bad for skin (Fig. 5.11c). To combat photoaging, marine organisms, particularly macroalgae, create a wide range of well-known photoprotective and anti-photoaging chemicals (Pallela et al. 2010). In addition to absorbing UV-A and UV-B rays, macroalgal bioactive compounds also have the ability to scavenge ROS that have formed and prevent the synthesis of MMPs.

5.5.10.3 Algae for Melanoma Treatments

A relatively frequent malignant tumor is skin cancer. Basal cell carcinoma, squamous cell carcinoma, and melanoma are the three primary kinds of skin cancer. Non-melanoma skin cancers include basal cell and squamous cell carcinomas. The most dangerous and prevalent type of skin cancer is melanoma, which is produced by melanocytes. The majority of melanomas range in color from brown to black, but they can also have more aggressive features including pink, red, or flesh color, as well as itchiness or bleeding. Skin cancer is brought on by both environmental and hereditary causes, such as pale skin, UV exposure, and numerous benign naevi (Garbe and Leiter 2009). Overexposure to UV radiation is the main risk factor for skin cancer. Repeated exposure to UV radiation can result in skin cancer, according to a number of experimental animal studies (Chiang et al. 2015; Wang et al. 2015; Cordeiro-Stone et al. 2016). Thus, wearing sunscreen and limiting your exposure to UV radiation are excellent ways to avoid skin cancer. Additional therapies are also required, such as surgery, chemotherapy, radiation therapy, and targeted therapy. The most often prescribed chemotherapy medications typically have higher cytotoxicity and side effects, which can injure other body organs, lower quality of life, and worsen medical conditions. For instance, autoimmune-mediated adverse effects such as colitis, hypophysitis, hepatitis, and iridocyclitis may appear in patients receiving CTLA-4 antibody therapy for metastatic melanoma (Kähler and Hauschild 2011). Treatment for metastatic melanoma with vemurafenib may result in facial palsy (Klein et al. 2013). There is an urgent need to research more suitable and efficient skin cancer treatments.

Marine algae have been shown to contain antitumor and cytotoxic substances, such as polysaccharides from *Sargassum fusiforme* that have anti-liver cancer activity (Fan et al. 2017). Spatane diterpinoids isolated from brown marine algae *Stoechospermum marginatum* can effectively inhibit malignant melanoma growth (Velatooru et al. 2016). Ascophyllan derived from brown seaweed *Ascophyllum nodosum* exhibits in vivo anti-metastatic activity on B16 melanoma cells (Abu et al.

2015). The anti-melanoma mechanisms of bioactive compounds derived from macroalgae usually rely on activating the caspase cascade such as caspase-3, -6, -9, and reducing the expression of cyclin-dependent kinase (cdk2, cdk4) and matrix metalloprotease family.

5.6 Challenges and Ways Forward

Compared to terrestrial biomass, the estimated costs for marine algae are now many times higher, but with better yields, scale, and operations, algae may eventually become cost-competitive with terrestrial crops (Warner et al. 2015). About 50 countries presently undertake seaweed cultivation, which has been expanding quickly (traditionally in Japan, the Republic of Korea, and China). Also gathered in 2014 were 27.3 million tons of aquatic plants, including seaweed, for a total value of $5.6 billion (Hasan and Rina 2009). The expense of upkeep for marine sensors, charging for autonomous underwater vehicles, charging for transport vessels, etc., may be another obstacle in the growth or production of marine algae.

The cost of production for marine algae is very high in comparison to conventionally cultivated crops: this eventually results in high final product cost. Simpler cultivation approaches, cheaper technologies for marine-based sensors, underwater vehicles, etc. should be adopted to cut the production cost of these essential marine algae.

Again, we must remember that there is limited or rare awareness of the general public to/on the importance of marine algae and the nutritional as well as medicinal value. It is very difficult to make people accept marine algae as an alternative food source as well as medicines seeing the general populace is accustomed with land cultivation food products. The way forward in this regard is to sensitize the general public via the Internet, televisions, radios, and other available media outlets on the authenticity, importance, as well as potential of marine algae important attributes and their potential to replace most conventionally cultivated agro-products and pharmaceuticals.

The improvement of microalgae’s growth and yield is one of the primary obstacles to expanding biotechnological applications of these organisms. The balance between actively dividing and dying cells determines the growth rate of a microalgal population. This “performance index” is primarily influenced by how well cells adapt to their culture environment and how expensive it is to regulate internal activities energetically. The latter is based on the strains’ physiological adaptability and how well their ecophysiological requirements match the surroundings. The “Photosynthetic Regulation Biotechnology” and the genetic engineering of strains are two of the most promising paths forward for increasing biomass or the creation of intriguing chemicals.

To maximize the fitness of farmed species and reduce production costs, the functional biodiversity of microalgae must be thoroughly investigated. In addition, the mass culturing conditions now in use must be reconsidered. For the reasons already covered in the regulated vs. uncontrolled environmental characteristics, indoor culturing systems would be preferred to outdoor systems (Barra et al. 2014). In addition, we advise using local microalgal species and saltwater from which the species has been isolated, i.e., growing plants close to aquatic habitats. This method could lower the cost of cultivation, permit the use of recently separated species and strains, and possibly also offer significant flexibility in the selection of species that have similar ecophysiological requirements. This might help address the potential decrease of growth efficiency brought on by sustained cultivation.

In addition, given that ammonium is digested more quickly than nitrate and thus allows for an increase in production efficiency, we advise using it as a nitrogen source to boost biomass production (see above). For the reasons described in the preceding sections, we propose using a sinusoidal-shaped intra-diel light–dark cycle as opposed to a quadratic distribution. Through a program of light variation frequency, turbulence, which reproduces coastal habitats, can be used to cultivate coastal species. We suggest using lighting technologies that enable control of the photon flux density and spectrum composition to better optimize photosynthetic productivity (Barra et al. 2014). To boost growth yield and lower production costs, green radiation may be eliminated from the light spectrum for several microalgal groups (such as diatoms or chlorophytes) (Wondraczek et al. 2013). We propose that genetic engineering should focus on the photophysiological response system, entering the field of "photosynthetic regulatory biotechnology," since the impact of light fluctuations on the physiology and growth of autotrophs is significant (Costa et al. 2013). However, when microalgae go through photophysiological pathway change, there is a strong possibility of developing a productive strain with a modest growth rate.

Diatoms, which include many coastal species, may be the ideal model to cultivate for biotechnological purposes due to their enormous variety and numerous ecological idiosyncrasies (see previous). To successfully cultivate diatom species for biotechnological applications, ecophysiological and process engineering researchers must communicate more effectively. Many diatom species thrive on benthic substrates, at least during one stage of their life cycle. In addition, they have sexual reproduction (Reed and Stewart 1988), which enables frequent gene recombination processes and significant physiological flexibility (Brunet et al. 2013). By adjusting the growth conditions, we could readily regulate their photophysiological reactions and biochemical pathways to boost the synthesis of specified compounds.

5.7 Conclusion and Future Prospect

The current study provides a succinct overview of marine algae with particular emphasis on marine algae in general: the chapter details the distribution of marine algae. Macroalgae are significant sources of the extraction of natural components, it can be said after summing the studies looking into the usage and composition of marine algae. These algae can be harvested and are used extensively in medicine and nutrition throughout the world. In addition, the biomass could be used to make adsorbents for water treatment filters or as a source of biofuel.

Marine algae if utilized efficiently can be a source material for many advanced materials for important applications. Thus, a variety of antioxidants, such as carotenoids, tocopherol, ascorbic acid, chlorophyll derivatives, phlorotannins, polyphenols, mycosporine-like amino acids, etc., can be produced using synthetic means. With careful investigation/research into these wonderful materials is highly essential to discover other beneficial antioxidants of high value for diverse applications.

As well as being a significant source of minerals, vitamins, proteins, fibers, and polyunsaturated fatty acids, marine algae are also known to include other nutrients. As was already noted, other studies have also shown their advantageous impacts on consumption. There is therefore a need to dig more into these valuable materials for better materials supplements (minerals, vitamins, proteins, fibers, etc.) as an awaited answer to diverse biomedical challenges.

Marine algae are projected as biorefinery to be a major source of industrially important chemicals for advanced materials synthesis like proteins, carbohydrates, polymers, oils, fats, aromatics, etc. Marine algae are underutilized at present though there is a new drive into their application by both researchers and industrialists globally: come on, let us make maximum use of marine algae.

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Antarctica Microbial Communities: Ecological and Industrial Importance

6

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Abstract

Prokaryotic life is continuously evolving under several extremes in the Antarctic landmass. Microbes exist in snow, lake, marine, and other habitats by developing unique strategies to withstand multiple extremes like cold stress, UV stress, metal stress, low nutrient stress, etc. The microbes present in the ocean have a great significance on the biological carbon pump. They reduce ~40% of CO_2 from the atmosphere. A large group of microbial communities has already been reported from both terrestrial as well as marine environments in Antarctica. Many microorganisms reported from snow are capable of producing extracellular enzymes like protease, amylase, lipase, esterase, etc., that help to mineralize the organic substances packed in the snow. Some microorganisms produce pigments that defend themselves from harmful radiation and oxidative injury. The microbes also secret cold active proteins that are functional in cold conditions and help to survive the microbes under cold stress. To overcome cold and radiation stress, some microbes develop stress responsive proteins, enzymes, extracellular polysaccharides, and other bioactive compounds. Thus, microbes from extreme environments possess a great interest in industrial and biotechnological applications like antioxidant, photoprotective, cryoprotective, antimicrobial, and emulsifying activities of the bioactive compounds. In the field of biomedical applications, these bioactive compounds also have fascinating activities like antitumor and anti-proliferating agents in drug manipulation. In this chapter, we will

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briefly discuss the microbial diversity of Antarctic land and its periphery and their biotechnological potentialities.

Keywords
Antarctica · Cold adaptation · Polymeric substance · Pigments · UV stress · Industrial impact

6.1 Antarctica: The No-Man's Land

The major part of the earth approximately >85% is the cold biosphere where the permanent temperature is below 5 °C. The major constituent of the cold biosphere is the Antarctic, Artic, glaciers, deep ocean and high-altitude mountains. The coldest, iciest, windiest, dry desert Antarctica is the world's one of the most extreme habitats (Chattopadhyay et al. 2014). The fifth-largest continent Antarctica is the least explored, least understood, and least great mystery on Earth. The Antarctic landmass is covered by 5.4 million square miles of frozen desert. Barely anything can survive here, no native people, or land mammals, not even a single tree in this driest place on Earth. The thick ice sheet totally covers the Antarctic continent except <2% of the total area is ice-free. This ice-free area does not support the growth of any vascular plant but constitutes the predominant biomass of bacteria, cyanobacteria, algae, fungi, lichens and a few mosses so this area considers the driest deserts on Earth (Reddy et al. 2002).

Continent Antarctica is divided by Walton 1984 into three regions: (1) no precipitation and permanently ice-free region, like Victoria land, the dry valley; (2) snow and ice cover mountains or exposed area of rock in mountains, like Theron mountain, the nunataks; and (3) the regions where the ice meld during summer and ice cover during winter, like Bunger and Schirmacher oases. During the summer season, free water is available in the oases (Shivaji et al. 1989). The planktons are growing on the surface of the ocean and both bacterioplankton and zooplankton are distributed by different major fronts like Polar Front, Southern Antarctic Circumpolar Current Fronts, Subantarctic Front and Subtropical Front (Gupta et al. 2015). Antarctic Circumpolar Current (ACC) is the series of eastward-flowing jets that is the strongest Polar Front. A distinctive biogeographical discontinuity is represented by the Polar Front because this is a strong barrier to free north-south exchange of water. The sharp change of surface temperature is visible at significant depths up to >1000 m (Clarke et al. 2005). The most biodiverse habitat of Antarctica is the marine benthic environment because it contains a majority of the regions of life. For scientific research purposes, permanent human settlement is begun in the continent in the mid-twentieth century. Non-native species are introduced by direct exposure to anthropogenic activity including national operators, military, private yachts, fishing, and tourist vessels. Thus, increasing of marine traffic in the water around Antarctica, lead to increase the non native species invasiveness. The Antarctic Circumpolar Current is the present considerable barrier of the geographically isolated Antarctica for species movement. The species survivorship is also reduced by physiological constraints (Hughes and Ashton 2017; Lynch et al. 2010).

6.2 Microbial Diversity of Antarctic Territory

Antarctica is the harshest, cold, and arid, remotely located, and frequently ice-covered barren, frozen landscape on Earth. Studying the soil, permafrost, and ice core samples reveals the presence of diverse masses of prokaryotes and eukaryotes organisms (Antony et al. 2012). Antarctica has a very robust divergence of species diversity in marine ecosystems as well as terrestrial ecosystems. The terrestrial ecosystem is species-poor in contrast to the marine ecosystem which is diverse of species richness in benthic communities (Rogers 2007). The habitats such as sea ice, permafrost, snow, glaciers, sediment, and oceanic water are provided by the cold biosphere. These habitats are helpful for the survival and reproduction of life forms. Diverse microorganisms colonize in this cold habitat such as bacteria, phage, archaea, algae, fungi, cysts, etc. (Suyal et al. 2018; Kumar et al. 2019). Those microorganisms are referred to as psychrophiles (Chattopadhyay et al. 2014). Despite the extreme climatic condition, the microorganisms are detected from different frozen land and seawater of the Antarctic continent. The microorganism differs in the range of different richness of nutrients, temperature, availability of water, etc. Terrestrial microbial diversity has been already explored from King George Island, Livingston Island, Ardley Island, Fildes Peninsula, Galindez Island, Queen Maud Land, Barrientos Island, Haswell Island, Victoria Land, Ellsworth Mountains, Schirmacher Oasis, Wilkes Land, Dronning Maud Land, Princess Elizabeth Land, James Ross Island, McMurdo Dry Valleys, Antarctica. As well as marine microbial community has also been explored from the Ross Sea, Bellingshausen Sea, Weddell Sea, Antarctic seawater, Antarctic sediment, etc. (Fig. 6.1).

6.2.1 Terrestrial Microflora

The terrestrial ecosystem accumulates divergence of microbial communities (Fig. 6.1). Microbial diversity is reported from King George Island, Nelson Island, Livingston Island, and Ardley Island; these are *Polaromonas* sp., *Deinococcus* sp., *Filibacter* sp., *Planococcus* sp., *Chryseobacterium* sp., *Flavobacterium* sp., *Arthrobacter* sp., *Lysobacter* sp., *Pseudomonas* sp., *Bacillus* sp., *Zobellia* sp., *Salinibacterium* sp., *Cryobacterium* sp., *Pedobacter* sp., *Sphingomonas* sp., *Burkholderia* sp., *Rhodanobacter* sp., *Hymenobacter* sp., *Streptomyces* sp., *Frigoribacterium* sp., *Paenibacillus* sp., *Janibacter hoylei, Marisediminicola antarctica, Arthrobacter flavus, Nocardioides* sp., *Phycicoccus jejuensis, Aeromicrobium tamlense, Angustibacter luteus, Hymenobacter glaciei, Subsaxibacter arcticus*, *Luteolibacter* sp., *Janibacter hoylei*, *Bacillus mojavensis, B. subtilis, B. mojavensis, B. safensis, B. licheniformis, Gordonia terrae*, etc. Some cyanobacteria, i.e., *Hassalia* sp., *Anabaena* sp., *Nostoc* sp., *Phormidium autumnale*

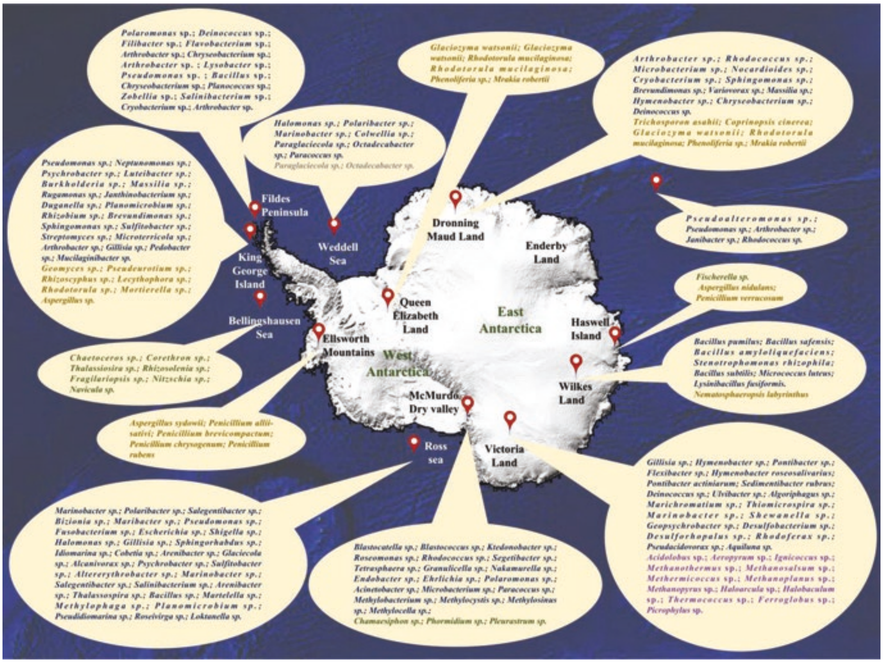

Fig. 6.1 The diversity of different species of bacteria, fungi, yeast, and archaea in Antarctic land and its periphery

are also reported. The fungal species identify as *Geomyces* sp. is reported from King George Island (Liu et al. 2021; Vila et al. 2019; Lo Giudice and Fani 2016; Pershina et al. 2018; Efimenko et al. 2018). Peninsula is a snow-free coastal area during the summer season but permanently it is covered by snow and ice. The Fildes peninsula is 4.3 miles long and present in the southwestern part of King George Island at the end of Antarctica. Bacterial species were reported from Fildes Peninsula by China's 27th and 31st Antarctic expeditions. Inhabitant species are *Pseudomonas* sp., *Neptunomonas* sp., *Psychrobacter* sp., *Luteibacter* sp., *Burkholderia* sp., *Massiliai* sp., *Rugamonas* sp., *Janthinobacterium* sp., *Duganella* sp., *Planomicrobium* sp., *Rhizobium* sp., *Brevundimonas* sp., *Sphingomonas* sp., *Sulfitobacter* sp., *Streptomyces* sp., *Microterricola* sp., *Arthrobacter* sp., *Gillisia* sp., *Pedobacter* sp., *Mucilaginibacter* sp. Some fungal species are also reported these are *Geomyces* sp., *Pseudeurotium* sp., *Rhizoscyphus* sp., *Lecythophora* sp., *Rhodotorula* sp., *Mortierella* sp., *Aspergillus* sp. (Cong et al. 2020). The region of Queen Maud land is about 1.0 million square miles long. It is reliant on the territory of Norway. Its appellation is after the Norwegian queen Maud of Wales (1869–1938). The novel bacterial species is isolated from Queen Maud Land, identify as *Marisediminicola senii* sp. nov. (Jani et al. 2021). The small, ice-free Barrientos Island is 1.71 by 0.54 km ranging. It is present on South Shetland Island, Antarctica. In Barrientos Island, the bacterial species represented by *Brevibacterium* spp., *Demetria* spp., *Gordonia*spp., *Janibacter* sp., *Kocuria* sp., *Lapillicoccus* sp., *Micromonospora* sp., *Nocardioides* sp., *Rhodococcus* sp., *Bradyrhizobium* sp., *Methylobacterium* sp., *Sphingomonas* sp. (Lo Giudice and Fani 2016). The rocky coastal Haswell Island is located in Mabus Point, Antarctica. In Haswell Island, the fungal species is represented by *Aspergillus nidulans, Penicillium verrucosum* and a reported algal species identify as *Fischerella* sp. (Gesheva and Negoita 2012; Lo Giudice and Fani 2016). The chain of Ellsworth Mountains is the highest mountain range in Antarctica. The mountains are 350 km long and 48 km wide present on the western margin of Marie Byrd Land. Reported fungal species from this mountain are identified as *Aspergillus sydowii, Penicillium allii-sativi, P. brevicompactum, P. chrysogenum, P. rubens* (Godinho et al. 2015; Lo Giudice and Fani 2016). The bacterial species represented by *Psychrobacter* sp. and *Rhizobium* sp. are reported from Galindez Island, Antarctica (Kirkinci et al. 2021). One of the most extreme deserts in the world is McMurdo Dry Valleys. These are the large snow-free and very low humidity valleys in Antarctica located in Victoria Land. The reported bacterial species are represented by *Blastocatella* sp., *Blastococcus* sp., *Ktedonobacter* sp., *Roseomonas* sp., *Rhodococcus* sp., *Segetibacter* sp., *Tetrasphaera* sp., *Granulicella* sp., *Nakamurella* sp., *Endobacter* sp., *Ehrlichia* sp., *Polaromonas* sp., *Acinetobacter* sp., *Microbacterium* sp., *Paracoccus* sp., *Methylobacterium* sp., *Methylocystis* sp., *Methylosinus* sp., *Methylocella* sp., *Janthinobacterium* sp., *Arthrobacter* spp. Some cyanobacteria identify as *Chamaesiphon* sp., *Phormidium* sp. an algal species reported as *Pleurastrum* sp. and a fungal species represented by *Phoma herbarum* (Mezzasoma et al. 2022; Sommers et al. 2019; Vishnivetskaya et al. 2018; Lo Giudice and Fani 2016; Onofri et al. 2000), Bacteria identify as *Flavobacterium* sp. and *Janthinobacterium* sp. are reported from Schirmacher Oasis (Mojib et al. 2010; Lo Giudice and Fani 2016). From Princess Elizabeth Land and Dronning Maud Land, the inhabited bacterial species are *Arthrobacter* sp., *Rhodococcus* sp., *Microbacterium* sp., *Nocardioides* sp., *Cryobacterium* sp., *Sphingomonas* sp., *Brevundimonas* sp., *Variovorax* sp., *Massilia* sp.,*Hymenobacter* sp., *Chryseobacterium* sp. and *Deinococcus* sp. Some reported fungal species are *Trichosporon asahii* and *Coprinopsis cinerea*. Yeast identifies as *Glaciozyma watsonii*, *Rhodotorula mucilaginosa*, *Phenoliferia* sp., *Mrakia robertii* (Sanyal et al. 2020; Ashok et al. 2019; Peeters et al. 2011). A dinoflagellate cyst *Nematosphaeropsislabyrinthus* and some bacterial species *Bacillus pumilus*, *Bacillus safensis*, *Bacillus amyloliquefaciens*, *Stenotrophomonas rhizophila*, *Bacillus subtilis*, *Micrococcus luteus*,*Lysinibacillus fusiform* are reported from Wilkes Land coast, Windmill Islands region, East Antarctica (Hartman et al. 2018; Tomova et al. 2014). The Victoria land is located in east Antarctica, westward from the Ross Sea in Antarctic Plateau. This region includes ranges of mountains, dry valleys (McMurdo Dry Valleys), and an active volcano Mount Melbourne about 9000 ft high. Different bacterial and archaeal species are reported from the Victoria land. The bacterial species are represented by *Gillisia* sp., *Hymenobacter* sp., *Pontibacter* sp., *Flexibacter* sp., *Hymenobacter roseosalivariusI*, *Pontibacter actiniarum*, *Sedimentibacter rubrus*, *Deinococcus* sp., *Ulvibacter* sp., *Algoriphagus* sp., *Marichromatium* sp., *Thiomicrospira* sp., *Marinobacter* sp., *Shewanella* sp., *Geopsychrobacter* sp., *Desulfobacterium* sp., *Desulforhopalus* sp., *Rhodoferax* sp., *Pseudacidovorax* sp., *Aquiluna* sp., *Pseudomonas* sp., *Psychrobacter* sp., *Shewanella arctica*, *Gelidibacter gilvus*, *Gelidibacter algens*, *Flavobacterium* sp., *Leifsonia* sp.,*Carnobacterium* sp., *Staphylococcus* sp., *Devosia psychrophile*, *Rhodobacter* sp., *Marinobacter* sp., *Pseudomonas* stutzeri, Psychrobacter *fozii*, *Aeromicrobium* sp., *Kocuria* sp., *Leifsonia* sp., *Rhodoglobus* sp., *Rothia* sp., *Alkalibacterium kapiis*, *Carnobacterium alterfunditum*, *Planococcus antarcticus*, *Sporosarcina* sp. The reported archaeal species identify as *Acidolobus* sp., *Aeropyrum* sp., *Ignicoccus* sp., *Methanothermus* sp., *Methanosalsum* sp., *Methermicoccus* sp., *Methanoplanus* sp., *Methanopyrus* sp., *Haloarcula* sp., *Halobaculum* sp., *Thermococcus* sp., *Ferroglobus* sp., *Picrophylus* sp. (Rizzo et al. 2020; Papale et al. 2019; Aislabie et al. 2006). The bacterial species represented by *Pseudomonas leptonychotis*, *Pseudomonas guineae*, *Pseudomonas peli*, *Achromobacter* sp., *Janthinobacterium* sp., *Rhodoferax* sp., *Sulfuricurvum* sp., *Sulfurimonas* sp., *Pseudomonas* sp., *Roseococcus* sp., *Deinococcus* sp. are reported from James Ross Archipelago, West Antarctic (Fernández et al. 2022; Nováková et al. 2020).

6.2.2 Marine Microflora

Beyond our naked eyes, the other microbial world is present on the ocean floor. They are invisible but have a great impact on our ecosystem. These microflorae include bacteria, archaea, fungi, diatoms, phage, etc. Those microbes adapt fundamentally to the changing Antarctic ecosystem. About 80% of ocean weight is covered by microbial diversity. The committee of the microflora is reported from the Weddell Sea, Bellingshausen Sea, Ross Sea, Antarctic seawater, and Antarctic sediment. The Weddell Sea is around 1200 miles widest and the area is around 2.8 million square kilometers large. It is part of the Southern Ocean embracing various ice shelves. The reported bacteria from the Weddell Sea are inhabited by *Halomonas* sp., *Polaribacter* sp., *Marinobacter* sp., *Colwellia* sp., *Paraglaciecola* sp., *Octadecabacter* sp., *Glaciecola* sp., *Pseudoalteromonas* sp., *Paracoccus* sp. and the reported phage species are identified as *Paraglaciecola* sp., *Octadecabacter* sp. (Luhtanen et al. 2018). The Bellingshausen Sea is present on the west side of the Antarctic peninsula. In 1821, Fabian Gottlieb Thaddeus von Bellingshausen, a Russian naval officer is the first to explore this area so it takes the name the Bellingshausen Sea. The depth of the sea near about 4.5 km. Many diatoms are reported from the Bellingshausen Sea, those are identified as *Chaetoceros* sp., *Corethron* sp., *Thalassiosira* sp., *Rhizosolenia* sp., *Fragilariopsis* sp., *Nitzschia* sp., and *Navicula* sp. (Meiners et al. 2004). The bacterial strains reported from Antarctic seawater are showing close similarity with *Pseudoalteromonas* sp., *Pseudomonas* sp., *Arthrobacter* sp., *Janibacter* sp., *and Rhodococcus* sp. (Fig. 6.1). The bacterial species on Antarctic sediment are Inhabitat by *Microlunatus* sp., *Nesterenkonia* sp., and *Nocardiopsis* sp. (Lo Giudice and Fani 2016). Between Victoria Land and Marie Byrd Land, the deep bay of the Southern Ocean is the Ross Sea. In 1841, after visiting this area, the British explorer James Ross delivered its name the Ross Sea. The Ross Sea is about 320 km from the south pole and has an area of 637,000 square kilometers. From the Ross Sea, the bacterial species are identified as *Marinobacter* sp., *Salegentibacter* sp., *Bizionia* sp., *Maribacter* sp., *Pseudomonas* sp., *Fusobacterium* sp., *Escherichia* sp., *Shigella* sp., *Halomonas* sp., *Gillisia* sp., *Polaribacter* sp., *Shewanella* sp., *Glaciecola* sp., *Sphingorhabdus* sp., *Idiomarina* sp., *Cobetia* sp., *Arenibacter* sp., *Alcanivorax* sp., *Psychrobacter* sp., *Sulfitobacter* sp., *Altererythrobacter* sp., *Salinibacterium* sp., *Arenibacter* sp., *Thalassospira* sp., *Bacillus* sp., *Pseudoalteromonas* sp., *Thalassobacter* sp.,*Martelella* sp., *Methylophaga* sp., *Planomicrobium* sp., *Pseudidiomarina* sp., *Roseivirga* sp., *Loktanella* sp., *Pseudomonas neustonica, Shewanella psychromarinicola* (Li et al. 2019; Jang et al. 2020; Hwang et al. 2019; Silvi et al. 2016).

6.2.3 Symbiotic Microbiome

The symbiotic relationship is a beneficial outcome where organisms are involved in a mutualistic association. Lichen is a composite organism where algal species are photobionts and fungal species are mycobionts both are involved in mutualistic relationships. The algal species provide food by photosynthesis and fungal species provide shelter and absorb nutrients from the soil then supply them to algal species (Bhatt et al. 2022). In the polar and alpine regions, lichen is used as a pollution indicator. Lichen is reported in Antarctica from Admiralty Bay, Schirmacher Oasis, Victoria Land, King George Island, Elephant Island, Deception Island, Livingston Island, Nelson Island, Barton Peninsula, etc. The identified lichen species are *Umbilicaria antarctica, Arthonia rufidula, Bacidia stipata, Sarcogyne privigna, Lecanora mawsonii* between Admiralty Bay and Schirmacher Oasis; *Buellia frigida, Lecanora aff. orosthea, L. aff. geophila, L. fuscobrunnea, Lecidea andersonii, L. cancriformis* between Schirmacher Oasis and Victoria Land, and *Buellia cladocarpiza*, *Tephromela atra* between Admiralty Bay, Schirmacher Oasis, Victoria Land. Lichen reported from Admiralty Bay are identified as *Cladonia* sp., *Pertusaria* sp., *Psoroma* sp., *Stereocaulon* sp., *Tephromela* sp., etc. (Sancho et al. 2019; Singh et al. 2015). Lichen reported from Barton Peninsula and King George Island identified as *Umbilicaria antarctica, Psoroma antarcticum*, *Usnea antarctica*, *Usnea aurantiaco-atra*, *Caloplaca regalis*, *Xanthoria elegans*, and *Ramalina terebrata* reported fungal species in lichen are *Phoma* sp., *Pseudogymnoascus pannorum*, *Chalara* sp., *Chaunopycnis alba*,*Cylindrocarpon* sp., *Phomopsis* sp., *Mortierella gamsii*, *Phaeosphaeria microscopica*, *Thelebolus microspores*, *Cladosporium herbarum*, *Monascella* sp., *Acremonium rutilum*, *Chalara constricta*, *Volucrispora graminea*, *Phialophora malorum*, *Pycnostysanus* sp., *Camarosporium* sp., *Libertella* sp., *Myrioconium* sp., *Phialophora* cf. *alba, Scytalidium* sp., *Chaetomium globosum*, *Phaeosphaeria eustoma*, *Acremonium butyri*, *Acremonium cerealis*, *Acremonium psychrophilum*, *Alternaria alternata*, *Alternaria* cf. *chlamydospore*. (Park et al. 2018; Carvalho et al. 2019) *Lecanora fuscobrunnea*, *Usnea antarctica*, *Lecanora* sp., *Lecanora fuscobrunnea*, *Lecidea cancriformis*, *Xanthoria elegans*, *Umbilicaria decussata*, *Rhizocarpon* sp., *Lecidea* sp., *Acarospora flavocordia* reported fungal species are *Elasticomyces elasticus*, *Friedmanniomyces* sp., *Endolithicus* sp. Lichen reported from Elephant Island, Deception Island, Livingston Island, and Nelson Island are identified as *Usnea antarctica*, *Ramalina terebrata* are identified as *Antarctomyces psychrotrophicus, Bensingtonia yamatoana, Capnobotryella* sp., *Cladosporium* sp. *Cryptococcus victoriae*, *Rhodotorula larynges*, *Bensingtonia yamatoana*, *Candida parapsilosis*, *Cryptococcus aquaticus*, *Naganishia friedmannii*, *Goffeauzyma gilvescens*, *Tremella* sp., *Solicoccozyma terricola*, *Vishniacozyma victoriae*, *Debaryomyces hansenii*, *Fusarium* sp., *Fusarium* cf. *globosum* (Carvalho et al. 2019; Duarte et al. 2016). The bioactive compound reported from lichen has antibacterial, antioxidant, anti-inflammatory, and cytotoxic activity (Londoñe-Bailon et al. 2019).

6.3 Role of Microbes on the Ecology of Antarctica

The Antarctic ecosystem is unfavorable to surviving any living organism. The coldest, driest, and harshest environment is uncomplimentary for survival and reproduction. The ozone hole in the stratosphere about 14 km above the continent is the door to the coming of non-ionizing ultraviolet radiation. UV radiation is of different types, which are classified according to their wavelength. UV radiation wavelengths are between 40 and 400 nm. These are divided into vacuum UV wavelength between 40 and 190 nm, far UV wavelength between 190 and 220 nm, UVC wavelength between 220 and 280 nm, UVB wavelength between 280 and 314 nm; and UVA wavelength between 314 and 400 nm (Núñez-Pons et al. 2018). This UV radiation causes genetic mutation and damages the DNA by producing thymine dimers. Continuously flowing cold wind 199 miles/h, snowfall, permafrost, and temperature below 0 °C, this climate has not favored the survival of living things. However, prokaryotic life has the ability to survive under multiple extremes. Microorganisms like bacteria, fungi, archaea, cysts, diatoms, etc., are adapted to functioning in this Antarctic ecosystem.

They are affected by different degrees of environmental conditions such as cold stress, heavy metal stress, non-ionizing radiation UV stress, etc., then evolved their community structure and functional gene diversity for fundamental to functioning in these habitats. This Antarctic habitat provided a model for the study of phenotypically diverse microbes under diverse environmental effects (Sinha and Krishnan 2013; Chattopadhyay et al. 2014). Antarctic lakes have very low species richness, low biomass, variables, and short food chains due to low temperature, nutrients, and constrained environments. In the McMurdo dry valley, it was observed that the lakes contribute to the growth of cyanobacterial mats. The contribution to the dynamic and nutrient, regional, and global biogeochemical cycle in the lake ecosystems by large biomass of heterotrophic bacterial association increases the prokaryotic dynamic function and structural interest. The food chain and biogeochemical cycles are influenced by the prokaryotes that are predominant in Antarctica; they also help in nutrient recycling (Shivaji et al. 2011; Chattopadhyay et al. 2014; Reddy et al. 2002; Antony et al. 2016).

6.3.1 Carbon Sequestration

A significant portion of the earth is covered by snow and it is an important part of the global climatic system. Antarctic snow is the reservoir of organic and inorganic carbon. The microorganisms are able to use this carbon reservoir and play an important role in the carbon cycle. Accumulation of labile and bioavailable organic carbon in substantial fraction from diverse sources to glacial ecosystem (Anesio et al. 2010). The dissolved organic matter (DOM) in snow packs is composed of material from in situ primary production as well as the long-range of anthropogenic organic matter and organic aerosol cause the deposition of organic carbon from the marine environment. In a recent study, it is observed that the production and consumption of dissolved organic matter by microbial communities play an important role in carbon fluxes (Antony et al. 2016). The Antarctic surface is covered by snow about 98% and the presence of metabolically functioning bacteria in the glacial ecosystem plays an important role in carbon sequestration. It converts the organic carbon into dissolved organic matter in snow. Organic matter mineralization has also been reported in freshwater and marine environments that have a major impact on atmospheric chemistry (Antony et al. 2011, 2012).

The major source and sink of carbon dioxide (CO_2) are the ocean. The phytoplankton that lives on the ocean surface reduce CO_2 by photosynthesis and release oxygen. However, the silencing mechanism behind CO_2 reduction by phytoplankton has an impact on the global climatic system due to the gradually increasing level of CO_2. The large amount of CO_2 is absorbed by the ocean and brings carbon molecules into the marine ecosystem. In very short time scales, the diffusion of CO_2 in the ocean system diffuses back into the atmosphere. Some carbon molecules of CO_2 are staying in the ocean and play a very important role in the carbon cycle and biological pump. Different dissolve chemicals that are present in the ocean water are also important for the carbon cycle and shell-binding living organisms including the group of Mollusca, that are present in the ocean. Some carbon atoms back to the atmosphere, some carbon molecules are linked with a carbon sequestration biological pump, and some carbon molecules are deposited down to the deep ocean sediment and stored for millions of years (Fig. 6.2). The CO_2 dissolves into the ocean and is combined with the H_2O molecule producing carbonic acid H_2CO_3. The carbonic acid then split into bicarbonate ion HCO_3^- and hydrogen ion H^+. The bicarbonate split into carbonate ion CO_3^{2-} and hydrogen ion H^+(Fig. 6.2). The carbonate ion CO_3^{2-} combines with calcium ion Ca^{2+} and produces calcium carbonate $CaCO_3$. Different types of ocean organisms such as oysters, sea urchins, sponges, coral, pteropods, lobster, and some species of plankton build their inner skeletons and shell (exoskeletons) by the use of calcium carbonate $CaCO_3$.

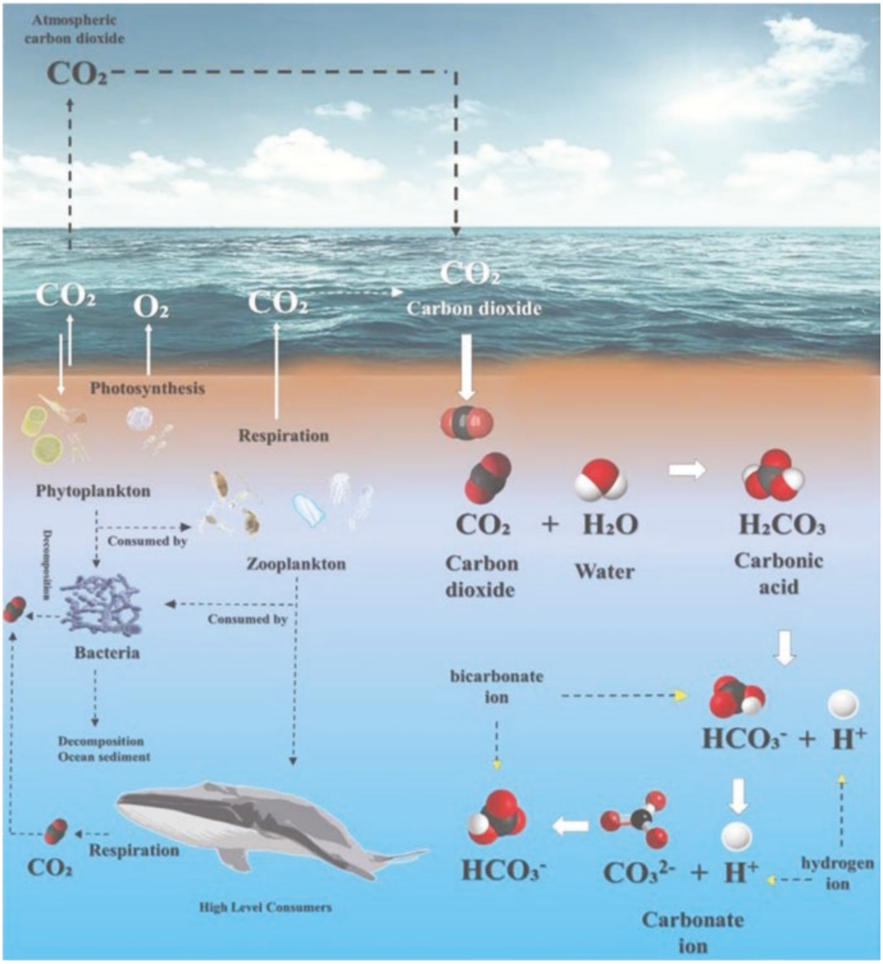

Fig. 6.2 Graphical representation of the role of microorganisms in ocean biological carbon sequestration

CO_2 (carbon dioxide) **+ H_2O** (water molecule) → **H_2CO_3**(carbonic acid).
H_2CO_3 → HCO_3^- (bicarbonate ion) **+ H^+** (hydrogen ion).
HCO_3^- → CO_3^{2-} (carbonate ion) **+ H^+.**
CO_3^{2-} + Ca^{2+}(calcium ion) → **$CaCO_3$** (calcium carbonate).

However, the phytoplankton is sequestered in about 40% of CO_2. The CO_2 reduction takes place both biotically by plankton and abiotically by micronutrient iron. It is observed that the CO_2 level is decreased when a low amount of iron is present in the water. Iron promotes the growth of phytoplankton bloom. Thus, the exogenous addition of iron may be overcome the iron deficiency and facilitates the phytoplankton bloom that sequestered CO_2 (Singh et al. 2015; Shivaji et al. 2015).

6.4 Microbial Survival Strategies

The microbes are developed different strategies to overcome the harsh environment. Those strategies allow the psychrophile to survive under extreme conditions. The microbial diversity and community are less characterized in the marine system. Under wide variations of pressure and temperature, there are different bacterial species present in the marine system. Those microbes have cold adaptive proteins, changes in cellular composition, production of extracellular polymeric substance (EPS), pigments, polyunsaturated fatty acids (PUFAs), antioxidants, sterols, other secondary metabolites, and biologically active compounds that help them to survive under multiple extreme conditions (Goel et al. 2022; Kirkinci et al. 2021; Flocco et al. 2019; Gupta et al. 2015).

6.4.1 Cold-Adapted Enzymes

Cold adaptive microbe psychrophiles are able to survive under a cold biosphere by the presence of some antifreeze proteins and enzymes inside of the cells. These enzymes are flexible and active in functioning in a cold environment. The proteins or enzymes contain a large amount of α-helix and thus fix with their substrate very easily.

These enzymes have high catalytic activity and huge plasticity than the enzymes obtained from mesophiles. The flexibility of the enzyme or protein structure is analyzed by the presence of fewer arginine and proline residues. The survival strategy of many microbes in this oligotrophic environment by the secretion of large amounts of extracellular enzymes such as esterase, lipase, protease, catalase, amylase, etc. The snow is a pocket of different organic matter reservoirs. The organic matters have a large polymer that is degraded into small molecules by those extracellular enzymes. Then the microorganisms are absorbed and metabolized by the small molecules. Microbial protease has a special contribution to the organic matter cycle in the Antarctic environment (Liu et al. 2021; Chattopadhyay et al. 2014). The functional active gene is present inside the bacteria that help them to sense the surrounding low temperature. The individual cell also serves as a primary sensor. The low-temperature signaling system of bacteria has a two-component signaling system.

The membrane-bounded sensor kinase and a response regulator which play a major role for bacterial cold adaptation (Fig. 6.3). The sensor kinase has two domains, a HAMP (histidine-kinase-adenylyl-cyclase-methyl-binding protein phosphatase) domain, the PAS (PER-ARNFSIM) domain, and a histidine kinase domain (H). The response regulator has a DNA-binding HMG domain and a transcriptional activation ARNT domain. Under cold stress, the membrane-bounded sensor kinase is activated by phosphorylation then it transfers the phosphate to the aspartic acid (D) residue of the response regulator. This response regulator act as a transcription factor that is triggered to activate the cold-responsive gene and form inducible proteins, the proteins accumulate and repair the damaged DNA of the cell (Shivaji and Prakash 2010; Sundareswaran et al. 2010).

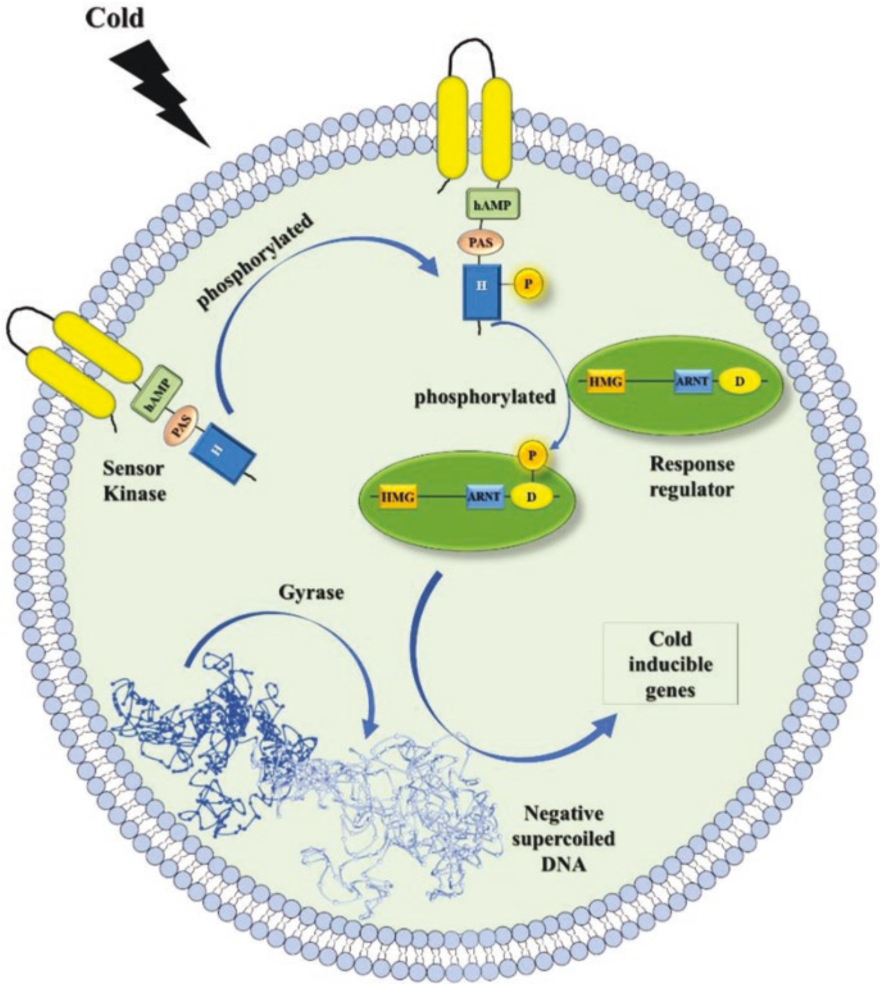

Fig. 6.3 Diagrammatic representation of low-temperature signaling pathway in bacteria

6.4.2 Pool of Pigment

The survival strategy of Antarctic bacteria in the cold biosphere by the production of pigments such as carotenoids help to regulate the membrane fluidity. However, in the earlier study, it was observed that the carotenoid-poor membrane is more fluid and the carotenoid-rich membrane is more rigid but the physiological significance behind is not known. Carotenoids are the isoprenoid pigment that includes bacterioruberin, decaprenoxanthin, violacein, zeaxanthin, melanin, astaxanthin, scytonemin, lycopene, etc. The pigment acts as a photoprotective, a quenching of excess UV-B radiation before it damages the internal mechanism of the cell. It also acts as an antioxidant by reducing the production of singlet oxygen species and protecting the cell from damage. The presence of antioxidant and coloring properties in the pigments are used in different industries such as pharmaceutical, cosmetic, etc. (Silva et al. 2021; Sajjad et al. 2020; Vila et al. 2019).

6.4.3 Ultraviolet (UV) Stress Tolerance Ability

The non-ionizing ultraviolet is very harmful radiation for living organisms. It causes a mutation by the formation of thymine dimer in the genetic material that halts RNA polymerase II during transcription. Bacteria have some mechanism to overcome UV stress condition by the evolving damage-repairing enzymes like CPD-photolyases, 6–4 photolyases, UvrABC proteins that responseble for cyclobutane pyrimidine dimers (CPDs) and 6–4 photoproducts, nucleotide excision repair respectively to remove DNA lesion. The UV resistance bacteria like *Hymenobacter coccineus*, *Lysobacter oligotrophicus*, *Janthinobacterium* sp., *Flavobacterium* sp., etc. have already been reported (Xu et al. 2021; Silva et al. 2021; Sajjad et al. 2020).

6.4.4 Extracellular Polymer Formation

Another cold adaptive strategy is the formation of EPS, i.e., extracellular polymeric substance. The extracellular polymeric substance is secreted by the psychrophiles and also other microbes under an extreme environment that covers and bound the microbes loosely to form slime. This EPS is fundamentally involved in a defense strategy that gives the protection of producing microorganisms under stressful conditions. The polymeric substance dispersed in the matrix of biofilm. These are classified into two types: CPS (capsular polysaccharides) and LPS (lipopolysaccharide) (Hong et al. 2021). The LPS is the major component of the outer membrane in gram-negative bacteria and CPS is closely associated with cells. The polymeric substance is linked by a glycoside bond. The polymers are either homopolymers, i.e., composed of the same types of monomers, or heteropolysaccharides, i.e., composed of more than one type of monomers. The polymers consist of carbohydrate substances or some non-carbohydrate substances. The carbohydrate substance composed of pentose sugar (ribose, arabinose, xylose etc.), hexose sugar (glucose, galactose, mannose, fucose, quinovose, rhamnose), and few are glucomannan type carbohydrate. Some non-carbohydrate substances (pyruvyl, acetyl, and succinyl groups) are also present. Sometimes sulfate, phosphate, acetates, acetate, and amino acid-like organic and inorganic substances are also reported (Halder et al. 2022; Lo Giudice et al. 2020; Caruso et al. 2019).

6.4.5 Antarctic Microorganisms: Bioplastic Formation, Microplastic/Plastic Degradation

The polymer of ethylene is referred to as polyethylene or polythene. Plastic is the polymer of ethylene, propylene, formaldehyde, ethylene glycol, styrene, phenol, vinyl chloride, etc. (Debbarma et al. 2017). Numerous prokaryotes (recombinant bacteria, algae, extremophiles) are produces microbial polyesters like polyhydroxyalkanoates (PHA) during stressful conditions. This PHA is renewable and biodegradable, which is generally used as a good alternative source of petrochemical polymer (Obruča et al. 2022). Many bacteria like *Pseudomonas putida*, *Cupriavidus nicato*r, *Halomonas bluephagenesis*, *Alcaligenes latus*, and *Burkholderia sacchari* are produces polyhydroxybutyrate (PHB) which is a linear polyester of 3-hydroxybutyrate. PHB is also an important commercial biomolecule that uses as a biomaterial in the medical and pharmaceutical industries (Kumar et al. 2021). Today plastic is the backbone of many industries. It uses for manufacturing different products like defense products, leather, pharmaceutical, cosmetics, etc., due to its durable and long-lasting nature. But a high amount of plastic is now accumulated due to its degradable resistance property. Now it captures interest in degradation from our natural planet. The degradation of plastic produces many small plastic particles. Plastic particle sizes of <5 mm are microplastic (MPs) and the size <100 nm range are nano plastics (NPs). These plastic particles form major health effects in both terrestrial and aquatic environments (Zeenat et al. 2021; Cappello et al. 2021). The microbes are associated with plastic particles and bear many metabolic activities it is called “plastisphere” (Cappello et al. 2021). Bacteria produce many enzymes like polyesterase, esterase cutinase-like enzyme, leucine aminopeptidase (LAP), β-glucosidase (β-GLU), and alkaline phosphatase (AP), polyester hydrolases, etc. are responsible for degradation of the plastic particles which are used by the bacteria and form CO_2 and H_2O aerobic digestion and CH_4, CO_2 from anaerobic digestion (Blázquez-Sánchez et al. 2022; Habib et al. 2020; Goel et al. 2008).

6.5 Industrial Prospecting of Antarctic Microorganisms

The dry desert of Antarctica is unfavorable to the survival and reproduction of any living organisms. However, microbes are fundamental for active functioning in this cold ecosystem. The microbes produce different types of substances like pigments, bioactive secondary metabolites, volatile compounds, extracellular active agents, extracellular polymeric substances, cold adaptive proteins, and enzymes that help the microbes survive under stressful climates. However, this microbial substance has now been of great interest due to the presence of some biological activities or potential like antimicrobial properties, antioxidant, photoprotective, antiproliferative effect, antitumoral, and cryoprotective activities, which are commercially applied in different industries and manufacturing of new drugs. The application of those microbial substances is represented by Table 6.1.

Table 6.1 Industrial application of bioactive molecules reported from Antarctic microorganisms

Bioactive molecules	Potentialities	Source	Industrial application	References
Pigments				
Carotenoid Phenazine Violacein	Antimicrobial	*Citricoccus zhacaiensis*, *Kocuria palustris*, *Brachybacterium rhamnosum*, *Agrococcus baldri*, *Iodobacter* sp., *Janthinobacterium lividum*	Food industry Pharmaceutical industry	Silva et al. (2021)
Carotenoid Bacterioruberin Decaprenoxanthin Melanin	Antioxidant	*Pedobacter* sp. *Arthrobacter agilis*, *Arthrobacter* sp., *Psychrochitiniphilus* sp., *Sphingomonas echinoides*, *Zobellia laminarie*, *Lysobacter oligotrophicus*, *Pedobacter terrae* **Fungi** *Sporobolomyces salmoni-color*	Cosmetic sectors	Silva et al. (2021) and Sajjad et al. (2020)
Violacein	Anti-mycobacterial	*Flavobacterium* sp., *Janthinobacterium* sp.	Food industry Pharmaceutical industry	Silva et al. (2021)
Carotenoid Bacterioruberin Nostoxanthin Zeaxanthin Cytochrome c3 Melanin Violacein Scytonemin Astaxanthin, Phoenicoxanthin	Photoprotective	*Flavobacterium segetis*, *Flavobacterium xinjiangense*, *Janthinobacterium* sp., *Arthrobacter agilis*, *Flavobacterium weaverense*, *Shewanella frigidimarina*, *Lysobacter oligotrophicus* **Fungi** *Xanthophyllomyces dendrorhous*, Cyanobacterial mats	Food, cosmetic sectors	Silva et al. (2021) and Sajjad et al. (2020)
Canthaxanthin Melanin	Photosensitive	*Chryseobacterium* sp. *Hymenobacter* sp., *Streptomyces fildesensis*	Food, cosmetic industry	Silva et al. (2021)

(continued)

Table 6.1 (continued)

Bioactive molecules	Potentialities	Source	Industrial application	References
Carotenoid Bacterioruberin Decaprenoxanthin α-carotene, Echinenone, Canthaxanthin Astaxanthin Zeaxanthin β-Cryptoxanthin β-Carotene Flexirubin Violacein 2-γ-carotene, Torulene, γ-Carotene, and Lycopene, Torulene, and lycopene OHK torulene Mycosporine	UV resistance	*Hymenobacter coccineus*, *Arthrobacter agilis*, *Microbacterium* Sp., *Cellulophaga fucicola*, *Flavobacterium* sp., *Janthinobacterium* sp., *Lysobacter oligotrophicus*, *Hymenobacter* actinosclerus, *Chryseobacterium chaponense*, *Pedobacter terrae* **Fungi** *Thelebolus microspores*, *Cystoflobasidium capitatum*, *Sporobolomyces ruberrimus*, *Sporobolomyces salmonicolor*, *Cryptococcus albidus*, *Cryptococcus laurentii*, *Rhodotorula mucilaginosa*, *Rhodotorula larynges*, *Dioszegia* sp., *Cryptococcus* sp., *Torrubiella* sp., *Arthrobotrys ferox*	Food, cosmetic sectors	Silva et al. (2021) and Sajjad et al. (2020)
Violacein	Anti-proliferative effect	*Janthinobacterium* sp.	Drug, pharmaceutical industry	Silva et al. (2021) and Alem et al. (2020)

(continued)

Table 6.1 (continued)

Bioactive molecules	Potentialities	Source	Industrial application	References
Bioactive secondary metabolites				
	Anti-microbial	*Bradyrhizobium* sp., *Methylobacterium* sp., *Paracoccus* sp., *Sphingomonas* sp., *Halomonas* sp., *Janthinobacterium* sp., *Mycobacterium* sp., *Streptomyces* sp., *Arthrobacter* sp., *Planococcus* sp., *Janibacter thuringensi*, *Nesterenkonia* sp., *Rhodococcus fascians*	Pharmaceutical industry, food sectors	Núñez-Montero and Barrientos 2018)
Proteinaceous, volatile, and soluble compounds				
	Anti-microbial	*Pseudomonas* sp., *Psychrobacter* sp., *Burkholderia* sp., *Pseudoalteromonas* sp., *Lysobacter oligotrophicus*, *Enterococcus* sp.	Including chilled-food preservation, food industry, pharmaceutical industry	Núñez-Montero and Barrientos (2018)
Extracellular active agents				
	Methicillin-resistant pathogens and other pathogenic bacteria	*Pseudoalteromonas* sp., *Nocardioides* sp.	Harmful diseases of rice, causing bacterial blight	Núñez-Montero and Barrientos (2018)
	Anti-microbial	*Gordonia terrae*, *Leifsonia soli*, *Terrabacter lapilli*	Food industry, Pharmaceutical industry	Núñez-Montero and Barrientos (2018)
	Anti-microbial compounds	**Cyanobacteria** *Pseudophormidium* sp., *Phormidium priestleyi*, *Leptolyngbya antarcticaI*, *Nostoc* sp., *Phormidium murrayi* **Fungi** *Aspergillus fumigatus*, *Cryptococcus neoformans*	Pharmaceutical, food industry	Núñez-Montero and Barrientos (2018)

(continued)

Table 6.1 (continued)

Bioactive molecules	Potentialities	Source	Industrial application	References
Extracellular polymeric substances (EPS)				
	Cryoprotective activity	*Colwellia* sp., *Marinobacter* sp., *Pseudoalteromonas* sp., *Pseudoalteromonas arctica*, *Pseudomonas* sp., *Pseudomonas mandelii*, *Winogradskyella* sp.	Food, pharmaceutical and cosmetic sectors	Lo Giudice et al. (2020) and Caruso et al. (2019)
	Heavy metal binding;	*Marinobacter* sp., *Pseudoalteromonas* sp.	Possible use in industrial sectors as agents for the disposal of pollutants as well as heavy metals	Lo Giudice et al. (2020) and Caruso et al. (2019)
	Anti-tumoral	*Pseudoalteromonas* sp., *Psychrobacter* sp.	Medicine sectors	Lo Giudice et al. (2020) and Zhang et al. (2018)
	Thixotropy and pseudoplastic	*Pseudomonas* sp.	Pharmaceutical sectors	Lo Giudice et al. (2020)
	Emulsifying activity	*Marinobacter* sp., *Pseudomonas* sp., *Winogradskyella* sp.	Emulsifying activity against many food and cosmetic oils Pharmaceutical sectors	Lo Giudice et al. (2020) and Caruso et al. (2019)
	High viscosity	*Halomonas alkaliantarctica*	Pharmaceuticals and mechanical devices	Lo Giudice et al. (2020)
	Cu-nanoparticles activity	*Bacillus altitudinis*	Drug industry	Halder et al. (2022)

(continued)

Table 6.1 (continued)

Bioactive molecules	Potentialities	Source	Industrial application	References
Antifreeze proteins (AFP)				
	Antifreeze activity	*Pseudomonas* sp., *Arthrobacter* sp., *Sporosarcina* sp., *Sphingomonas* sp., *Plantibacter* sp.,	These proteins have potential in frozen food industry avoiding the damage in the structure of animal or vegetal foods	Muñoz et al. (2017)
Enzyme				
	Mo-reducing capability	*Arthrobacter* sp.	Bioremediation, ability and potential in remediating Mo pollution in Antarctic soil.	Darham et al. (2021)
Cold-active enzymes alkaline pectate lyase	Biocatalysts activity	*Massilia eurypsychrophila*	Textile industry	Tang et al. (2019)
Cellulase-secretome enzyme	Carbohydrate-degrading activity	*Flavobacterium* sp.	Biofuel industry	Herrera et al. (2019)
Extracellular cold active amylases	Starch hydrolysis activity	*Carnobacterium iners*, *Arthrobacter* sp., *Psychrobacter luti*, *Marinomonas* sp., *Primoryensis* sp., *Pseudoalteromonas* sp.	Pharmaceutical, textile, detergent, or food industries	Ottoni et al. (2020) and Wang et al. (2018)

(continued)

Table 6.1 (continued)

Bioactive molecules	Potentialities	Source	Industrial application	References
Cold-active lignocellulolytic enzymes	Cellulose, hemicellulose, and lignin degradation activity	*Streptomyces* sp., *Streptosporangium* sp., *Nocardiopsis* sp., *Geodermatophilus* sp., *Hermobispora* sp., *Amycolatopsis* sp., *Frankia* sp., *Actinoplanes* sp., *Amycolicicoccus* sp., *Kitasatospora* sp., *Micromonospora* sp., *Mycobacterium* sp., *Pseudonocardia* sp., *Rhodococcus* sp., *Thermomonospora* sp., *Saccharopolyspora* sp., *Saccharothrix* sp.	Biotechnological production of biofuel	Oh et al. (2019)
Cold adapted lipases	Triglycerides degrading activity	*Moraxella* sp., *Pseudoalteromonas* sp., *Psychrobacter* sp., *Vibrio* sp., *Psychrobacter okhotskensis*, *Psychrobacter immobilis* **Fungi** *Candida antarctica*	Medical and pharmaceutical application, fine chemical synthesis, food industry, domestic and environmental application	Babu et al. (2007)
Polyesterase	MoPE hydrolyzes non-biodegradable plastics	*Moraxella* sp.	Plastic bioremediation	Nikolaivits et al. (2022)
Esterase cutinase-like enzyme	Biodegradable plastic-degrading enzymes	*Pseudozyma antarctica*, *Paraphoma* sp.	Agricultural industry	Sato et al. (2017) and Watanabe et al. (2014)

(continued)

Table 6.1 (continued)

Bioactive molecules	Potentialities	Source	Industrial application	References
Leucine aminopeptidase (LAP), β-glucosidase (β-GLU) and alkaline phosphatase (AP)	Plastic-degrading enzymes	*Arthrobacter* sp., *Brevibacterium* sp., *Curtobacterium* sp., *Janibacter, Knoellia*, *Rhodococcus* sp., *Streptomyces* sp., *Thermoleophilum* sp., *Alcanivorax* sp., and *Marinobacter* sp.	Plastic bioremediation	Cappello et al. (2021)
	Plastic degradation activity	*Pseudomonas* sp., *Rhodococcus* sp.	Remove microplastic	Habib et al. (2020)
Polyester hydrolases	Polyethylene terephthalate degradation activity	*Moraxella* sp., *Oleispira antarctica*	Microplastic degradation	Blázquez-Sánchez et al. (2022)

6.6 Conclusion

The iciest, windiest, driest, coldest Antarctic headland is an extremely punitive and stressful climate for survival. However, the prokaryotes evolved to survive under multiple extremes. They develop some adaptive features for tolerance of this stressful condition. Microbes are reported from the different habitats of this landmass and they have tricks to withstand the worrying ecosystem. The microbes bear large diversity in both terrestrial as well as marine ecosystems. The marine is the large source and sinks of CO_2. The microbes present in the water reduce large amounts of CO_2 and help the global carbon cycle by maintaining carbon sequestration. Snowpacks have different kinds of organic and inorganic matter that are mineralized by microbial enzymes such as protease, lipase, esterase, etc. The microbes are a source of the pigment pool that protects from UV radiation and photooxidative damage, UV-resistant proteins and enzymes help them to continue functioning under non-ionizing UV radiations, and some polymeric substances like EPS or CPS have been produced, which helps the microbe defense against stressful conditions. This adaptive substance of microbe is useful in various ways. These microbes are captured great interest due to the presence of this substance that has commercial applications in cosmetic, food, nutraceutical, textile, medicine, biofuel industries, and biotechnological industries. Antimicrobial active compounds are also used to develop new drugs in this antibiotic-resistant era.

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7

Crustaceans: Microbes and Defense Mechanisms

Asha Pillai

Abstract

Crustaceans are one of the most diverse arthropods that have successfully inhabited the aquatic ecosystems including freshwater, estuarine and marine. They have enormous benefits for the economy and human health and are of great significance to the aquaculture industry. Crustaceans are a rich source of protein and hence contribute to more than 70% of the world's economy through aquaculture. These animals are prone to many pathogenic species of microbes, especially bacteria. Most of the bacterial diseases in crustaceans are due to pathogenic strains belonging to *Vibrio* spp. *Pseudomonas* spp. and *Aeromonas* spp. that are known to cause blackspot diseases, necrosis and other shell diseases. To combat such infectious pathogens, crustaceans have developed simple but strong innate defense strategies. These innate immune mechanisms are activated upon specific recognition of molecules such as lipopolysaccharides, glycoproteins, glycolipids and peptidoglycans present in the invading microbes. An array of immune components both cellular and humoral are involved in the crustacean effector defense processes including phagocytosis, antibacterial activity, encapsulation and agglutination. This chapter presents an overview of the microbes infecting the crustaceans, especially the decapods and focuses on the various defense mechanisms adopted by them.

Keywords

Crustaceans microbes · Lectins · Phagocytosis · Encapsulation · proPO

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7.1 Introduction

Crustaceans are a key source of protein represented by a variety of edible species distributed throughout the world. The growing demand for wild or captive crustaceans has uplifted crustacean aquaculture as one of the most promising and commercially viable businesses to meet the requirements of the ever-growing human population. The expansion of crustacean aquaculture has always been accompanied by increased incidences of several diseases caused by infectious microbes, notably of bacterial or viral origin. To combat such pathogenic microbes, the crustaceans have developed simple but strong defense mechanisms. Several studies on these innate immune mechanisms of crustaceans have been made with great progress since the late 1970s (Hauton 2012). In the past two decades, this progress has accelerated with the isolation, characterization (Asha and Arumugam 2017; Chen and Wang 2019) and functional analysis (Asha and Arumugam 2021) of new classes of effector molecules.

In normal conditions, crustaceans maintain a healthy state and keep infections under control. Externally, they are covered by a hard, rigid exoskeleton that functions as an efficient physiochemical barrier against mechanical injury and microbe invasion. The cuticular coat, in combination with an acid environment rich in digestive enzymes, is able to inactivate and degrade most viruses and bacteria and forms the first line of defenses of the crustaceans. However, they lack the complex and highly specific adaptive immune system of vertebrates, which is based on lymphocytes, immunoglobulins and immunological memory.

Crustaceans possess an open circulatory system that distributes the essential nutrients, hormones, oxygen and cells through the hemolymph. The circulating cells of crustaceans known as hemocytes are functionally analogous to leukocytes of vertebrates and are mainly concerned with the recognition and elimination of non-self-molecules (Sritunyalucksana and Söderhäll 2000). The defense mechanisms of crustaceans become activated based on the nature and characteristics of the invading pathogen (Vazquez et al. 2009). Accordingly, different effector molecules are activated, such as proPO system, phagocytosis and encapsulation, which are mediated by proteins such as lectins, antimicrobial proteins and pattern recognition proteins (Luis et al. 2021).

This chapter provides a brief but broad overview of our current understanding of the crustacean immune system, especially of the decapods. This enables innumerable pathways that enable us to venture into new avenues for solutions to ensure global food security through crustacean aquaculture. Although the details of the immune pathways and their interrelations are not known, the limited data provide information on the disease combat in crustacean aquaculture through prophylaxis.

7.2 Crustaceans and Their Importance

Crustaceans are primarily marine organisms and compose a large, ancient and diverse animal group that includes many well-known, commercially exploited members, such as shrimps, crabs, crayfish and lobsters that form a vital link in food web (Szaniawska 2018). They are the most abundant animals inhabiting the world's oceans and include freshwater, terrestrial and semi-terrestrial species. Crustaceans form the fourth largest diverse group among animals comprising approximately 75,000 species, including barnacles and beach fleas. They are mostly aquatic in habitat with majority of the species inhabiting marine waters. Diversity of crustaceans is also witnessed in freshwaters but on land the diversity is low. Studies have estimated a total of 14,756 species and 2725 genera of extent decapods and about 3047 species of shrimps and prawns worldwide (Susanto 2021).

Crustaceans possess a hard exoskeleton due to the deposition of calcium carbonate in the cuticle. The body and appendages are specialized into the thoracic and abdominal region with the head bearing the feeding and sensory organs. In a few crustaceans, the head and thorax are merged to form the cephalothorax secured by an expansive carapace. These animals possess remarkable survival due to the presence of a large number and assortment of joint appendages occurring in every segment of their body.

Crustaceans being rich in proteins, serve as valuable food sources and are therefore of substantial economic importance in aquaculture. They are the highest-valued seafood next to freshwater fishes with a global production of 8.5 million tonnes (Boenish et al. 2021; Azra et al. 2021).

Malacostracans (crabs) are the most cultivated class of crustaceans accounting for 20% of the marine crustacean species captured, reared, and used worldwide. This is followed by shrimps and prawns, the majority of the penaeid species (*Penaeus monodon, Litopenaeus vannamei*) that are mainly cultivated in Asia (Pratiwi 2008). Among the freshwater prawns, *Macrobrachium is* the only species that is cultured.

Apart from these, the shells of crabs and other crustaceans are used in the treatment of inflammatory disorders. Crustaceans also serve as bioindicators as they successfully inhabited numerous environments. *Palaemonetes argentinus* serves as a bioindicator for pollution in freshwater bodies (Bertrand et al. 2018). Besides, copepods are used as live feeds in aquariums, baits for game fishing and for compost production.

7.3 Disease-Causing Microbes of Crustaceans

The expansion of aquaculture has always been accompanied by an increase in the incidence of diseases caused by microbes. The crustacean aquaculture industry is no exemption and faces heavy losses due to disease-causing microbes. An insight into the various pathogenic strains of microbes infecting crustaceans revealed that bacterial strains dominated the scenario. Studies have shown that crustaceans inhabiting freshwater as well as marine habitats are equally prone to such infectious microbes (Odeyemi et al. 2021; Rowley 2022). These microbes invade a series of host tissues and organs including gut (Zhao et al. 2018; Le et al. 2019; Cicala et al. 2020), hemolymph, hepatopancreas (Gainza et al. 2018; Landsman et al. 2019) gills, muscles as well as gonads.

Accordingly, a highly pathogenic bacterium *Aeromonas hydrophila* has been isolated from the freshwater crayfish, *Pacifastacus leniusculus* (Jiravanichpaisal et al. 2009), which causes necrosis of the gill, heart, hepatopancreas and the circulatory system. Endotoxins released by these bacteria were found to be the causative agent for the crayfish mortality. In the bluecrab *Callinectus sapidus* gram-negative bacteria viz., *Vibrio sps (*gut, hepatopancreas, gills) and *Clostridium botulinium* (hemolymph) were observed to cause necrotic centers in arteries, heart and hemal sinuses resulting in fatalities (Sizemore and Davis 1985). Several bacteremias in crabs have been attributed to *Vibrio sps*., *Aeromonas sps*., and Rhodobacteriales like chitinolytic and chitinoclastic bacteria which may cause shell diseases in these crabs (Wang 2011). Novel pathogens like *Spiroplasma sps* identified in the Chinese mitten crab (Wang et al. 2004) have inflicted a huge loss to the crustacean aquaculture industry. Potential spoilage bacteria have also been identified in the farmed shrimp *Litopenaeus vannamei* (Don et al. 2018). In the freshwater prawn *Macrobrachium rosenbergii* more than 186 bacterial isolates related to 7 bacterial genera causing midcycle disease and black spot disease which are highly infectious have been identified. These bacterial spp. were *V.alginolyticus, A.hydrophila, P.fluorescens, C.freundii* and *E.aerogens* (Stephen 2022) which caused a heavy loss to the freshwater aquaculture industry.

Infectious diseases among the cultivated penaeid prawns in the Northeast Asian countries of Hawaii and Japan and along the coasts of Central America were caused by viruses. Most of them belonged to the baculovirus species that infected the midgut, exoskeleton and the hepatopancreas of the penaeid prawns (Lee et al. 2022). In India, the myxobacterial infection, reported in *Penaeus indicus, Penaeus monodon, Metapenaeus affinis* and *Metapenaeus dobsonii*, is caused by *Chondrococcus* spp. and *Flexibacter succicans* (Rao and Soni 1986). Several chitin-destroying bacteria, such as *Pseudomonas* spp. and *Aeromonas* spp., known to cause brown spot disease have been identified in the juveniles as well as adults of *Penaeus indicus*. Vibriosis caused by *Vibrio anguillarum* is the most important disease of *Penaeus indicus* cultivated in brackish water fields (Nilla et al. 2012; Newman 2022) while *Escherichia coli* infects the larvae of these penaeids.

7.4 An Overview of Crustacean Immunity

Invertebrates, unlike vertebrates, do not possess true adaptive immune mechanisms and are hence totally dependent on their innate immune system to defend themselves against invading pathogens. In these animals, an inquiry into the nature of basic immune mechanisms underlying the defense network against invading pathogens has led to the identification of an apparently simple and primitive defense system consisting of humoral and cellular components (Smith and Chisholm 1992; Cerenius and Söderhäll 1995; Zhang et al. 2019). It is notable that crustaceans have been one of the most extensively studied group of arthropods and are capable of maintaining a healthy state by mounting exclusive defense mechanisms against potential pathogens (Kulkarni et al. 2020). A complex system of innate immune mechanisms involving cellular and humoral components is triggered in the host upon entry of a pathogen.

In crustaceans, the cellular immune components primarily include certain fixed maintaining cells such as branchial podocytes, nephrocytes, and circulating blood cells or hemocytes (Smith and Ratcliffe 1980; Johnson 1987; Stara et al. 2018). Various soluble substances detected in the hemolymph of these animals constitute the humoral immune system in crustaceans. These include β-1,3 glucan binding proteins (bacterial lipopolysaccharides (LPS)-binding proteins) (Yu and Kanost 2002; Chaosomboon et al. 2017), antimicrobial peptides (Destoumieux et al. 1997; Vazquez et al. 2022), lectins or agglutinins (Maheswari et al. 2002; Asha and Arumugam 2017), hemolytic components (Milochau et al. 1997), antifungal proteins (Iijima et al. 1993) and a series of cytotoxic molecules. These innate immune mechanisms are activated upon specific recognition of molecules such as lipopolysaccharides, glycoproteins, glycolipids and peptidoglycans present in the invading microbes (Chen and Wang 2019).

7.4.1 Cellular Immunity in Crustaceans

The circulating cells (coelomocytes or hemocytes) represent the primary effector component during the immune response of crustaceans (Beck et al. 1994; Söderhäll and Junkunlo 2019). There are three types of circulating hemocytes found in crustaceans viz., (1) hyaline cells—which are small, spherical and lesser in number which varies among different crustacean species; (2) semigranular cells—which possess small eosinophilic granules and also contain prophenoloxidase activators; (3) granular cells—which are filled with numerous granules in their cytoplasm and can attach and spread on the foreign bodies (Wu et al. 2019; Liu et al. 2021; Li et al. 2021; Asha and Arumugam 2021). These cells have been have shown to interact with a range of foreign materials and mediate different types of immune reactions such as phagocytosis, nodule formation, encapsulation, cytotoxicity and exocytosis of immune-reactive substances (Leippe and Renwrantz 1988; Liu et al. 2018; Junkunlo et al. 2018, 2020). In addition, crustaceans possess fixed cells in their gills (Smith and Ratcliffe 1978), which plays a vital role in cellular defense mechanisms.

7.4.2 Phagocytosis

In crustaceans, intracellular destruction of microbes is accomplished through phagocytosis and the mechanism is similar to other animals. In the process, the microbe is trapped, ingested, destroyed and eliminated from the host with the help of phagocytic cells. Fixed phagocytic cells are seen in pericardial sinuses, gills and the base of appendages, whereas circulating/mobile phagocytes are observed in the hemolymph in continuous circulation (Liu et al. 2020). Phagocytosis is a phenomenon in which hemocytes recognize and ingest foreign bodies such as bacteria, spores or dead cells of the organism itself. Most of the circulating pathogens such as Gram -ve *Pseudomonas* spp. and *Escherichia coli* in the hemocoel of the freshwater crab *Parachaerabs bicarinatus* and *Cherax destructer* are destructed and eliminated by the phagocytic hemocytes (McKay and Jenkin 1970). A similar situation has been observed in the American lobster *Homarus americanus* (Mori and Stewart 2006). Although in the blue crab *Callinectus sapidus,* both Gram +ve as well as Gram −ve bacteria are eliminated by the hemocytes (Cassels et al. 1986). The rate of phagocytosis by the hemocytes of crustaceans also appears to vary with other parameters including diet as observed in the larvae of tiger shrimp *Penaeus monodon* where the resistance due to infection was high in larvae fed on *Vibrio* spp. (Bechteler and Holler 1995).

7.4.3 Encapsulation

Pathogens of larger size like parasites cannot be phagocytosed and hence need to be eliminated through a different mechanism. The phenomenon of encapsulation involves the formation of compact layers of hemocytes especially the semigranular cells that congregate and surround the invader, thereby preventing them to enter into the muscle or hemocoel. Typically, a hemocyte capsule comprises several layers (10–30) of hemocytes tightly packed without intercellular spaces. The layer of hemocytes immediately in contact with the surface of the foreign particle lies extremely flattened on it almost resembling a myelin sheath. Interestingly the granular hemocytes rupture upon adherence to the foreign particle surface thereby releasing the granular material. The material also contains enzymes that signals the hemocytes to flatten and conjoin to form capsules and are referred to as encapsulation-promoting factors (EPF) (Ratner and Vinson 1983). In vitro encapsulation of foreign particles by separated hemocytes from several crustaceans have been reported including crayfish *Pascifastacus leniusculus* (Söderhäll et al. 1984), *Astacus leptodactylus* (Persson et al. 1987) and the horseshoe crab *Carcinus maenas* (Söderhäll et al. 1986).

7.4.4 Clottable Proteins

These are defense molecules with multifunctional properties mostly found in the hemolymph plasma rather than hemocytes. The formation of intravascular and extracellular clots prevents the loss of hemolymph as well as the entry of invading pathogens by rapid sealing of wounds in injured animals (Morales et al. 2019). In some malacostracans, a hemocyte-derived clotting cascade is triggered by the lipopolysaccharide of bacteria which activates the clotting enzyme that catalyzes the conversion of a soluble coagulogen into insoluble clot, coagulin (Kawabata et al. 1996). In crustaceans like shrimps, lobsters and cray fishes, the transglutaminase released from hemocytes upon entry of a pathogen, catalyzes the linking of clottable protein into insoluble aggregates in the presence of calcium, which results in clotting and trapping of pathogens. Clottable proteins with sequence identities have been found in freshwater crayfish, *Penaeus leniusculus, Littopenaeus setiferus* and *Penaeus monodon*. Although crustacean clottable proteins exhibit similarities in function, they do not share structural similarities (Hall et al. 1999).

7.4.5 Cytotoxicity

Similar to the cytotoxic mechanisms accomplished by the mammalian NK cells, the semigranular and granular cells of crustaceans destroy the tumor cells present in the host by attaching themselves to the target tissue and releasing the cytotoxic chemicals (Parrinello and Arizza 1992).

7.4.6 Humoral Immunity in Crustaceans

The innate defense mechanisms of crustaceans also involve several factors, occurring in the serum or plasma, which act against foreign bodies. Naturally occurring bioactive molecules are activated to bring about certain important immunological phenomena such as agglutination (Sima and Vetvicka 1993; Bouallegui 2021), lysis, precipitation and stasis (Boman et al. 1991) of nonself particles. These molecules that form the humoral defense of crustaceans include lectins, β-1,3 glucan-binding proteins (Duvic and Söderhäll 1990; Chai et al. 2018), proPO system, antifungal proteins (Chen et al. 2018) and antimicrobial proteins (Destoumieux et al. 1997; Vazquez et al. 2022). Although most of these humoral factors belong to the category of genuine humoral defense, some of them are derivatives of the circulating hemocytes that operate in conjunction with the cellular network. Agglutinins/lectins, antimicrobial peptides and prophenoloxidases are the most widely studied humoral components mainly due to their universal distribution, opsonophagocytic properties and well-known antimicrobial activity.

7.4.7 Agglutinins/Lectins

Agglutinins/lectins are proteins or glycoproteins capable of specifically binding to a whole sugar, a part of the sugar, a sequence of sugars, or their glycosidic linkages (Goldstein et al. 1980; Gabius 1994). In crustaceans, agglutinins have been detected against vertebrate erythrocytes (Hall and Rowlands Jr 1974a, b; Ratanapo and Chulavatnatol 1990), bacteria, invertebrate sperm, protozoans and other cells. In general, agglutinins do not occur in every crustacean species (Smith and Chisholm 1992) and compared to other invertebrates, the titers are often quite low. Several agglutinins may be present in any one species but levels of activity may differ considerably between individual animals (Adams 1991).

Most of the lectins reported in crustaceans belong to the C type as they are dependent on calcium for their functioning (Runsaeng et al. 2018; Snigdha et al. 2022). Identification of a 9.5 kDa lectin with N/O acetylated sugar specificity, in the freshwater prawn *Macrobrachium rosenbergii,* have been found to be produced in the hemocytes and remain on their membrane and participate in the recognition of foreign bodies (Vázquez et al. 1997). Although lectins from decapod crustaceans have been found to exhibit heterogeneity in molecular mass and subunit conformation, they possess preserved carbohydrate-binding specificities for N/O acetylated sugars, thus indicating the conservation of such sugar-binding specificities throughout evolution (Kilpatrick 2002; Alpuche et al. 2005; Vasta et al. 2007). Bacterial species like *Aeromonas* spp. and *Bacillus cereus*, which possess O- acetyl groups of sugars in their cell wall is recognized by lectins in *Macrobrachium rosenbergii* (Vázquez et al. 1994, 1996). In a similar way, LPS from *Escherichia coli* is identified by a lectin from *Carcinoscorpius cauda* (Dorai et al. 1982). Studies have also shown that the synthesis of lectins in different organs of the crustaceans might be activated by the entry of the pathogenic bacteria or viruses. Thus, in the swimming crab *Portunus trituberculatus,* the main source of lectins is the hepatopancreas followed by the gills, hemocytes and ovary (Kong et al. 2008). In the case of postlarvae of *Penaeus monodon* infected with White Spot Syndrome Virus (WSSV), the expression of lectin gene has been traced to the muscle, eye stalk and cuticle apart from hepatopancreas (Leu et al. 2007). Lectins have also been observed to show structural and functional diversity within the same species as reported in *Litopenaeus vannamei* (Viana et al. 2022).

In addition, many experimental studies have attributed a variety of biological functions to lectins including feeding, symbiosis, larval settlement and diverse endogenous functions, such as cell aggregation, embryonic development, metamorphosis, wound repair and transport of carbohydrates (Yeaton 1981; Beck et al. 1994; Ahamed et al. 2022). Agglutinins present in the hemolymph of crustaceans are proposed to act as carriers for sugars, glyco-conjugates, or antibody-like molecules, or as opsonins involved in humoral responses against pathogens. These agglutinins present on hemocyte surfaces as in crayfish and bluecrab have been proposed to participate in cellular recognition and trigger phagocytosis, nodulation and encapsulation responses. Naturally occurring agglutinins serve as opsonins and facilitate the phagocytic uptake of target cells precoated with the serum-purified agglutinins (Kondo et al. 1992; Maheswari 2000; Ogutu 2003). It has also been demonstrated (Asha and Arumugam 2016) that the ability to agglutinate target cells is also associated with one of the most important physiological phenomena in crustaceans viz., stages of moult cycle.

7.4.8 Antimicrobial Peptides

Antimicrobial peptides (AMPs) are one of the major components of the innate immune defense of crustaceans. AMPs are primarily known as natural antibiotics, which play a role in host defense response including self or non-self-recognition, cell-to-cell communication, superoxide anion activity, melanization, phagocytosis, cytotoxicity and encapsulation (Xiao et al. 2018). Penaeidins, a family of antimicrobial components, have been identified in shrimps and they have been shown to possess both antibacterial and antifungal properties (Vazquez et al. 2022). In crabs, a low molecular weight antimicrobial peptide called crustin which is immunologically effective against Gram-positive bacteria has been isolated (Du et al. 2019). The pathological and clinical backgrounds have prompted researchers to investigate novel and potent antioxidant peptides from crustaceans that are of therapeutic use. The regulation of expression and distribution of penaeidins during microbial challenges is done through hemocyte reactions and hemocyte proliferation process. Thus, these antimicrobial peptides in penaeid shrimps protect the tissues from infections and aid in wound healing (Xiao et al. 2020).

7.4.9 Prophenoloxidase System

Phenoloxidase (PO) is a copper-containing enzyme capable of catalyzing the hydroxylation and oxidation of phenols into quinones and a series of steps leading to the synthesis of melanin. It is the terminal enzyme of proPO which is a cascade that is activated by the extremely low levels of microbial cell wall components such as LPS and β-1,3 glucans. This stimulation of immune responses by the proPO system is achieved by specific interactions with receptors on the hemocytes (Li et al. 2018). The concept of pattern recognition receptors (PRPs), which are a group of germlines encoded receptors, recognize surface antigens on microbes like LPS, peptidoglycans, mannans and β-1,3 glucans (Habib and Zhang 2020; Tran et al. 2020). This results in the production of melanin pigment which is seen accumulated as dark spots in the cuticle of arthropods. The toxic metabolites that are formed during melanin formation are known to exhibit antifungal activity. Enzymes of the proPO system are localized in the hemocytes of penaeid shrimps, especially in the semigranular and granular cells (Perazzolo and Barracco 1997). Studies on *Penaeus monodon* hemocytes have confirmed this showing the expression of proPO mRNA only in the hemocytes. The proPO cascade culminating in melanization plays a vital role in preventing the bacteria from proliferation and becoming deleterious to the host. Studies on the kuruma shrimp *Marsupenaeus japonicus* have shown that the inactivation of proPO results in a significant increase in the amount of bacteria and a sharp increase in shrimp mortality (Fernand et al. 2009). A bacteria-induced β-1,3-glucan binding protein has been isolated from red swamp crayfish *Procambarus clarkii*, which appears to protect the host from *Aeromonas hydrophila* infection (Chai et al. 2018). Furthermore, it has been demonstrated that the expression of genes encoding LPS binding protein in hemocytes of the white shrimp *L.vannamei* is upregulated in infections caused by *Vibrio sps* (Cheng et al. 2005) and viruses (Roux et al. 2002).

7.5 Future Perspectives

Crustaceans as a source of animal protein are much relied upon by the increasing global population demanding an expansion of their aquaculture. However, this demand for the expansion of crustacean aquaculture has to be met along with several challenges, especially the spread of potential disease-causing microbes. Opportunistic pathogens cause diseases that severely affect crustaceans resulting in irreparable economic losses. The simple but strong innate immune mechanisms possessed by these animals have enabled them to overcome an unfavorable and life-threatening attack by the microbes. Several physiological factors like developmental stages, ecdysis and stress conditions also play a vital role in modulating these immune mechanisms. In recent years, there has been a lot of progress in the understanding of the crustacean defense systems. Novel defense molecules, some of which have been found to be homologous proteins to C Reactive Proteins (CRPs) and complement factors in human immune systems, have been discovered in crustaceans. This chapter highlighted the general immune mechanisms adopted by some of the disease-sensitive species of crustaceans, especially decapods. At this juncture, it is of utmost importance and relevance to focus on the modifications in the immune mechanisms of crustaceans to avoid losses in aquaculture production sectors.

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Part III

Marine Microorganisms and Environmental Bioremediation

Pollution in Marine Ecosystem: Impact and Prevention

8

Madhumita Ghosh Datta

Abstract

Marine ecosystems are aquatic environments with high levels of dissolved salt, such as those in or near the ocean. People's direct and indirect introduction of pollutants into the maritime environment has a detrimental effect on the marine environment's water quality, marine life, human consumption of seafood, and services. Marine pollution comes in many different forms, including trash pollution, plastic pollution (particularly microplastics), ocean acidification, nutrient pollution, poisons, and underwater noise. Additionally, modifications to the ocean biota, habitat, and food chains all affect productivity trends. Given all of these factors, it is logical to conclude that the most reliable and efficient way to remove xenobiotics from water is by microbial decomposition. This is due to the fact that they consume biological components as food before converting them into final goods. The International Union for Conservation of Nature (IUCN) defines nature-based conservation as methods to safeguard, sustainably manage, and improve natural or modified ecosystems while addressing a range of environmental, climatic, and development-related issues.

Keywords

Marine ecosystem · Marine pollution · Bioremediation · Nature-based conservation

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8.1 Introduction

Aquatic habitats with high levels of dissolved salt, such as those in or near the ocean, are referred to as marine ecosystems. The coastal and marine habitats approach the continental shelf and can be found up to 100 km inland. They also feature ocean systems that can reach a depth of 50 m. Seagrass beds, coral reefs, oyster reefs, marshes, mangroves, sandy beaches, dunes, and dune systems can all be found in these places (Barbier 2017).

Marine ecosystems are distinguished by their distinct biotic (living) and abiotic (non-living) components. Abiotic components in the ecosystem include the amount of oxygen and nutrients dissolved in the water, the distance from land, the depth, and the temperature. Biologic factors include plants, animals, and microorganisms. For marine ecosystems, sunlight is an essential abiotic element. Scientists must classify up to three components of the marine ecosystem based on how much light they receive.

The euphotic zone is the uppermost layer of a marine ecosystem, extending down to 200 m (656 ft) below the surface. There is enough light at this depth to support normal photosynthetic activity. This zone is home to the vast majority of marine species. The dysphotic zone exists beneath the euphotic zone and can extend from 200 to 1000 m (656–3280 ft) below the surface. At these depths, sunlight is still present, but only enough to allow for some photosynthesis. Below the dysphotic zone is the aphotic zone, which receives no sunshine. Bacterial production was minimal below the euphotic layer, even with sufficient availability of inorganic nutrients. The bacterial production rate in the surface waters was about 7 times and over 50 times higher than that of the aphotic zone in the winter and the summer seasons, respectively (Mathew et al. 2021).

The ocean is the world's largest and most significant body of water, and because of its enormous capacity to store water, it has long been home to the planet's most stable ecology. Whale, tuna, and sharks live in the oceanic zone, which is a vast open area of the ocean. Many invertebrates live on substrates in the benthic zone, which is located below the water's surface. The region between high and low tides is known as the intertidal zone. Seagrass meadow, mudflat, mangrove, salt marsh, coral reef, rocky intertidal system, and lagoon are some more nearshore (neritic) habitats. The foundation of the food chain in hydrothermal vents in the deep ocean is chemosynthetic sulfur bacteria. The biological community of species that they are linked with and their physical surroundings serve to define marine ecosystems. Sharks, echinoderms, corals, dinoflagellates, brown algae, and other species can all be found in marine habitats. For nutrition, employment, and other ecological services, a large number of people rely on marine ecosystems all throughout the world. The stability of these ecosystems is severely threatened by human use of maritime areas and contamination of marine ecosystems. While the many elements that enter the water from the land are accepted, the ocean itself does not alter significantly. However, in recent decades, as global industry has grown, there has been an increase in ocean pollution, which has had a significant impact on the local marine ecosystem and is continuing to do so.

Marine ecosystems are impacted by environmental issues such as unsustainable resource extraction (such as overfishing of some species), marine pollution, climate change, and coastal development. The ocean also absorbs a sizeable portion of the carbon dioxide that contributes to global warming and the heat it produces. Ocean acidification is one process that is affecting the chemistry of the ocean and endangering marine habitats. Due to the opportunities for humans in marine ecosystems as well as the issues that humans have caused, the international community has designated "Life below water" as Sustainable Development Goal 14. The objective is to protect, preserve, and maintain the oceans, seas, and waterbodies in a sustainable manner in order to support sustainable development.

Humans introduce substances into the maritime environment that harm living resources, pose a health risk, obstruct marine activities, degrade the quality of the seawater, and reduce the availability of facilities (Wilhelmsson et al. 2013). Worldwide marine ecosystems may be quite resistant to particular kinds of contamination of normal magnitudes on average, despite the fact that pollution has significant negative effects on species and habitats in numerous areas and regions (Beiras 2018). On the other hand, nothing is known about the synergy between various types of pollutants and the long-term lasting effects of, say, chemicals. The effects of pollution may weaken an ecosystem's resistance to additional stresses like ocean acidification and rising sea-surface temperatures. Since future global growth can take many different paths in terms of, for example, production, transportation, and consumption patterns of commodities, services, and energy, it is impossible to make reliable and concise projections of the future volume and impact of marine pollution on a global scale.

It is possible to describe marine pollution as the direct or indirect introduction of substances to the marine environment by humans, which results in adverse impacts on water quality, marine biota, and the consumption of seafood by humans, as well as a reduction in the number of amenities available (Landrigan et al. 2020). There are around 45% of people on Earth who live within 150 km of the coast, and marine pollution is a result of increases in population density as well as industrialization. The difficulties caused by marine pollution are typically confined to nearshore waters rather than the open ocean. The primary regions impacted by marine pollution are estuaries, fjords, and rias, in addition to the shelf seas that are next to these water bodies. Chemicals, radiation, solid waste, sedimentation brought on by humans, forms of energy (such as heat and noise), oil spills, and "biological pollution" are some of the contributors to marine pollution (pathogens, parasites, and invasive species). The purpose of this chapter of the book is to present a condensed review of the many different types, histories of sources, processes, and risks to marine ecosystems in order to describe the existing and anticipated worldwide dangers linked with marine pollution. In this overview of ocean pollution cures, the natural marine bioremediation and nature-based approaches to treating both marine pollution and climate change were discussed. Both of these treatments are potentially effective.

8.2 Forms of Marine Pollution

Litter pollution, plastic pollution (including microplastics), acidification of the oceans, nutrient pollution, poisons, and underwater noise are all examples of marine pollution. Marine pollution is caused by chemicals and debris, the bulk of which originates on land and is dumped or blown into the water. This pollution harms the environment, the health of all living creatures, and global economic institutions. Marine pollution is becoming more of a problem in the modern world. Chemicals and trash are the two main sources of pollution in our ocean (Table 8.1).

Chemical risks, commonly known as nutrient pollution, are a source of worry for health, the planet, and the economy as a whole. This type of pollution occurs when human activities, most notably fertilizer use on farms, produce chemical runoff into streams, which eventually drain into the ocean (Kumar et al. 2021). Increased concentrations of chemicals in coastal water, such as nitrogen and phosphorus, promote the growth of algal blooms, which can be toxic to species and harmful to humans. These nitrogen and phosphorus nutrients, which are also found in fertilizers, stimulate phytoplankton and macroalgae growth, which can lead to eutrophication and severe algal blooms that are detrimental to both humans and marine life. The negative effects of algal blooms on human health and the environment hurt the local fishing and tourism industries. Concerns have also been raised about fertilizer (nitrogen and phosphorus) runoff from intensive agriculture, as well as the discharge of untreated or poorly treated sewage into rivers and, eventually, the oceans. The negative effects of algal blooms on human health and the environment hurt the local fishing and tourism industries. Fertilizer (nitrogen and phosphorus) runoff from intensive agriculture, as well as the flow of untreated or poorly treated sewage into rivers and, eventually, the oceans, are also causes for worry (Giri et al. 2017; Dash et al. 2021). The breakdown of algal blooms can lead to the depletion of oxygen in coastal waters, a problem that could intensify as climate change lowers vertical mixing of the water column. Additionally, unrestrained algae growth endangers coral health and species diversity by choking coral reefs. Oil and gas industries have

Table 8.1 Direct and indirect drivers of modification in marine ecosystems

Indirect interaction	Direct interaction
Migration of people	Algal bloom (diatoms, dinoflagellates)
Transect	Eutrophication
Organization	Pollution (metals, oil, pesticides, radionuclides, gasoline additives)
Manufacturing technology	Overfishing
Urbanization	Nutrients
Carbon dioxide emission	Invasive species
Regional and global issues	Diseases
	Seawater temperature
	Acidification of ocean
	Marine toxins (Ciguatoxins, saxitoxins, etc.)

dumped oil spills and waste gases into the sea, causing marine contamination. It is still difficult to conduct research into acceptable and dynamic solutions to the problem of oil spill cleanup.

Metals with no metabolic activity, such as Hg, Cd, Pb, and Sn, are examples of xenobiotics. Cu, Zn, Ni, and Co, on the other hand, are examples of important metals with restricted internal levels. These metals are required in trace amounts and are known as essentials. Both can be harmful in quantities larger than a specified limit. Metals including Fe, V, Cu, and Zn are needed for a variety of biochemical activities in the marine environment, yet even in mildly polluted estuaries, these metals cause stress in marine biota. The amount of Hg and Pb in the atmosphere has increased due to human activities, which is then deposited in the oceans. The increase in Hg concentrations in the atmosphere has resulted in a 300% increase in deposition and a 230% increase in surface marine waters. Deeper marine waters show only 12–25% growth (Outridge et al. 2018). Riverine inputs are the primary sources of Cu and Cd to the sea. Metals gradually build in the sediments in both circumstances. Tributyltin (TBT), Cd, Hg, Ni, Pb, and similar compounds are evaluated in water quality assessments due to their hazardous and bioaccumulative properties. Although tolerated in minute amounts, these elements have little to no biological function and demonstrate hazardous consequences above threshold concentrations. Depending on mineralogical parameters, background concentrations of trace metals in marine sediments range from 0.1 mg/kg DW for Hg to 0.5 mg/kg for Cd, and 50 mg/kg for Pb and Cu. Anthropogenic Cu and Zn are commonly found in wastewater and can reach concentrations of 1 mg/L. Mercury can occur in water in several forms, including inorganic mercury, biologically complexed mercury (with naturally dissolved organic carbon), dissolved gas mercury (Hg0), and the methylated species monomethyl mercury (MMHg), and dimethyl mercury (DMHg). MMHg and DMHg are both found in sediments, the water column, and the tissues of marine organisms (Bravo and Cosio 2020). Cadmium and lead are by-products of the industry. The chloralkali industry is the principal source of Hg. Global Pb pollution decreased as leaded gasoline was phased out, as evidenced by dated ice cores. Because of the great accessibility of CH_3-Hg, which quickly crosses membranes, and the difficult excretion of Hg species that bind to proteins, anaerobic bacteria methylate mercury in sediments and biomagnify it in trophic webs. In gastropods, TBT from antifouling coatings caused imposex. As a result, metals will have an impact on diverse portions of the marine food chain depending on their physicochemical state or bioavailability. Because of the ease with which CH_3-Hg traverses membranes and the difficulty with which Hg species that bind to proteins are excreted, anaerobic bacteria methylate mercury in sediments and biomagnify it in trophic webs. TBT from antifouling coatings produced imposex in gastropods. As a result, depending on their physicochemical state or bioavailability, metals will have an impact on various parts of the marine food chain. In rare cases, bioaccumulation and/or bio-magnification may occur, exposing humans to a potential health risk by consuming seafood. Microplastic, or very small bits of degraded plastic, is eaten by small organisms, which absorb the toxins in the plastic into their tissues.

Microplastics, which have a diameter of less than 5 mm, have been detected in a range of marine animals, including plankton and whales (0.2 in.). When larger animals consume microscopic organisms that absorb microplastics, the harmful substances become a part of their tissues. Microplastic contamination thus goes up the food chain and eventually finds up in the food that people eat. Plastic pollution in the ocean is a type of marine pollution generated by plastics of various sizes, ranging from big starting materials such as bottles and bags to microplastics formed through plastic fragmentation.

Microplastic, or very small bits of degraded plastic, is eaten by small organisms, who absorb the toxins in the plastic into their tissues. Microplastics, which have a diameter of less than five millimeters, have been detected in a range of marine animals, including plankton and whales (0.2 in.). When larger animals consume microscopic organisms that absorb microplastics, the harmful substances become a part of their tissues. Microplastic contamination thus goes up the food chain and eventually is found in the food that people eat. Plastic pollution in the ocean is a type of marine pollution generated by plastics of various sizes, ranging from materials such as bottles and bags to microplastics formed through plastic fragmentation. The vast bulk of marine debris is human garbage that floats or is suspended in the ocean. Plastic pollution is hazardous to marine life. Plastic contamination is widely acknowledged to be a problem in the maritime environment (Villarrubia-Gomez et al. 2018). Its associated chemicals are less well known and understood. Unreacted monomers, oligomers, and additives in plastics can leach into water over time. Furthermore, plastics absorb organic and inorganic pollutants from the surrounding waters, such as polychlorinated biphenyls (PCBs) and metals. By collecting and removing plastics from the oceans, the amount of chemical pollutants entering or re-entering them can be minimized. The reduction in the pH of the Earth's seas is referred to as ocean acidification. This operation could take decades or even centuries to finish (Osborne et al. 2019). The principal cause is the absorption of carbon dioxide from the environment. As a result, CO_2 levels in the water are rising. Between 23 and 30% of the CO_2 in the atmosphere is absorbed by the seas, rivers, and lakes. Acidification is one of the many effects of rising CO_2 on the ocean. Other chemical changes can also cause water acidification. The chemistry of seawater changes as a result of CO_2 absorption by the ocean, affecting the habitats of marine animals. Many species are affected, especially those that rely on calcium carbonate for their skeletons and shells, such as corals, oysters, and mollusks.

Microplastics are plastic fragments smaller than 5 mm in size that are found in marine habitats. The main sources of microplastics are primary and secondary microplastics. They are identified using a number of analytical procedures due to their potentially harmful effects, such as ingestion and entanglements. Microplastics are formed from plastic trash by oxidation, thermal breakdown, biodegradation, and thermooxidative degradation. During degradation, polymers vary in density, surface morphology, crystallinity, color, and other properties (Fig. 8.1).

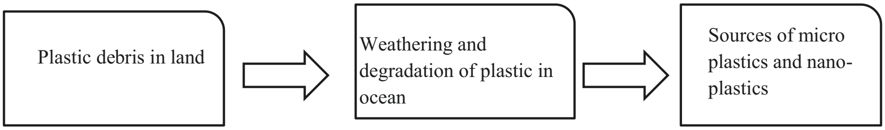

Fig. 8.1 Sources of plastic pollution

Numerous studies have found microplastics in marine vertebrates, such as seabird species and fin whales, which may indicate a trophic transfer to the top of the food chain or direct ingestion. Different food types and sources had different microplastic contents. The prevalence of microplastics in aquatic animals is closely connected with food consumption, showing a transfer of microplastics through the food chain rather than being directly absorbed or broken down by ingested microplastics. Significant issues include comprehending how particles are transmitted between trophic levels and how microplastics affect wild animals (Choudhury et al. 2022). Long-term dangers to marine life and food webs come from newly emerging human-made poisons including microplastics, polychlorinated biphenyls, and radionuclides like cesium-137 emissions from the Fukushima nuclear catastrophe in March 2011. For instance, in the "Age of Plastics," plastic pollution and microplastics pose some of the greatest threats to sea health and pollution. This is due to the emergence and explosive growth of the "Plastic era" over the previous 50 years, as well as the initial discovery of plastics in the marine environment in the early 1970s.

The majority of the previously occupied territory has been taken over by invasive species as a result of their invasion of recently discovered continents. Examples of invasive species include plants, mammals, birds, fish, amphibians, reptiles, arthropods, mollusks, and diseases of plants and animals. A ship needs ballast water to maintain stability and seaworthiness. Aquatic invasive species discharge from ballast water is a serious problem in the maritime environment. Aquatic species are most at risk from species transfers. Invasive species also possess characteristics including excellent dispersal abilities, rapid rates of development, rapid generation times, and broad climatic tolerances. Fast range extension is made feasible by these characteristics in response to climate change.

The term "ocean acidification" describes the lowering of the oceans' pH levels. This process could take decades or longer to finish (Osborne et al. 2019). The main cause is the uptake of carbon dioxide from the atmosphere. CO_2 levels in the ocean increase as a result. Between 23% and 30% of the CO_2 in the atmosphere is absorbed by the seas, rivers, and lakes. Acidification is just one of the effects that more CO_2 has on the water. Other chemical changes can also cause acidification of the water. The ocean's absorption of CO_2 causes changes in seawater chemistry that have an impact on the habitats of marine animals. Numerous species are affected, especially those like corals, oysters, and mollusks that depend on calcium carbonate for their skeletons and shells. Particular components of this organism's anatomical structure have trouble developing in increasingly acidic ocean environments. The main tactic for addressing the current ocean acidification issue is reducing atmospheric CO_2

through climate-change mitigation. When carbon dioxide is absorbed by water, it quickly splits into bicarbonate (HCO_3) and hydrogen (H^+) ions to form carbonic acid. Acidification results from the free hydrogen ions (H^+) in the ocean lowering pH levels (this does not mean that seawater is acidic yet: it is still alkaline with a pH higher than 8). Since carbonate ions are the main constituents of calcium carbonate ($CaCO_3$) shells and skeletons, lowering the pH lowers their concentration. Additionally, it lowers the saturation state of carbonate minerals. Ocean alkalinity is unaffected by ocean acidification, but it may increase over time as a result of carbonate breakdown and a decline in the formation of calcium carbonate shells.

"Eutrophication" is the term used to describe an increase in chemical nutrients, frequently compounds containing nitrogen or phosphorus, in an ecosystem. It leds to other effects like shortage of oxygen and drastic losses in water quality, fish populations, and other animal populations, in addition to increasing the ecosystem's primary production (excessive plant growth and deterioration). Water contamination due to excessive nutrient inputs is referred to as fertilizer pollution. It is a key contributor to the eutrophication of surface waters by encouraging the growth of algae with excess nutrients, typically nitrates or phosphates. Such blooms are common in nature, but they may be becoming more common because of anthropogenic inputs or maybe because they are being watched over more carefully. The primary offenders are rivers that empty into the ocean, carrying with them a variety of fertilizers used in agriculture as well as wastes from people and animals. Hypoxia and the creation of a dead zone can result from excessive amounts of oxygen-depleting chemicals in the water.

Estuaries usually exhibit eutrophic conditions by nature because land-derived nutrients are concentrated when runoff enters the marine environment in a constrained channel. The World Resources Institute has identified 375 hypoxic coastal zones, with the majority of them being in coastal areas of western Europe, the eastern and southern coasts of the United States, and East Asia, particularly Japan. Red tide algae blooms are widespread in the ocean, and when they approach the beach, they can kill fish and marine mammals as well as cause respiratory problems in people and some domestic animals (Gobler 2020). In addition to drainage from the land, air nitrogen fixed by humans can also infiltrate the open ocean. This could account for up to 3% of the annual new marine biological production and around one-third of the external (non-recycled) nitrogen supply to the ocean, according to a research (Terhaar et al. 2021). Similar effects to those of atmospheric carbon dioxide have been connected to the buildup of reactive nitrogen in the environment. Re-establishing shellfish populations, like those of oysters, is one suggested strategy for combating estuary eutrophication. Oyster reefs reduce the likelihood or severity of harmful algal blooms or anoxic conditions by filtering out suspended particles and removing nitrogen from the water column. By reducing phytoplankton density and sequestering nutrients, which can be removed from the system by shellfish harvesting, buried in sediments, or lost by denitrification, filter feeding activity is assumed to improve water quality. In Sweden, Odd Lindahl et al. employed mussels to develop the concept of improving marine water quality through shellfish farming (Lindahl 2011). Other dangerous contaminants, besides plastics, also present unique problems since they either do not decompose or do so very slowly in the marine environment. Some examples of persistent toxicants are PCBs, DDT (dichloro-diphenyl-trichloroethane), TBT, pesticides, furans, dioxins, phenols, and radioactive waste (Suyal and Soni 2022). Heavy metals are metallic chemical elements with a relatively high density that are hazardous or dangerous at low doses. Examples include mercury, lead, copper, and cadmium. Some toxicants can accumulate in the tissues of several aquatic animal species thanks to a process called bioaccumulation. They have also been observed to accumulate in benthic environments including estuaries and bay muds, which operate as a geological record of human activities from the previous century.

A new, emergent problem that needs to be investigated and dealt with right now is the interaction of climate change (i.e., ocean warming and acidification) with marine pollution. Sea-level rise, ocean warming, ocean acidification, increased upper ocean stratification, increased salinity contrasts (salty areas becoming saltier and fresher areas becoming less salty), oxygen loss, an increase in marine heatwaves, and changes to ocean currents, including a potential slowdown or shutdown of thermohaline circulation, are the main physical effects of climate change on the world ocean. Two chemical effects are oxygen depletion and ocean acidification. There is increased stratification of water temperature as a result of the warmer ocean surface brought on by warmer air temperatures. As ocean layer mixing decreases, warm water collects near the top while cold, deep water circulation is reduced. The reduced up-and-down mixing reduces the ocean's ability to absorb heat, which causes a greater share of future warming to be absorbed by the atmosphere and land. While the nutrients available to fish in the higher ocean layers and the oceans' capacity to store carbon are anticipated to drop, it is predicted that tropical cyclones and other storms will be able to gain an increase in energy. The gas exchange balance shifts as a result of warmer water's inability to hold as much oxygen as colder water, lowering ocean oxygen levels while raising atmospheric oxygen levels. Increased temperature stratification may accelerate the respiration of organic materials, thereby reducing the oxygen content of the water. The whole water column of the ocean has already lost oxygen, and oxygen minimum zones are expanding globally (IPCC 2019; Freedman 2020).

These modifications affect coastal fishing and tourism as well as destabilize marine ecosystems, which can result in extinctions and population expansions. Additional warming of the ocean's surface waters will be harmful to some ocean ecosystems, including coral reefs. Coral bleaching on these reefs is the immediate result, and because they have a narrow temperature range, even a small temperature increase has a big effect there. The production and distribution of species, as well as changes to fisheries and marine ecosystems, will be impacted by ocean acidification and temperature rise. The creatures of the Artic region that depend on sea ice habitat loss as a result of warming will feel the effects greatly. The demands on the climate system and ocean ecosystems are increased by the interactions between many of these climate change components.

8.3 A Quick Overview of Marine Contamination

Marine pollution and anthropogenic climate change have affected and reconstructed the oceans on a variety of scales since the start of the Holocene. Man started to have a big impact on both geological and ecological processes in the late 1700s and early 1800s. Growing human populations, food dependence, industrialization, and worldwide transportation with related emissions of chemical pollutants are all having a negative impact on how nature functions. Earth's temperatures are currently 1.0 °C higher than preindustrial levels as a result of increasing CO_2 emissions. Both old and new pollutants have affected the health of the world's oceans and posed a threat to the survival of marine animals. Climate change is also a major factor contributing to the enhanced effects of environmental and anthropogenic contaminants on marine food webs and ecosystems.

The first instances of chemical ocean contamination by toxins appeared in the 1500s (sixteenth century) as a result of human-caused mercury leaks from mining. Recently, manmade radionuclides (such those from 1930 to 1945) and persistent organic pollutants (POPs) have contributed to ocean pollution. Although some POPs, such as polychlorinated biphenyls (PCBs) and dichloro-diphenyl-trichloroethanes (DDTs), were outlawed in developed and industrialized nations during the 1970s, some organochlorine pesticides (OCPs), such as DDT, are still used in developing nations to control pests that damage crops and act as carriers of diseases like malaria as well as newly emerging POPs, like polybro. The countries with temperate climates where POPs were produced, stored, or dumped into the ocean continue to have the highest concentrations of PCBs and DDT (such as Palos Verdes in the Southern California Bight). In her 1962 book *Silent Spring*, Rachel Carson raised awareness of the potential effects of man-made chemicals, particularly pesticides, on wildlife populations, including raptors and songbirds, and human health at the height of the "Organochlorine era," or the period of OCP use from the late 1940s to the early 1980s. However, the effects of those negligent and dangerous industrial chemicals and agricultural insecticides continue to deface our beautiful globe. The world's oceans contain radionuclides, POPs, currently in use insecticides, heavy metals, microplastics, personal care and pharmaceutical products (PCPPs), and polycyclic aromatic hydrocarbons (PAHs). POPs and organic mercury, which are bioaccumulative and fatal, are two examples of a type of stressor that particularly affects creatures at the top of marine food webs (such as seabirds, marine mammals, huge pelagic fish, and humans). As a result of climate change, numerous POPs that were deposited in sinks like oceans and ice over the past 20 years were unexpectedly remobilized and volatilized into the atmosphere from repositories in Arctic regions, according to recent research and modeling work. The sustainability of coastal resources, food security, and sustainable resource development are all under threat from climate change and environmental pollution, the two main human-caused factors of the twenty-first century. The effects of pollution on marine food webs and climate change will both be accelerated by rising greenhouse gas emissions (Alava 2019).

8.4 Marine Pollution's Effects

Marine pollution affects both the ecosystem and human health since it disturbs the biota and surroundings of the ocean. Pollutants in the environment of the ocean can have a variety of biological impacts, including mortality, metabolic imbalance, and genetic harm. Productivity patterns are also impacted by changes to the food chain, habitat, and ocean biota. Stress is mostly caused by environmental factors that affect the health of aquatic life. Marine life can be stressed by a variety of things, including physical, chemical, and biological ones. All stress is a result of human activity. Some of the consequences of human activity on the environment are not environmental, but others are. Human activity is responsible for a variety of novel pressures, including as bioaccumulation, biomagnification, medications, noise, microplastics, and climate change. As a result, invasive species, habitat loss, and toxicant-induced climatic vulnerability take place. Monomers and additives are the root causes of the negative consequences of microplastics. They can take in hydrophobic contaminants from water due to their high area to volume ratio. Noise is considered by the World Health Organization as a major pollutant with well-known adverse effects. Chronic noise exposure disrupts sleep permanently. Marine life is being found to contain toxic chemicals as a result of bioaccumulation and biomagnification. Damage to tissue cells and other biochemical illnesses are both possible outcomes. They aid in the decline of biodiversity as well. Identification of the pollutants that are common in the environment or have been isolated is crucial given all of these implications. Both the global climate and natural resources are impacted by climate change.

8.5 Pollution's Current and Future Effects on Marine Ecosystems Worldwide

Pollutants are substantial stressors in many environments, and they have a visible impact on marine species, habitats, and ecosystem services. The availability and quality of food, as well as threats to human health, are greatly increased by marine pollution in many areas. Furthermore, it is clear that ocean pollution may harm marine species in a variety of locations and that there is a risk that these effects may be widespread at the ecosystem level (Ryan 2016). Comparing the effects of marine pollution to other global problems like ocean acidification, overfishing, and an increase in sea surface temperatures makes the significance of these effects on a global scale less clear. Global marine ecosystems, however, may be quite resilient to pollution and have a reasonably quick recovery time, and total pollution input in an area may be rare to occasional and have only minor effects on ecosystems (depending on the type of pollution and affected ecosystem components). Individual effects of trash dumping, shipping (oil spills and other pollution), sewage discharge, underwater noise, dumping of oil/gas structures, radioactive waste disposal, and other effects have been suggested to be minimal and local to regional. These effects have also been investigated.

Although the extent of oil spills and chemical pollution (such as mercury from coal burning) will change as a result of the continued use of fossil fuels, it is only speculative to assume that factors such as population growth and rising global GNP per capita will result in increased marine pollution. Changes in temperature zones may bring new pests to new areas, raising the need for insecticides. It is anticipated that rising temperatures and changing precipitation patterns will alter the behavior, pathways, and effects of pollutants, with increased or decreased impacts depending on the substance and the region. Other categorical findings include the possibility that the expansion of offshore wind and wave energy would result in increased noise pollution in the ocean (e.g., Wilhelmsson et al. 2013). However, as the use of fossil fuels declines, there will be fewer offshore oil rigs, less shipping, and less seismic exploration of the ocean floor, all of which will result in reduced noise pollution from these industries.

8.6 Laws and Regulations

There are numerous ways for the ocean to become polluted, which has resulted in the adoption of numerous laws, regulations, and treaties. International regulations have been created to safeguard the ocean from marine pollution. At the Stockholm United Nations Conference on the Human Environment in 1972, marine pollution was a hot topic. In addition, that year saw the signing of the London Convention, which prohibits the discharge of waste and other materials into the ocean. The London Convention established black and gray lists of substances that should be forbidden (black) or under the scrutiny of national authorities, although it did not explicitly forbid marine pollution (gray). The London Convention created black and gray lists for substances that ought to be controlled (gray) or banned (black) by national authorities but did not outright prohibit marine pollution (gray). For instance, high-level radioactive waste and cyanide were added to the list of forbidden substances. Since the London Convention only applied to waste discharged from ships, it did not regulate waste released as liquids through pipes.

The 1982 United Nations Convention on the Law of the Sea (UNCLOS) was created to protect the marine ecosystem by mandating states to restrict their ocean pollution. It established restrictions on the amount of toxins and pollutants that can be discharged from all ships that sail internationally. The National Oceanic and Atmospheric Administration (NOAA) created the Marine Debris Research, Prevention, and Reduction Act in 2006 to track down, identify, reduce, and prevent marine debris. To address marine plastic pollution, the UN Environmental Agency (UNEA) established the Ad Hoc Open-Ended Expert Group on Marine Litter and Microplastics in December 2017.

8.7 The Fourteenth Objective of the Sustainable Development Goals

Goal 14 of the Sustainable Development Goals, created by the United Nations in a resolution passed in 2017, includes the elimination of marine pollution as one of its measurable objectives. The world has agreed that cleaning up the oceans should be a top concern. This is being kept an eye on as part of Sustainable Development Goal 14, which aims to mitigate the impact of human activities on the oceans. The definition of Objective 14.1 is that until 2025, all forms of marine pollution, including floating debris and nutrient contamination, should be avoided or drastically reduced. The Aichi Biodiversity Strategic Goals of the Convention on Biological Diversity aimed to significantly decrease habitat degradation and increase biodiversity as part of the UN 2030 Agenda for Sustainable Development. Preserving and managing the maritime environment sustainably, especially in regard to vulnerable habitats like estuaries, coastlines, and oceanic islands, is a top concern on the international environmental agenda. It is crucial for coastal communities and the marine sector to achieve sustainable oceans to preserve the future viability, prosperity, and condition of seas, as stated by United Nations Sustainable Development Goal 14 (Life below Water). In turn, the Nereus Program recognized certain ocean goals as additional gains from progress toward SDG 14 (https://en.wikipedia.org/wiki/Marinepollution). Protecting and preserving marine environments, minimizing ocean acidification, managing marine ecosystems sustainably, and recovering damaged ecosystems are all important goals. Especially for less well-known small-scale fisheries (i.e., local, subsistence, recreation, and ceremonial) in poor nations, the growth of the "Blue Economy" is projected to have a major impact on fisheries sustainability. Particularly at risk are commercially and ecologically significant species in the ocean.

8.8 Monitoring and Hazard Identification

Ecotoxicologists, marine biologists, oceanographers, ocean engineers, and environmental scientists are launching comprehensive studies with a focus on finding solutions in response to the major anthropogenic problems of marine pollution that affect marine biodiversity and fisheries in the global ocean. Research and competence in ecotoxicology are required in order to identify and control the risks posed by anthropogenic contaminants in response to changes in the global environment. Both the prevention of and responses to marine pollution are possible. Today's society makes extensive use of disposable and one-time-use plastic products, such as shopping bags, shipping containers, and plastic bottles. To shift people's mentality about the utility of plastic in society will require both time and financial investment. However, there can be some things that make it impossible to clean up. Many types of rubbish, including some plastics, cannot float and are thus buried deep below the water. This results in a significant amount of trash being dumped there. Plastics that are buoyant have a propensity to collect in large “patches” within the gyres of the ocean. One such accumulation is known as the Pacific Garbage Patch, and it is made up of larger pieces of plastic as well as smaller pieces of plastic that may be found both on the surface of the water as well as further down in its tumultuous currents. These clumps, as described by the National Oceanic and Atmospheric Administration, are more comparable to microplastic pepper flecks floating across an ocean soup as opposed to garbage islands. In the fight against marine pollution, there are very few strategies that are even remotely viable. Plastics that are sold under the guise of being “biodegradable” typically only break down at extremely high temperatures, which the ocean can never reach. Nevertheless, several nations are beginning to take action. According to a report published by the United Nations in 2018, more than 60 countries have enacted legislation that places restrictions on or outright bans on the use of disposable plastic products (https://education.nationalgeographic.org). Monitoring is the process of assessing a pollutant either directly or indirectly in order to determine the levels at which it is present and to control the effects it has on both humans and the environment. Marine monitoring programs can be carried out by either national or international organizations and consist of a sampling strategy, a selection of measurements, and an established methodology. In the current monitoring procedures, the effects on organisms, including biomarkers and bioassays that give ecological relevance, are evaluated using biological equipment and chemical analyses. The use of biological techniques has the potential to uncover newly emerging pollutants that are not being picked up by conventional chemical monitoring. Insight into the influence processes can be gained from lower levels of biological order, while ecological significance can be gleaned from higher levels. It is possible to classify a monitoring program as either surveillance, in which the goal is to identify geographical patterns and temporal trends, or investigative, in which the emphasis is placed on problem areas with the intention of motivating remediation. The high and consistent quantities provided by sediments make them the ideal matrix for maritime monitoring, but only the presence of biota can provide information on the fraction of the sediment that is bioavailable. The biomonitors used in passive monitoring are native creatures, whereas the biomonitors used in active monitoring are organisms that have been transplanted. In many nations, mussels serve as sentinels for the monitoring of coastal environments, despite the fact that seasonal fluctuations, genetic differences, and environmental shifts between populations might have an impact on the outcomes of biomonitoring. International treaties with regional relevance, such as Oslo and Paris Conventions (OSPAR) for the North Atlantic and the Cartagena Convention for the Caribbean Sea, provide incentives for the assessment of environmental quality and the protection of the marine environment. It is essential to have a solid understanding of the origin, location, and ultimate destination of pollutants before selecting a treatment or preventative measure to put into effect. There are several different methods of detection available to choose from when searching for contaminants in water.

Several of them make use of spectroscopy, Fourier transform infrared spectroscopy, Raman spectroscopy, sensors, remote sensing, thermal analysis, simulation models, and Raman spectroscopy. The aforementioned pieces of apparatus are able to detect the analyte in water. The primary criteria for categorizing pollutants are the physical and chemical properties of the contaminants. These tools are utilized in order to distinguish between organic and inorganic components that are present in the target that has been specified. Finding contaminants can have both positive and negative effects, particularly in terms of chemical analyses. At this time, both physical and chemical methods are merged and put to use all over the world. Recent advances include things like simulation models and remote controls. According to Gaston (2000) and Joanna (2006), biotas are widely used to characterize the characteristics of a given biosphere and are utilized on a regular basis to evaluate the extent to which ecological changes have taken place (Zaghloul et al. 2020). In addition to the elimination of pollutants, the maintenance of the ecosystem's health should be a secondary objective of any remediation method. For this reason, accurate evaluations of biological health are required for determining whether or not a certain remediation technique is successful. In today's world, a variety of organizations, such as the World Conservation Union and the International Union for the Conservation of Nature, use biological indicators as tools for biological monitoring and 17 provide support for this practice. It seems reasonable to assert that the benefits of using biological markers demonstrably outweigh the drawbacks of doing so. In conclusion, the use of a single species or a small group of biotas to evaluate the state of a particular ecosystem and how it changes over time is the primary objective of biological indicators. This may be an oversimplification of an intricate ecosystem, but this is the general purpose of biological indicators. Biological indicators, according to Nkwoji et al. (2010), continuously integrate knowledge from the genetic, physical, and chemical components of their ecosystem. This results in changes in individual fitness, population density, community organization, and ecosystem processes. When viewed from a managerial perspective, biological metrics differentiate between things that are and are not biologically stable. Using biological criteria, it may be possible to recognize the impact that human interference has had before it is too late to prevent it. Planktons are found in the vast majority of aquatic settings on a regular basis. Planktons eventually grow to the point where they are able to swim against the currents. They can be as small as a fraction of an inch or as large as certain kinds of crabs and jellyfish, despite the fact that most of the time they are extremely minute. Scientists classify planktons according to a number of different criteria, some of which include size, kind, and the amount of time spent drifting. However, the most fundamental classifications divide plankton into two primary groupings: phytoplankton, which consists of plants, and zooplankton, which consists of animals. They are a type of bacteria that are unable to swim against the flow of water and can be found in a variety of aquatic environments. Planktons are an essential component of the diet of a wide variety of large and small aquatic biota. Both chlorophyll and planktons can be found in significant quantities when they are biologically produced in aquatic environments such as rivers, reservoirs, streams, and swamps. According to the findings of Offem et al. (2013), communities of planktons moving near rivers and tides frequently combine massive amounts of energy, which is then finally transferred to higher trophic levels. They are able to integrate, and one of the most popular uses for them is to detect the level of contamination in an aquatic habitat. Because they are so sensitive to the effects of the natural world on their surroundings, planktons provide an invaluable indicator of the state of aquatic habitats. Because both phosphorus and nitrogen are contaminants that have an effect on other biotas found in aquatic environments, planktons could be used to monitor aquatic ecosystems with high amounts of both contaminants (Thakur et al. 2013). The presence of healthy plankton populations in an aquatic environment can be interpreted as an indication of the general health of the ecosystem. According to Saber et al. (2015), plankton can therefore be seen as a gauge of the sustainability of an ecosystem in addition to serving as a primary food source for a variety of different species. The production of blooms of cyanophyta is a well-known and powerful plankton biological indicator that reveals rapid eutrophication of aquatic ecosystems (Thakur et al. 2013). The use of phytoplankton as a biological indicator in the successful monitoring of the contamination of aquatic ecosystems has shown positive results. Pollutants have the potential to change the dynamics of the interactions that exist between the growth rate and each of these factors. For instance, a colorful effluent from an industrial process that contains suspended solid light may be filtered or absorbed, which would lead to a reduction in the growth rates of the organisms. In addition, phytoplankton is a key contributor to the transport of pollutants from the ocean to the upper tropics' human population. This class of biological indicators is also known as microalgae; as they include chlorophyll, just like land plants, they are dependent on sunlight for their continued existence and growth. The majority of them are found swimming in the more elevated parts of aquatic ecosystems. According to Zannatul and Muktadir (2009), zooplanktons are microscopic animals that dwell near the surface of aquatic habitats and are affected by the tides and currents in those environments. They consume phytoplankton, bacterial planktons, and sea ice, among other things. Zooplanktons are essential biological markers that help to determine the degree to which aquatic ecosystems have been polluted. Along with shrimp, these are the principal sources of nutrition for a great number of other marine species that make their homes in freshwater environments. The growth of zooplanktons is dependent on a number of factors, including the effectiveness of aquatic systems, the accumulation of nutrients in the water, and an increase in the size of freshwater bodies. The zooplanktons are significantly affected when there is a shift in the weather. According to Ramchandra et al. and Zannatul et al., the capacity of zooplankton as biological indicators is strong when associated with some other biotic parameters, such as food shortage, predation, and competitiveness, affecting their production, as well as some abiotic elements such as temperature, saltiness, stratification, and pollutants. In other words, the capacity of zooplankton as biological indicators is strong when associated with some other biotic parameters Trichotria tetrat, Alona guttata, Moscyclopesedex, Cyclips, and Aheyella which were all found in aquatic settings that included a high concentration of phosphorus and potentially toxic elements (PTEs), which led researchers to hypothesize that these organisms could be utilized as pollution indicators. According to Ramchandra et al., the growth of zooplankton in the aquatic ecosystem was constantly restricted by phosphorus and PTEs as well as by high aggregate alkalinity, hardness, and high conductivity. These factors combined to make the water extremely acidic. Seasonal zooplankton intensities in India indicated increased abundance during the rainy seasons and a drop throughout the summer due to the high temperatures. Copepods (Cladocer), Rotifers, and Ostrocoda are the three types of zooplankton communities that are found most frequently (Zannatul and Muktadir 2009).

8.9 New Methods to Combat Marine Pollution

In light of all these factors, it can be concluded that the best and most trustworthy method for removing pesticides from water is microbial breakdown. This is due to the fact that they consume organic components as food and convert them into finished goods. Modern technology is utilized to remove these chemicals from water by enzymatic microbial breakdown. The two trustworthy processes that convert these molecules into smaller by-products are hydrolysis and oxidation. Although there are treatments available, it is strongly advised to eradicate or control pests in order to stop their release into water bodies. It can stop the contamination of aquatic biota (Kumar 2021a) (Table 8.2).

According to its synthesis process, nanometals can be used to fabricate nanomembranes, which can be used to remove pollutants such as algal toxins and micropollutants. Vermiculite, clays, sands, polymers, and composites are some of the materials that are used more often to clear algal-contaminated water. These substances typically adhere to the major principles of coagulation, flocculation, netting, and electrostatic interaction between them (Kumar 2021b).

Contrarily, oil spills can be cleaned up with the help of cutting-edge technologies like magnetic foams and skimmers. Using contemporary developments like seabin, catchers, and floaters, plastic garbage is cleaned up all over the world. However, the biodegradation of materials made of plastic might offer a proactive and resource-saving alternative. Biodegradation can also be used to recover resources. Environmental elements like pH, temperature, soil structure, redox potential, moisture, oxygen and nutrient availability, as well as the presence of inhibitors, have an impact on the deterioration. Distribution, quantity, diversity, and activity of microbes all have the potential to alter how quickly plastics degrade. Large-scale innovations are necessary, underscoring the significance of removing plastic from the ocean. Every design is based on how useful and effective it is. The innovators will be assisted in bringing their ideas to the real world by the cost-benefit analysis (Kumar 2021c, d).

Covalent bonds that transition metals like Hg, Cd, Cu, Pb, and Zn create with macromolecules give them a unique level of toxicity. These metals combine to form divalent cations in water, which bind to two SH– groups on proteins. By inhibiting enzymes, for instance, this binding changes the structure and functionality of the impacted protein. These cations may be bound by humic matter, which lessens their

Table 8.2 Remediation and strategies applied in treating marine pollutants

Thermal remediation	Injection of energy into the subsurface to mobilize and recover volatile and semi-volatile organic contaminants
Bioremediation	Application of living or dead systems
In situ	Onsite biological treatment
Bioventing	Oxygen
Biosparging	Air is injected below the groundwater table or subsurface
Biostimulation	Nutrient
Bioattenuation	Natural microorganism
Bioaugmentation	Specialized microorganisms
Bioleaching	Fe/S oxidizing bacteria
Ex situ	Offsite biological treatment
Aerobic and anaerobic bioremediation	High molecular weight PAHs and most chlorinated organic compounds, e.g., DDT, dioxins, furans, and PCBs
Phycoremediation	Algae
Mycoremediation	Fungi
Bacterioremediation	Bacteria
Bio-surfactants	Marine bacteria producing rhamnolipids, increasing bioavailability of persistent organic pollutant
Phytoremediation	Hyper-accumulators plants effective against hydrocarbons
Strategies	
Phytostabilization (or phytoimmobilization)	Immobilization of pollutants by plants and their storage in underground tissues, e.g., Spartina maritima
Phytofiltration (or rhizofiltration)	The pollutants are absorbed or adsorbed to plant roots and thus their movement is minimized
Phytodegradation (or phytotransformation)	Plant enzymes such as dehalogenase and oxygenase
Rhizoremediation (or rhizosphere bioremediation)	Rhizosphere and root-associated bacteria and fungi
Phytovolatilization	Release of pollutants into the atmosphere through transpiration
Phyto-excretion	Salt marsh plants and halophytes uptake pollutants through the roots and their translocation to above-ground tissues, such as leaves and shoots, where they are accumulated

toxicity. Mussels can control Cu but they can also collect Hg, Cd, and Pb. Cu is also accumulated by oysters. Levels of Hg, Cd, and Pb in food are regulated due to their bioaccumulation in many organisms and potential health risks to humans. Radionuclides (isotopes of uranium, cesium, chromium, strontium, tritium, plutonium, radium, etc.) produced during nuclear fission, explosion, and research activities are harmful to human health as well as continuously contaminating the ecology and upsetting the food chain. Only a few kinds of plants, bacteria, fungi, and algae can degrade radionuclides or change their solubility, bioavailability, and mobility. Biotransformation, bioaccumulation, biosorption, bioprecipitation, biofilm development, and siderophore-mediated remediation (biomineralization/immobilization) are just a few of the mechanisms used by microorganisms to carry out bioremediation. Rhizofiltration, phytoextraction, phytovolatilization, phytostabilization, and phytostimulation are all components of the efficient green technique known as phytoremediation. Native or genetically altered microbes and plants, referred to as "hyperaccumulators," that include novel genes and proteins (such as NiCoT, ChrR6, merA, fccA, ctyc3, PpcA, czc, and phoK) can collaborate to enhance radionuclide cleaning on a large scale (Koul and Adlakha 2021).

Technologies for adsorption, absorption, and remediation have all been created (O'Brien et al. 2018). However, factors like oil-removing capability, ease of use, and cost effectiveness determine how effective a strategy is. These innovations have demonstrated their ability to withstand environmental factors including ocean currents and other environmental factors without producing secondary contamination. Some of the criteria are structural features, removal mode, weathering effect on oil removal capacity, buoyancy, hydrophobic property, and surface tension. Materials such as natural waste, agro waste, microorganisms, and polymers that can be converted into oil sorption devices such as floating foams, sponges, and membranes have recently been designed to address this ecological issue.

Due to the activity and half-life of radionuclides, the existence of radioactive waste in the environment may have long-term consequences, with the impact increasing over time. These particles exist in various oxidation states depending on their origin and routes of liberation, and can be identified as oxides, precipitates, or organic or inorganic complexes. They are most commonly seen oxidized, which makes them more water soluble and so transportable (Francis and Nancharaiah 2015). They cannot be destroyed, unlike organic toxins, and must instead be converted into a stable form or collected from the environment. Tritium (3H), cesium-137 (137Cs), strontium-90 (90Sr), plutonium-239 (239Pu), and plutonium-240 (240Pu) are all abundant in ocean waters due to anthropogenic influences. Technetium-99 (99Tc), carbon-14, strontium-90, cobalt-60 (60Co), iodine-129 (129I), iodine-131 (131I), americium-241 (241Am), neptunium-237 (237Np), and other types of radioactive plutonium and uranium are the most prevalent radionuclides in soils. Bacterial species' metabolic conversion of radionuclides into stable isotopes differs substantially from the metabolism of organic compounds produced from carbon sources. They are extremely energetic radioactive forms that can be transformed indirectly via the microbial energy transfer mechanism. Radioisotopes can be directly transformed via valence state changes by acting as acceptors or cofactors to enzymes. They can also be altered indirectly by microbe-produced reducing and oxidizing chemicals, which cause changes in pH or redox potential. Another two mechanisms include precipitation and complexation of surfactants, or chelating agents that bind to radioactive elements. Human intervention, on the other hand, can improve these processes by using omics and genetic engineering, as well as introducing nutrients or microorganisms into the treatment zone. Aside from the structural and functional links that these genes, proteins, and enzymes share with other metabolites, omics, particularly genomics and proteomics, enables the identification and evaluation of the genes, proteins, and enzymes involved in radionuclide bioremediation. When Rhodopseudomonas palustris and Novosphingobium aromaticivorans are cultivated in medium containing radioactive cobal, the NiCoT gene is highly overexpressed, according to genome sequencing of distinct microorganisms. Geobacter sulfurreducens also possesses over 100 coding areas for c-type cytochromes implicated in radionuclide bioremediation. Plants are used in phytoremediation to remove poisons from the environment or to make them less hazardous. It is a viable approach for radionuclides when decontamination periods are long and waste concentrations are low. Some plant species can alter radioisotope status (without becoming harmful) by concentrating them in different sections of their structure, pushing them via the roots, making them volatile, or stabilizing them on the ground. Plant genetic engineering and biostimulation, also known as phytostimulation, have improved and accelerated these processes, especially in fast-growing plants. For example, *Rhizobium rhizogenes* is frequently utilized and significantly increases radioactive uptake by roots.

8.10 Natural Remedies to Marine Pollution

The International Union for Conservation of Nature (IUCN) defines nature-based solutions as actions to safeguard, sustainably manage, and restore natural or modified ecosystems by addressing issues related to the environment, the climate, and development in general. The same guiding principles supporting just and equitable policies should also apply to it. There are some marine-based natural remedies for marine pollution: large protected region with replenishment of marine life stocks (plants, algae, and animals), low trophic aquaculture, restoration of seagrass and seaweed, protection of shorelines with boulders, shellfish, reefs, and seagrass, shipping powered by wind energy, and antifouling agents on ships (Riisager-Simonsen et al. 2022).

8.11 Conclusion

Despite the scarcity of real-world examples of biological approaches in marine environments, the data provided so far is sufficient to conclude that bioremediation and phytoremediation are potential techniques for the remediation of both organic and inorganic pollutants in those ecosystems. More research in the field of new biological technologies, such as vermiremediation, is also desirable. Vermiremediation is the employment of earthworms to remove contaminants from soil, and it could be a promising strategy in intertidal soils damaged by both metals and oil. Additionally, research on novel contaminants, such as pharmaceutical chemicals, is expanding, and it is important to consider biological remediation of such pollutants. Furthermore, more study is required to comprehend the methods used by microbes, particularly plants, to combat pollution and act as effective agents of remediation.

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Source and Effect of Oil Spills on Associated Microorganisms in Marine Aquatic Environment

9

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Abstract

Crude oil is majorly used and is transported globally via waterways. Consequently, the occurrence of oil spills has increased due to anthropogenic and natural activities. In the event of an oil spill, the oil can either remain on the surface of the water column or can get dispersed into the littoral zone. The oil that does not get dispersed gets carried to the coastal region by the water currents and causes harm to the coastal organisms. When the oil gets dispersed, it affects a wide range of marine organisms such as marine microorganisms, fishes, sea birds, marine mammals etc. These organisms bring about the bioaccumulation and the biomagnification of the toxic components of crude oil up the food chain. Oil spills affect the production of eggs in fishes and serves lethal to the fish larvae. Oil also causes the smothering of fur and feathers in sea otters and sea birds and cause them to drown. Various coastal and offshore ecosystems are also highly impacted by the presence of oil, which causes damage to the surrounding vegetation and the organisms living there. In the case of marine microorganisms, oil seems to cause a change in composition of the normal flora in the environment. Only microbes that have the potential to survive in the presence of oil will grow, and these microbes secrete biosurfactants and specific degradative enzymes. This property of the microorganisms can be utilized for the treatment of marine oil spills. This review discusses various sources of oil spills in the marine and coastal ecosystems. It also explains the impacts of oil spills on various marine microorganisms, their adaptive responses and current status of research work on this

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aspect. The application of marine microorganisms to treat oil spills is also emphasized upon.

Keywords

Oil spills · Biosurfactants · Offshore ecosystems · Bioremediation

9.1 Introduction

Petroleum is a general term used to describe liquid mixtures majorly composed of carbon and hydrogen with trace amounts of oxygen, nitrogen and sulphur. It is generally formed as a result of organic matter decomposition over millions of years. It is present in the sedimentary rocks formed and buried beneath the marine and terrestrial environments. On the earth, petroleum naturally exists in three states of matter, namely: solid—asphalt, liquid—crude oil, gas-natural gas. According to a study it was reported that most petroleum products are derivatives of crude oil that are majorly constituted by hydrocarbons, and the components of petroleum can be divided using absorption chromatography into four fractions: aromatics, asphaltenes, resins and saturates (Harayama et al. 1999; Suyal and Soni 2022). Aromatics and saturates mainly contain hydrocarbons, whereas resins and asphaltenes contain non-hydrocarbon compounds where in addition to carbon and hydrogen, trace amounts of sulphur, oxygen and nitrogen are also present. Crude oil is subjected to a fractional distillation process, in which various fractions are formed and various petroleum products are produced by this process called refining (Harayama et al. 1999). The rising demand for petroleum hydrocarbons has led to the exploitation of oil reserves in deep marine sediments, and this exploitation has led to pollution in this region. Most of the light oil residues of petroleum hydrocarbons that enter the marine environment are weathered by natural processes such as spreading, evaporation, aerosolization, photooxidation, emulsification, dissolution, natural dispersion etc. (Mendelssohn et al. 2012). The increasing rate of pollution associated with the exploitation of oil reserves has, in turn, caused an upsurge in the urgent requirement for remediation.

There are several incidences in the past that have reported major oil spills. The most common cause of an oil spill in marine environment is due to the operating activity of ships and this process is termed as operational oil spills. It can either occur during a drilling activity, during fuelling of a ship or when oil is being transported via water route. Some of the few case studies are mentioned below, one taken place in the recent past:

1. Amoco Cadiz, France, 16 March 1978

   Amoco Cadiz was a supertanker containing 223,000 tons of Iranian and Arabian crude oil that grounded off from the shore of Portsall, Northern Brittany, and is one of the greatest single oil spill up till then (Conan 1982; Nounou 1980). The crude oil that the supertanker was carrying was light and had low viscosity, containing 30–35% of highly toxic aromatic hydrocarbons (Conan 1982). About 40% of the total composition consisted of volatile fraction (Nounou 1980). High spring tides and strong onshore winds brought the oil onto the beaches, estuaries and marshes along the coast of Brittany and intensified the mixing of oil into sea water by processes such as emulsification and dissolution (Conan 1982; Nounou 1980). This incident ended up killing up to 15,000–20,000 sea birds, 54% of limpets, populations of bivalves, peracarid crustaceans, heart urchins etc. (Conan 1982).

2. Exxon Valdez, Alaska, 24 March 1989

   The Exxon Valdez oil spill incident occurred on the Bligh reef in the Prince William Sound of Alaska when the tanker ran ashore and spilled around 40 million tonnes of crude oil. After a period of 2 days of the oil spill incident, a powerful storm with winds blowing at a speed of 70 mph hit the coast of Alaska, that transformed the spilt oil into tar balls and mousse. This massive release of crude oil contaminated almost 1900 km of the shoreline (Peterson et al. 2003). About 250,000 sea birds, 2800 sea otters, 22 killer whales, 300 harbour seals and many fish eggs were killed. Since most Alaskans rely on fishing as their major fishing activity, the Alaskan economy suffered heavy loss after the spill as many salmon eggs failed to hatch post the incident of the oil spill. Following the oil spill, there was also a collapse in the fishing industry, tourism and also community-based health (Lee et al. 2016).

3. Gulf War Oil Spill, Persian Gulf, Kuwait, 19 January 1991

   During the Gulf War in the year 1991, millions of barrels of crude oil were released intentionally into the 700-km long coast between Abu Ali Island and Kuwait (Krupp 1997). The coral reefs in certain areas of Persian Gulf were severely affected by the oil spill and showed signs of severe stress (Ahmed et al. 1998). The oil spill caused enormous decline in the production of fishes (Joyner and Kirkhope 1992; Pashaei et al. 2015). The shrimp industry of the Gulf was also found to be heavily affected (Joyner and Kirkhope 1992). A study conducted in 2006 in one of the bays of the Gulf oil-spill-affected region revealed that, 20% of the sites that were under investigation were still yet to recover from the residual impact of the oil (Joydas et al. 2012). Polycyclic aromatic hydrocarbons (PAHs) were found to be persistent in the shore sediments from the southern shore of Saudi Arabia (Bejarano and Michel 2010). The water in this region is warm, the level of toxicity in the spilt oil is high and the weathering of oil takes place at a much faster rate, making the Persian Gulf the most polluted marine basin in the world (Pashaei et al. 2015).

4. The Deepwater Horizon Oil Spill, Gulf of Mexico, 20 April 2010

   The Deepwater Horizon oil drilling rig underwent a mechanical failure at the continental shelf of the Gulf of Mexico. The rig was capped only in September 2010 and until then it ended up releasing about 430,000–500,000 tonnes of oil making it the largest spill in the history of the petroleum industry (Zhang et al. 2019). According to a satellite imagery, the estimated volume of ocean that was affected came up to 180,000 km^2 (Martin et al. 2014). The Deepwater Horizon sank into the ocean after continuously burning for approximately 2 days (Mansir and Jones 2012). According to a study it was found that up to 95% of coral damage at about 6 km away from the spill. Impaired reproduction, reduced growth, disease, impaired physiological health and mortality were found in populations of fishes, birds, sea mammals, invertebrates, planktons etc. (Zhang et al. 2019).

9.2 Oil Spill Event: The Fate of Oil

Hydrocarbon pollution in the marine environment can occur through various anthropogenic and natural sources (Mahjoubi et al. 2017). Natural sources are the seepage of natural gas and oil especially at locations such as faults on the plates on the earth's surface (Mahjoubi et al. 2017). The oil upon entering the marine waters can undergo various processes leading to compositional changes, and finally, its weathering. Crude oil undergoes weathering where it will start to degrade under the influence of many simultaneous processes such as evaporation, photochemical reaction, chemical oxidation and microbial degradation, that will cause a change in its characteristics (Rout and Sharma 2013; Mishra and Kumar 2015). More volatile hydrocarbons in the oil evaporate as soon as the spill event occurs. Hence different processes of weathering start first at the surface. The process of weathering differs between light and heavy hydrocarbons, where light-weighted hydrocarbons undergo rapid weathering caused by evaporation, while heavy-weighted hydrocarbons remain unchanged for a long period of time and undergo processes such as microbial degradation and chemical oxidation (Mishra and Kumar 2015). Properties of the oil and the environmental conditions play a major role in the process of weathering. The series of events involved in weathering is spreading, evaporation, dispersion, dissolution and emulsification. The spilled crude oil in the sea is spread due to the action of winds and waves, and it gets mixed with seawater due to the rolling action of waves, by ingression of seawater into the oil droplets and then release through the thin oil layer or ingression of oil into water causing floating. The higher viscosity and lower surface tension of the oil than the seawater creates emulsions that are water-in-oil (W/O) type, i.e., seawater dispersed in crude oil. With the repeated ingression and release, there is repeated expansion and shrinking of the oil layer on water surface, with evaporation of volatile components taking place simultaneously adding to the viscosity of the oil. Thus, the water content of emulsion remains steady but viscosity increases, looking dark brown in colour and forming the highly viscous mousse which drifts on the sea surface (https://www.pcs.gr.jp/doc/EMousse/text.htm#PROCESS3). The mousse contains high molecular weight compounds. The formation of tar balls is the next phase, where non-degraded hydrocarbons are found at different locations of the marine environments.

Photosensitive hydrocarbons such as PAHs oxidize by solar radiation forming more water soluble but recalcitrant compounds. Small alkanes and monoaromatic hydrocarbons solubilize in seawater. Aerosolization and loss of volatility occur upon evaporation. All these results in reduced toxicity of spilt oil but increasing the toxicity of overlying or surrounding air. Progressive volatile loss affects the density of the spilled oil, leading to density-driven stratification within the water column.

Oil starts spreading in the form of a thin sheet away from the slick and this spreading is density-gradient-driven. The emulsions formed after spreading of thin layer of oil across the waters and mixing with it due to wave action, turbidity etc. remain stable for a long time. These stable emulsions increase the area and viscosity of spilled oil and as they disperse, they come in contact with particulate matter, living microorganisms etc. in the water column, and further cling onto this helpless marine life causing a threat to them (Mishra and Kumar 2015) (Fig. 9.1). This contact of oil with suspended particulate matter leads to formation of oil-particulate aggregates (OPA) that tend to sink forming marine tar residuals (Zhang et al. 2019). Weathering processes also include microbial degradation. There are several indigenous hydrocarbon-degrading microorganisms in the aquatic environment that bring about the process of biodegradation; however, this process occurs at a very slow rate and is insufficient to tackle the problem of the rising toxicity of oil spills on marine life. It is generally thought that these organisms are normally present in very small numbers, and by providing conditions that allow them to take advantage of the hydrocarbons as a carbon and energy source, they grow and multiply rapidly (Head et al. 2006).

PAHs that contain four rings and more, and their associated metabolites cause chronic toxigenicity and mutagenicity, and remain in the soil for a very long time period. During the event of a marine oil spill, the water currents and winds bring about rapid spreading of the oil. Aquatic habitats such as salt marshes, coral reefs and mangroves are considered to be highly sensitive to oil contamination (Macreadie et al. 2017). These ecosystems aid in a number of processes such as carbon sequestration, flood mitigation, pollution removal etc. (Beland et al. 2017).

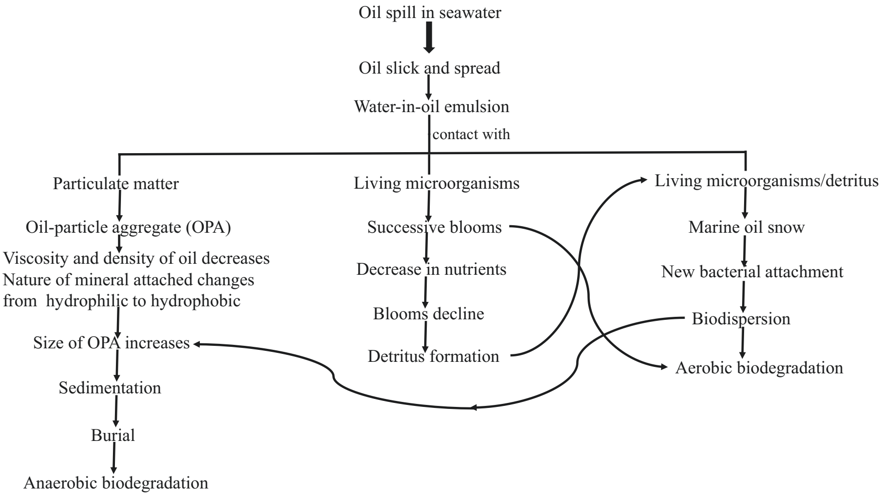

Fig. 9.1 Schematic representation of fate of oil spill from emulsions to aggregates and degradation by microorganisms

9.3 Impact of Oil Spill on Marine Life

The crude oil that is released, enters into various marine and aquatic ecosystems where it will persist for a long time, causing contamination and mortality of the organisms living there.

9.3.1 Planktonic Organisms

Phytoplankton, the basis of food chain, are an important group of organisms that determine the productivity of any water body (Tweddle et al. 2018). The growth of phytoplankton community differs in response to environmental conditions such as varying temperatures, availability of nutrients, presence or absence of pollutants etc. (Quigg et al. 2021). With an increase in the nutrient input, the productivity shows a stepwise change by increase in the phytoplankton biomass (Zhao et al. 2015). As the phytoplankton biomass increases due to the uptake of these nutrients, it will further influence the biogeochemical cycling of hydrophobic organic compounds (HOC) such as PAHs and organochlorine pesticides (OCPs) (Skei et al. 2000). The contaminants will accumulate in the phytoplankton and will be transported to the higher organisms of the pelagic food chain when it is grazed upon or it will be carried to the sediments by live or dead cells and other sinking aggregates (D'Costa et al. 2017). Naphthalene, fluorene, phenanthrene, acenaphthylene, acenaphthene, anthracene, pyrene, chrysene, benzo(*k*)fluoranthene, benzo(*a*)pyrene, indeno (1,2,3-*cd*) pyrene, dibenzo(*a*,*h*)anthracene, benzo(*g*,*h*,*i*)perylene, are considered as PAHs of primary environmental concern according to the U.S. Environmental Protection Agency (USEPA) (Jayasiri et al. 2014).

The abundance of auto/mixotrophic bloom-forming toxic dinoflagellate species such as *Prorocentrum texanum*, *Prorocentrum triestinum* and *Scrippsiella trochoidea* increased in abundance after an exposure to oil or oil dispersants (Almeda et al. 2018). *Noctiluca scintillans* and *Gyrodinium spyrilae* ingested 17–28% of dispersed crude oil droplets in surface waters when these dinoflagellates were present in high numbers (Almeda et al. 2018). Harmful algal bloom-forming species such as *Karenia brevis*, *Prorocentrum micans* and *Hetrosigma akashiwo* are also affected by oil spills (Özhan and Bargu 2014). Toxin profiles of these algae in the presence of different concentrations of crude oil showed that higher concentrations of crude oil caused a decline in the growth rate of the algae as well as in the toxin productivity. At the lowest concentration of crude oil, the toxin productivity was found to be relatively higher (Özhan and Bargu 2014). Crude oil when combined with oil dispersants exerts higher toxicity on diatoms (Hook and Osborn 2012).

Zooplankton is an important food source for larger fishes and can control the rate of primary productivity. Exposure of zooplankton to the oil-in-water column prevents them from moving against the water currents, leading to high mortality due to its reduced physiological functions. Copepods such as *Oithona* and *Paracalanus* are highly affected by oil spills in comparison to the larger planktonic assemblages and hence serve as an indicator for the assessment of oil spills in marine environment

(Yuewen and Adzigbli 2018). Zooplankton are capable of accumulating and metabolizing hydrocarbons (Ifelebuegu et al. 2017). Hence zooplankton play a major role in the cycling of hydrocarbons within aquatic systems (Almeda et al. 2013). Therefore, though there was a notable impact of PAHs on the plankton community, there were no severe toxic effects that were noted (Ifelebuegu et al. 2017).

9.3.2 Fishes

Fishes are affected by marine oil spills causing increased rates of mortality, reduced growth rates of fish larvae and eggs, reduced rates of feeding, increased rates of starvation, morphological deformities and increased risks of attack from predators (Yuewen and Adzigbli 2018). The early stages of fish are very susceptible to the presence of PAHs even when it is present at low concentrations and can cause death, circulatory failure, stunted development, low appetite and growth and malformities of morphological structures etc. Carcinogenic properties of PAHs have also been observed in English sole (*Parophrys vetulus*) and Flounder (*Platichthys stellatus*). Some fishes such as the rainbow trout and medaka serve as well-established sensitive models for assessing the chemical carcinogenic effects caused by various endogenous and exogenous factors (Honda and Suzuki 2020; Varanasi et al. 1987). PAHs have relatively shorter half-lives as compared to other pollutants, and are excreted rapidly (Huang et al. 2014). Continuous exposure and contamination of PAHs in fishes is happening worldwide. But, fishes have a well-developed hepatic mixed function oxygenase (MFO) system that helps in bioconversion of PAHs and thus they do not store up and retain large amounts of PAHs within them. Thus, they are not at a serious threat and are not likely to transfer hydrocarbons to their predators (Saadoun 2015). Due to the quick metabolism in fishes, the occurrence of biomagnification of PAHs in the trophic level of the food chain is not considered (Honda and Suzuki 2020).

9.4 Impact of Oil Spill on Coastal Organisms

9.4.1 Macroorganisms

Corals and shellfishes in coastal areas are at higher risk of exposure to oil as they are immobile organisms and unable to escape the oil slick (Rout and Sharma 2013). Hard and soft corals in their early life stages are negatively affected by dispersed oils and the oil dispersants that are used to remove them (Shafir et al. 2003). High toxicity of dispersants is lethal to coral nubbins as well (Saadoun 2015).

Humpbacks, dolphins, bottlenose, fins, sperm whales, sei whales, sea otters, cetaceans and pinnipeds are some of the marine mammals which suffer many health defects such as low immunity, eyes and adenoidal tissue damage, lung and adrenal diseases when exposed to oil on sea surface and shoreline (Saadoun 2015; Kuzhaeva and Berlinskii 2018). Oil fouls the fur of sea otter, which in good condition is required for their insulation, and therefore these animals have to consume more food to maintain core body temperature (Helm et al. 2015). This results in a huge challenge for the sea otters, as they normally consume just 25% of their body weight. Though sea lions and seals are less affected by oil spills as compared to sea otters, their eyes, ears, mucous membranes, internal organ systems and external genitalia show negative effects when exposed to the oil. External oil does not affect their ability to maintain body temperature (Helm et al. 2015). This has been observed in walruses too. Bottlenose dolphins suffered health disorders such as lung and adrenal ailments when exposed to oil (Schwacke et al. 2014; Carmichael et al. 2012). In the polar regions, the spilled oil accumulates in polynyas, breathing holes, in leads, and ice edge which are the resting sites for organisms such as belugas, narwhals, walruses, ringed seals, and polar bears and thus these organisms get exposed to oil (Geraci and Aubin 1988).

Sea birds exposed to oil experience impaired health defects, including ulcerations, haemolytic anaemia etc. (Yuewen and Adzigbli 2018). Birds ingest oil while feeding and inhale oil droplets during frequent interactions at oil water interfaces. This causes to severe internal poisoning leading to high mortality (Peterson et al. 2003). PAH levels in their plasma increases causing disturbance to their resident flora (Yuewen and Adzigbli 2018; Antonio et al. 2011). In addition, when oil comes in contact with their feathers, the feathers get tangled into a thick mass (matted) losing the property to fly and float, thus increasing the risk of drowning (Rout and Sharma 2013). In the temperate and Arctic regions, a large number of oil coated birds freeze to death (Nounou 1980). Even weathered oil in small amounts is detrimental to the embryos of certain birds such as mallard duck *Anas platyrhynchos* (Finch et al. 2012).

9.4.2 Microorganisms

The marine microbial community is immensely diverse in their composition and hence they exhibit a wide range of effects on the occurrence of an oil spill (Zhang et al. 2019) (Fig. 9.2). In response to an oil spill, the microbial community of both marine and fresh water environments undergo certain changes such as, an increase in the abundance of the bacterial population, alteration of enzyme kinetics and other physiological changes (Ghanavati et al. 2008; Hara et al. 2003; Hassanshahian 2014; Labud et al. 2007). There seem to be a temporal succession of different bacteria. There is increase in the abundance of *Alcanivorax* spp., followed by *Cycloclasticus* spp. (McGenity et al. 2012). An assessment conducted on the toxicological impacts of oils on marine microbial communities by means of their genomic analysis revealed both short-term and elevated levels of damage caused due to contamination in addition to adaptive responses of microbes to these exposures (Das et al. 2015). The toxicity of most hydrophobic organic hydrocarbons is caused by general, non-specific accumulation of the hydrocarbons in the lipid bilayer of the organism's cell membrane causing non-specific chemical associations that produce toxic effects (Cabral et al. 2003; Ferrante et al. 1995). Organic solvents

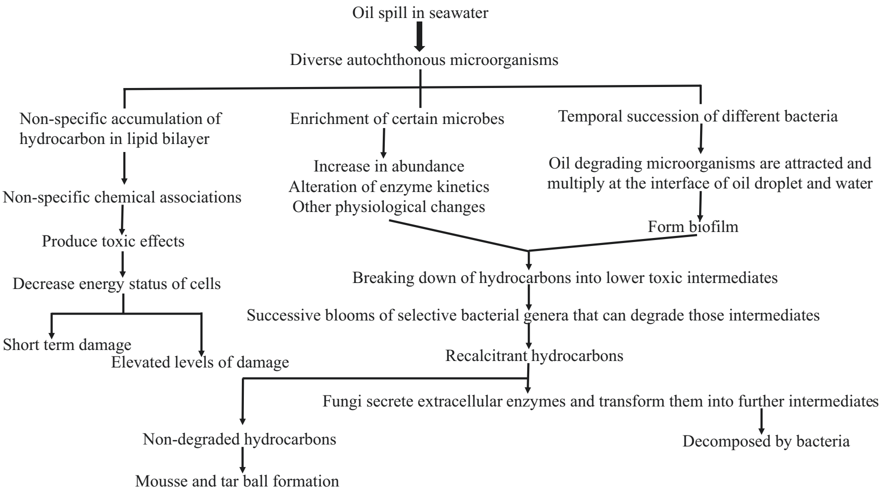

Fig. 9.2 Schematic representation of the effect of oil spill on autochthonous microorganisms in seawater

decrease the energy status of the cells (Heipieper and Martínez 2010). *Escherichia coli* cells when treated with phenol released potassium ions and adenosine triphosphate (ATP) (Heipieper et al. 1991). The toxicity of hydrocarbon increases with addition of aldehyde or epoxide groups, and this increases membrane toxicity (Duldhardt et al. 2007). Anaerobic bacteria are three times more sensitive to the chlorinated phenols than the aerobic strains (Duldhardt et al. 2007).

Crude oil contaminants remain in the environment for a long time because of their non-degradable nature and will affect the chemical, physical and biochemical behaviour of contaminated water/soil (Das et al. 2015). Oil degrading microorganisms usually are attracted and will multiply at the interface of oil droplet and water (Abbasnezhad et al. 2011).

9.5 Impact of Oil Spill on the Coastal Ecosystem

9.5.1 Beaches

Tar balls were observed to be present on the shores of Karnataka, Goa and Gujarat during monsoon season (Suneel et al. 2019). Tar balls is the phase after the formation of mousse as an effect of oil contamination. Oil spills are therefore a major threat to the shorelines (Asif et al. 2022). Crude oil spreads like an oil slick on the water surface and affects nearby beaches. High amount of oil gets stranded on the beach during onshore winds and falling tide, and it gets deposited on the intertidal zone (Bejarano and Michel 2016). When oil gets trapped in the shoreline sediment, it can wash away beach's fine sand or silt (Asif et al. 2022). Sediment-oil agglomerates, which are a few centimetres to a metre in length, are formed on the beaches by the interaction of the sediment with oil mousse. When the length of the agglomerates exceeds a metre, they form sediment-oil mat. The agglomerates and the mat can persist on the beach for a long time (Asif et al. 2022). Various factors such as beach biochemistry and hydrodynamics, properties of beach and oil affect the distribution and persistence of oil within the matrix of the beach (Boufadel et al. 2019). Rocky shores serve as a habitat to many organisms, but they are extremely vulnerable to oil spills as they do not absorb oil and the oil that is stranded ends up killing many species (Fales and Smith 2022; Balogun et al. 2021). Wide range of waste substances are dumped on the beaches and plastic is one such waste that does not decompose. The larger pieces of plastic breaks into tinier pieces called microplastics (Huang et al. 2021). The plastic pellets that are formed as a result of plastic pollution have high affinity towards the highly hydrophobic organic pollutants (Amelia et al. 2021). Highest concentrations of PAHs are adsorbed on to microplastics in highly polluted regions (Frias et al. 2010). These organic contaminants are transported to the beach through the atmosphere in the form of gas or particle or distributed between the two (vapour-to-particle) based on the vapour pressure of the respective organic contaminant (Bidleman 1988). Oil spill also causes damage to nearshore sediments due to deposition and penetration of the oil into the sediments. After the occurrence of the Deepwater Horizon oil spill, the weathered oil washed ashore with concentrations of total petroleum hydrocarbon (TPH) scaling approximately up to 2 kg per m of the beach. Due to the action of waves and storms, the oil was buried into the sand and a migratory oiled layer of the sand was formed within the sediment column (Huettel et al. 2018). Since the oil takes many years to completely dissipate, it can still persist in the soil even after cleanup process has been carried out (Wang et al. 2020). The impact of oil spill on the shorelines and the extent to which various treatment techniques are successful can also be influenced by seasonal variations (Asif et al. 2022).

9.5.2 Estuaries

Estuaries act as biogeochemical barrier, and trap the terrigenous materials which contain pollutants formed as a result of various anthropogenic activities. This property of estuaries is attributed to their hydrodynamic properties such as high salinity gradient. Estuaries are at high risk from human activities including marine oil spills (Whitehead 2013). The Deepwater Horizon oil spill that took place in 2010 contaminated about 650 square miles of coastal habitat of Northern Gulf of Mexico, over a period of 87 days (Crone and Tolstoy 2010). This oil spill affected many coastal and deep-water species (Silliman et al. 2012; White et al. 2012a, b; Dubansky et al. 2013). A study was conducted on the seasonal changes of PAH in the estuaries of the rivers Partizanskaya and Tumen of the Sea of Japan (Chizhova et al. 2020). It was observed that the fraction of dissolved polycyclic aromatic hydrocarbon (DPAH) is larger than that of the particulate polycyclic aromatic hydrocarbon (PPAH), hence indicating that the estuaries are effective geochemical barriers where there is deposition of a significant amount of PPAH. In the Partizanskaya river estuary, there was a noticeable rise in the levels of total polycyclic aromatic hydrocarbons (TPAHs) concentrations during winter, due to the emission of significant amounts of PAHs from residential and municipal heating systems. During summer the concentration of TPAHs in river Tumen estuary were comparatively higher and this was attributed to the heating during summers and anthropogenic activities.

Winds govern the coastal currents and therefore, the circulation within an estuary. Tidal forces also govern the water flow in an estuary. These factors may cause the oil to flush out of the estuary and disperse elsewhere along the coast under the influence of alongshore currents (Feng and Hodges 2020). As tidal range governs the transport of oil slicks upstream or downstream an estuary, the time of oil release and the tidal cycle are important factors to determining the fate of the oil spill. Oil slicks released during heavy river discharge leave the estuaries quickly than otherwise. During spring tide, oil slicks travel larger distance than during neap tide. The distance between the mouth of an estuary and the point of release of the oil also determines the impacted area. Stronger currents at the mouth of an estuary causes the oil to spread larger distance further upstream whereas if the oil is released upstream, then the impacted area decreases due to decreasing current velocities (Eke et al. 2021).

9.5.3 River Delta

The Deepwater Horizon oil spill adversely affected wetlands of Louisiana's Mississippi River Delta ecosystem (Mendelssohn et al. 2012). About 24% of Yellow River Delta is contaminated with crude oil due to the Gudong oil field (Gao et al. 2022). The Niger Delta and the Nun River of Nigeria are also contaminated with oil pollution. The Bhagirathi-Hooghly River bed is also contaminated with oil (Roy et al. 2022). One of the studies on the impact of oil pollution on water quality assessment showed that certain physicochemical parameters such as turbidity, total dissolved solids, total suspended solids, dissolved oxygen, conductivity and heavy metals were in breach of national and international limits of drinking water and ecological health. The diversities of planktonic and microbial populations and diversities in all freshwater samples were also observed to be impacted by minor spills that were reaching the river (Ifelebuegu et al. 2017). Concentration of heavy metals seems to be enhanced due to oil spill destructing the habitat (Roy et al. 2022).

9.5.4 Salt Marsh

Salt marshes host a broad range of plant, animal and mammal life. These coastal wetlands are drained with salt brought in by seawater during high tides. With the introduction of fresh light oil into marsh regions, the vegetation there is easily damaged (Rout and Sharma 2013). According to this study, salt marshes have a positive influence on microbial community and increases the abundance of hydrocarbon degrading microbial populations in the rhizosphere. Discharges from municipal wastes, industrial waste water, urban runoff and oil leakage from boats and ships are possible sources of total petroleum hydrocarbons (TPHs) (Ribeiro et al. 2011). The impact of oil spill in salt marsh areas depends on the period of plant growth. Long-term damage can be caused due to repeated or chronic oiling which occurs over several years. Cleaning up a salt marsh is difficult and in this process plants will be damaged and hence marsh plants are let to clean up naturally (Kuzhaeva and Berlinskii 2018). Salt marshes are highly vulnerable to floating oil, as they are exposed to high tide waters. The oil impacts the shoreline as it causes erosion along it and also causes disturbance to the food web (Challenger et al. 2021). Overton et al. (2022) conducted a study on the fate of petroleum in the sediment and water samples of the marsh area, along the Gulf of Mexico that was affected by the Deepwater Horizon oil spill. It was observed that, some of the recalcitrant residues are present in very low concentrations until today (Overton et al. 2022). The weathering of oil residues in the marshes showed two distinct patterns. The samples that were coated with high levels of solid oil residues displayed a lesser rate of weathering when compared to that of the samples that contained low levels of dispersed oils (Overton et al. 2022). It was also noted that, a part of the thick emulsified oil that were originally present on the marsh surface was also buried under lower plants and oiled vegetation mats. A part of this oil residue has been observed to get re-burrowed overtime into soil/sediments by the action of fiddler crabs (Zengel et al. 2016).

9.5.5 Mangroves

Mangroves get severely affected with the increase in oil tanker trafficking. The major route for oil contamination to occur in a mangrove ecosystem is by oil pollution incidents that are either accidental or deliberate (Duke 2016). Four major types of oil spills that affect mangrove ecosystems include: pipeline rupture, vessel incidents, shore tank facility disruptions and wellhead damage. The oil adheres to the root surfaces of mangrove shrubs and trees, when they are exposed to tidal water flow. This is harmful to the plants and animals that live within the mangrove ecosystem (Duke 2016). Mangroves have the ability to tolerate high concentrations of salt and can remain planted in currents and surfs due to their unique root system that is submerged. The submerged and the aerial roots make them more susceptible to floating oil and hence it may lead to the respiratory and osmoregulatory damage of the mangrove plants (Getter et al. 1981). The occurrence of an oil spill can block the air passage that is present on the lenticels of the mangrove roots and hence will cause its death. The sensitivity of mangroves depends largely on the substrate on which the mangrove is growing (Kuzhaeva and Berlinskii 2018). Riverine and overwash mangrove forest types have the least potential to biological damage, outer fringe and dwarf mangrove forests have moderate susceptibility to oil exposure because they are exposed to waves and tides thus reducing the duration of oil exposure. Basin and inner fringe mangrove forests are highly susceptible to exposure to oil because of their low exposure to waves and tides but high exposure to oils (Kuzhaeva and Berlinskii 2018). Certain investigators outlined that when certain mangrove species, like the seedlings of red mangrove *Rhizophoram angle*, were transplanted onto oil spilled areas, they did not show production of new leaves (Saadoun 2015).

9.5.6 Impact on Offshore Ecosystem

Devastating effects of oil spill in the offshore waters is seen immediately, but these subside within a year. Following the *Argo Merchant* (1976) spillage that released about 50,000 tons of oil, the death of cod, cod eggs and herring was observed in the middle of a fishing area. The revival of a few of these species was seen within 1 year of the spill. Fishes present in offshore waters are found to have the ability to avoid oil-impacted areas, and the larval and adult immigrants will rapidly replace the eliminated individuals. Birds are highly sensitive to oil spills and as a result some species are near to extinction or are lost. Disruption of feathers, ingestion of toxic oils, starvation etc. will cause the death of these organisms similar to near shore regions (Nounou 1980).

9.6 Oil Degrading/Oil Resistant Microorganisms

The occurrence of an oil spill negatively affects the coastal ecosystems. Naturally, the environment exhibits mechanisms such as photooxidation, degradation, evaporation to degrade/weather and remove spilled oil from the environment (Bacosa et al. 2022). The microbial community are able to adapt to contamination by the enrichment of certain microorganisms that can either utilize the contaminant or that can resist the toxic effects (Hassanshahian 2014). Distinct species of microorganisms have been known to accelerate the degradation of oil at contaminated sites (Lee et al. 2018; Chen et al. 2020) (Table 9.1). Marine bacteria utilize petroleum hydrocarbons as carbon and energy source for their growth and reproduction and thus, degrade them diminishing the physiological stress caused due to the presence of petroleum hydrocarbons (Xu et al. 2018). It was observed that, when oil or its constituents were introduced into the seawater, successive blooms of selective bacterial genera were formed (Yakimov et al. 2007). The bacterial genera included *Alcanivorax*, *Cycloclasticus*, *Marinobacter*, *Oleispira*, *Thalassolituus* and few others. Prior to the polluting event, these bacterial species were present at low or undetectable levels (Yakimov et al. 2007). Marine sediments collected from the coast of Labrador in Canada after oil spill incidences comprised genera *Oleispira* and *Thalassolituus* that were capable of degrading alkanes from diesel while *Zhongshania* and a novel bacterial lineage PGZG01 having capability to degrade alkanes from crude oil. *Amphritea*, *Marinobacter* and *Pseudomans* were found to exhibit degradative properties for aromatic hydrocarbons present in diesel. *Paraperlucidibaca* was also found to demonstrate degradative properties for alkanes (Murphy et al. 2021). In the Arctic areas, bacterial genera that belonged to *Alcanivorax*, *Colwellia*, *Cycloclasticus*, *Octadecabacter*, *Oleispira*, *Marinobacter*,

Table 9.1 A few examples of species of microorganisms which demonstrate biodegradative properties for various components of crude oil

Species of microorganisms	Site of isolation	Result	References
Bacterial Species			
Paraperlucidibaca *Marinobacter* *Pseudomonas_D* *Amphritea*	Marine sediments collected from the coast of Labrador in Canada	Degradation of Alkanes	Murphy et al. (2021)
Pseudomonas *Stenotrophomonas* *Paenibacillus* *Alcaligenes* *Sporosarcina* *Lysinibacillus*	Odisha, Eastern costal region of India	Forms biofilm and brings about degradation of polycyclic aromatic hydrocarbon such as pyrene and phenanthrene	Mangwani et al. (2019)
Bacillus cereus	North coastal waters of Sungai panking and South coast of Bengakalis island, Indonesia	Produces biosurfactants and degradation of crude oil	Nursyirwani et al. (2019)
Pseudomonas aeruginosa	Khor Musa sediments, Persian Gulf, Iran	Produces rhamnolopids which can form stable emulsions with crude oil and kerosene	Shahaliyan et al. (2015)
Acinetobacter calcaoceticus, Acinetobacter oleivorans	Canadian North Atlantic	Produces bioemulsifiers when grown on petroleum hydrocarbon as sole carbon source	Cai et al. (2014)
Achromobacter denitrificans, *Bacillus cereus*, *Corynebacterium renale*, *Cyclotrophicus* sp., *Moraxella* sp., *Mycobacterium* sp., *Burkholderia cepacia*, *P. fluorescens*, *P. paucimobilis*, *P. putida*, *Brevundimonas vesicularis*, *Comamonas testosteroni*, *Rhodococcus* sp., *Streptomyces* sp., *Vibrio* sp.	–	Degradation of naphthalene by the process of mineralization	Dash et al. (2013)
P. aeruginosa	Cultured in Erlenmeyer flask in the presence of crude oil, that was collected from Chennai refineries, Chennai, India	Demonstrated about 93% of cell adhesion to crude oil, which serves as an indication of biosurfactant production	Thavasi et al. (2011)

(continued)

Table 9.1 (continued)

Species of microorganisms	Site of isolation	Result	References
Antarctobacter strain TG22	Antarctica	Extracellular water-soluble glycoprotein-type polymer, that were found to produce stable emulsions with vegetable oil	Gutiérrez et al. (2007)
Oleispira *Thalassolituus* *Zhongshania* Novel strain PGZG01	Marine sediments collected from the coast of Labrador in Canada	Degradation of alkanes	Yakimov et al. (2007)
Alcanivorax brokumensis	Cultured on hydrocarbon containing media	Produces low molecular weight anionic glycolipid biosurfactant when grown on hydrocarbons	Schneiker et al. (2006)
Fungal species			
Aureobasidium, *Candida*, *Rhodotorula* and *Sporobolomyces*	Port of Oran, Algeria	Degradation of recalcitrant hydrocarbon by secreting extracellular enzymes, that transform them into lower toxic intermediates	Steliga (2012), Maamar et al. (2020)
Aspergillus oryzae, *Mucor irregularis*	Isolated from contaminated crude-oil fields of Nigeria	Degradation of hydrocarbon	Asemoloye et al. (2020)
Aspergillus sp., *Penicillium* sp., *Acremonium* sp., *Fusarium* sp.	Mangrove sediments from Divar, Goa, offshore sediments from the Arabian Sea and tar balls from Betul beach, South Goa, India	Degradation of long-chain *n*-alkanes	Barnes et al. (2018)
Aspergillus scelrotiorum CBMAI 849	Brazilian Cnidarian samples (scleractinian coral *Mussismilia hipsida*)	Degradation of 99.7% of pyrene and 76.6% of benzo[*a*]pyrene	Passarini et al. (2011)

(continued)

Table 9.1 (continued)

Species of microorganisms	Site of isolation	Result	References
Mussimilia racemosus	Brazilian Cnidarian samples (zooanthid *Palythoa variabilis*)	Degradation of more than 50% of benzo[*a*]pyrene	Passarini et al. (2011)
Archaeal species			
Haloarcula, *Halobacterium*, *Halococcus*, *Haloferax*	–	Degradation of alkanes	Bejarano and Michel (2010), Al-Mailem et al. (2010), Tapilatu et al. (2010), Fathepure (2014), Wegener et al. (2022)
Archaeglobi	Marine sediments	Produces fumarate-adding enzyme (FAE) that encodes anaerobic hydrocarbon degradation via fumarate addition	Zhang et al. (2021)
Ferroglobus placidusbis	Hydrothermal vent sediment	Carries out benzene degradation at 85 °C coupled to Fe(III) reduction	Cruz et al. (2017)

Pelagibacter, *Sulfitobacter* and *Thallassolittus* were associated with degradation of oil compounds (Yakimov et al. 2007; Kube et al. 2013; Krolicka et al. 2019; Peeb et al. 2022). For the first time, a report on intracellular signal transduction pathway associated with PAH degradation in *Cycloclasticus*, a ubiquitous PAH degrading bacterium in marine environments was investigated. The key genes involved in intracellular PAH sensing, signal transduction and regulation of degradation pathways were pyrene degrading dioxygenase genes *dpr-1* and *dpr-2* (DPR-1 and DPR-2 proteins, respectively) which encode for regulatory proteins in the CRP/FNR family. Both proteins were upregulated in the presence of naphthalene, phenanthrene or pyrene (Wang and Shao 2021).

Apart from bacteria, over 100 fungal genera are found to carry out biodegradation of hydrocarbons (Prince 2005; Maamar et al. 2020). *Aureobasidium*, *Candida*, *Rhodotorula* and *Sporobolomyces* are some of the most common marine-derived fungi capable of degrading petroleum oil. These fungi aid in bringing about the degradation of recalcitrant hydrocarbon by secreting extracellular enzymes, that transform these compounds into lower toxic intermediates that can further be decomposed by bacteria (Steliga 2012; Maamar et al. 2020).The fungi belonging to the genera *Aspergillus* and *Cladosporium* are aliphatic hydrocarbon degraders, whereas *Fusarium*, *Mucor*, *Cunninghamella*, *Penicillium* and *Aspergillus* are representative genera that exhibit the ability to degrade aromatic hydrocarbons that are recalcitrant (Amend et al. 2019). Barnes et al. (2018) demonstrated the ability of marine fungi to degrade crude oil from Arabian Sea sediments, tar balls and mangrove sediments, in which six out of the ten isolates that degraded crude oil belonged to *Aspergillus* sp., one each to *Penicillium* and *Acremonium* and two to *Fusarium*. Ligninolytic fungi produce enzymes known as lignin-modifying enzymes (LMEs) which include lignin peroxidase (LP), manganese-dependent peroxidase (MnP), laccase etc. which can be used for degradation of PAHs. Most of the non-ligninolytic fungi cannot completely mineralize PAHs but uses cytochrome P450 monooxygenase to transform parent PAHs into less toxic PAHs (Vala et al. 2018).

All the three domains of life are now recognized to possess the ability to carry out alkane degradation (Zobell 1946; Bertrand et al. 1990; Wang et al. 2010; Wegener et al. 2022). Although among them, the role of marine archaea in the process of oil degradation is not completely understood (Krzmarzick et al. 2018; Miettinen et al. 2019). Nonetheless, archaea such as *Bathyarchaeota* and *Halobacteriaceae* might have oil degradative properties (Jeanbille et al. 2016; Yan et al. 2018; Miettinen et al. 2019). Halophilic archaea such as *Haloarcula*, *Halobacterium*, *Halococcus* and *Haloferax* show degradative properties towards alkanes (Bertrand et al. 1990; Al-Mailem et al. 2010; Tapilatu et al. 2010; Fathepure 2014; Wegener et al. 2022). *Archaeglobi* present in the marine sediments, produced fumarate-adding enzyme (FAE) that encodes anaerobic hydrocarbon degradation via fumarate addition. Besides this, genomes of *Lokiarchaeota*, *Heimdallarchaeota*, *Thermoplasmata* and *Thorarchaeota* also revealed the presence of FAE, implying that these archaeal species may also play a role in oil degradation (Zhang et al. 2021). *Ferroglobus placidusbis*, a hyperthermophilic archaea isolated from a hydrothermal vent sediment, is capable of benzene degradation at 85 °C coupled to Fe (III) reduction (Cruz et al. 2017).

9.7 Treatment of Oil Spills

Sources of crude oil entering the marine environment include sea-based, land-based and natural. The recovery and cleanup of the spilled oil is tough and depends on various factors such as temperature of the spilled site, the climatic conditions, the type of shoreline etc., (Garapati 2012). Oils can generally be cleaned up by three methods: Physical, Chemical and Natural methods.

1. Physical Methods

   These methods are used as they function as barriers to control and prevent the spreading of oil spill and slick (Hoang et al. 2018). The commonly used physical barriers are skimmers, booms, sorbents, sediment relocation and tilling, in situ burning, washing etc. (Garapati 2012). Although the methods involved in the physical treatment of oil spills are effective, they are expensive and complex in nature as they require technological devices, and it is necessary to treat the devices before usage (Hoang et al. 2018).

2. Chemical Methods

   It is another approach to clean up oil from spilled areas. Chemical methods bring about the cleanup of an oil spill by usage of various chemicals such as dispersants (Type I, Type II, Type III) (Garapati 2012) and solidifiers (Hoang et al. 2018). The use of chemical means was highly efficient and more cost effective than physical means. Although their biggest disadvantage was that recovery of the oil of high viscosity, emulsion and oil slick was not shown as the contact time between the dispersant and the oil slick is less due to other processes, such as evaporation, dispersion etc. However, the effect of dispersants on viscous oils on shorelines is better due to prolonged and better penetration of dispersants into the oil (Garapati 2012).
3. Natural Cleaning Methods

   These are the natural processes happening to the oil in the environment that include evaporation, photooxidation, dispersion, emulsification, dissolution, adsorption and biodegradation. Since no external treatment is done to hasten the process by any means, and it occurs naturally using indigenous organisms, cleaning by this method is very slow and therefore usually carried out in areas where the impact of oil spill will not be very drastic to the ecosystem, i.e., when the environment is not very vulnerable to the toxic effects of oil spill, either due to the distance or due to the concentration (Radermacher 2008).

9.7.1 Treatment of Oil Spills Using Microorganisms

Crude oil or hydrocarbons being hydrophobic in nature, their degradation remains a challenge. There are many bacteria which grow on the surface of oil droplet and stick to them by forming biofilm. *Pseudomonas*, *Stenotrophomonas*, *Paenibacillus*, *Alcaligenes*, *Sporosarcina* and *Lysinibacillus* were found to be potential biofilm forming polycyclic aromatic hydrocarbon (phenanthrene and pyrene) degrading bacteria from marine environments (Mangwani et al. 2019). *Pseudomonas aeruginosa* produces phenazine-1,6-dicarboxylic acid (PDC) which helps the bacterium to degrade crude oil components by producing about nine times thicker biofilm than otherwise (Dasgupta et al. 2015). Thus, biofilm formation is the first step towards degradation of crude oil. Biofilm facilitates greater degradation than the planktonic cells (Mangwani et al. 2016). Biofilm-mediated bioremediation is regulated by the biofilm architecture and quorum-sensing amongst the biofilm bacteria and the surrounding cells (Balan et al. 2021). The degradation process is actually carried out by production of biosurfactants. Bioemulsifiers and biosurfactants are surface active molecules that absorb to interfaces of air-water (foam) or oil-water (emulsion) and assist in the formation and stabilization of foams and emulsions (Salek and Euston 2019). The two are differentiated from each other based on their physico-chemical characteristics and structure. Emulsifiers are usually composed of biopolymers, such as proteins and polysaccharides, that are capable of reducing surface tension, to form emulsion droplets and foam bubbles, that form a steric stabilizing absorbed layer at the interface (Salek and Euston 2019). Biosurfactants are amphiphilic molecules that contain a hydrophilic head and a hydrophobic tail (Salek and Euston

2019). They are low molecular weight molecules that are capable of absorbing at newly formed interfaces and bring about the stabilization of oil droplets and air bubbles when they are still small and more stable (Salek and Euston 2019). *Pseudomonas aeruginosa* is the most commonly studied marine bacteria for its biosurfactant producing abilities (Nikolova and Gutierrez 2021). *P. aeruginosa* are capable of growing on diverse hydrocarbon and non-hydrocarbon containing substrates and produce rhamnolipids which can form stable emulsions with crude oil and kerosene (Shahaliyan et al. 2015). Another study of *P. aeruginosa* also revealed that this bacterial species demonstrated about 93% of cell adhesion (high affinity) to crude oil, which serves as an indication of biosurfactant production (Thavasi et al. 2011). Many species from the genus *Acinetobacter* are found to be hydrocarbon degraders which are capable of producing exopolymeric substances (EPS) (Hassanshahian et al. 2012; Nikolova and Gutierrez 2021). The common high molecular weight bioemulsifiers, Emulsan and Alasan are produced by *Acinetobacter* species *A. calcaoceticus* and *A. radioresistensis* (Nikolova and Gutierrez 2021). *Antarctobacter* sp. strain TG22, another marine bacterium was capable of producing an extracellular water-soluble glycoprotein-type polymer, that were found to produce stable emulsions with vegetable oil (Gutiérrez et al. 2007). *Alcanivorax brokumensis*, an obligate hydrocarbonoclasic bacteria when grown on hydrocarbons was found to produce a lower molecular weight anionic glycolipid biosurfactant (Schneiker et al. 2006). *Alcanivorax borkumensis* SK2, dominant in oil-polluted open ocean consists of three P450 systems that have hydrocarbon degradation capacity. Its genome accounts for unique combination of systems involving scavenging of nutrients, biofilm formation at oil-water interface, biosurfactant production and stress response that gives the bacteria competitive edge in oil-polluted environment (Schneiker et al. 2006). *Achromobacter denitrificans*, *Bacillus cereus*, *Corynebacterium renale*, *Cyclotrophicus* sp., *Moraxella* sp., *Mycobacterium* sp., *Burkholderia cepacia*, *Pseudomonas fluorescens*, *Pseudomonas paucimobilis*, *P. putida*, *Brevundimonas vesicularis*, *Comamonas testosteroni*, *Rhodococcus* sp., *Streptomyces* sp. and *Vibrio* sp. were capable of degrading naphthalene which is a type of PAHs by the process of mineralization (Dash et al. 2013).

Biodegradation by biofilm formation and biosurfactant production is an important route to treat an oil spill. Biodegradation is a biological method of removing oil spill residues, by which organic chemicals are broken down by a living organism. Bioremediation of oil spills is considered a natural method as microorganisms utilize hydrocarbons as energy source (Ławniczak et al. 2020). Fungi, yeast, bacteria and archaea are the chief microbiological bioremediators (Strong and Burgess 2008; Alvernia et al. 2021). The major factors impacting the efficiency of bioremediation are the composition of marine microorganisms in the water column, characteristics of the spilled oil, bioavailability, physico-chemical characteristics surrounding the site of the oil spill (Mathew and Abraham 2020). Aromatic hydrocarbons and hydrogen-rich alkanes are chemicals that contain highly reduced carbon backbones and hence serve as potential electron donors (Ławniczak et al. 2020). Even though the microorganisms that contain genes related to hydrocarbon degradation are ubiquitous, they exhibit a low abundance (Ławniczak et al. 2020). For this reason, bioremediation technology can be enhanced by two interrelated approaches namely, bioaugmentation and biostimulation (Nikolopoulou and Kalogerakis 2010).

Biostimulation is a process in which, the growth of indigenous hydrocarbon degraders is stimulated by the addition of growth-limiting nutrients such as nitrogen and phosphorous (Nikolopoulou and Kalogerakis 2010). At the site of an oil spill, the concentration of carbon source is much higher than that of nitrogen and phosphorous and hence nitrogen and phosphorous are limiting nutrients for the process of oil biodegradation (Knezevich et al. 2006; Nikolopoulou and Kalogerakis 2008). Xia et al. (2006) demonstrated that the addition of nitrogen source in the form of nitrate, ammonium and phosphorous sources in the form of phosphate in the N/P ratio of 10:1 and 20:1 stimulated the degradation of oil by a certain extent. These nutrients were shown to enhance the growth of petroleum-degrading bacteria and heterotrophic bacteria. Addition of ammonium showed more success rate than the addition of nitrate. Moreover, water-soluble inorganic nutrients undergo rapid dilution in sea water. Hence, using oleophilic fertilizers would overcome this problem, as they provide nutrients for the growth and metabolism of oil degrading bacteria (Gertler et al. 2015). One such study was conducted by using uric acid as a hydrophobic nitrogen source. The results demonstrated that, *Halomonas* spp. carried out the conversion of uric acid to ammonium, that stimulated the growth of marine microbial consortia which was dominated by *Alcanivorax* spp. and *Pseudomonas* spp. (Gertler et al. 2015).

Bioaugmentation is a process that enhances the rates of biodegradation by the addition of hydrocarbon degrading bacteria (Nikolopoulou and Kalogerakis 2010). Indigenous and non-indigenous petroleum degrading bacteria have the ability to produce intracellular enzymes which aids the bacterium in utilizing petroleum hydrocarbons as a food source (Sayed et al. 2021). Few microbes that were isolated from contaminated sites produced biosurfactants (Singh et al. 2020).

9.7.2 Current Status of Research Work

In the recent times, marine oil spills are one of the most significant environmental impacts (Singh et al. 2020). They cause severe damage to ecosystems and biodiversity while also causing a loss in ecosystem process (Vasconcelos et al. 2020). An increase in the occurrences of marine oil spill events during the past few decades has inspired scientists to create new improved tools and technologies to detect oil spills in the sea (Beyer et al. 2016; Li et al. 2016; Vasconcelos et al. 2020). Remote sensing tools such as oil spill detection and mapping (OSPM) is used to carry out monitoring, surveillance, risk assessment and management of the remotely detected data that is received (Vasconcelos et al. 2020). Application of nanotechnology in the treatment of oil spills is a very recent approach where magnetic nanomaterials have been developed to carry out treatment of oil spills (Singh et al. 2020). Nanoparticles behave as sorbents or emulsifiers, increasing the interfacial area between water-oil, leading to more surface area for adsorption of microorganisms and thus more biodegradation (Pete et al. 2021). Bioremediation is an extremely organized microbiological activity, that is applied to carry out the breakdown or conversion of toxic pollutants to lesser toxic compounds in order to obtain energy and produce biomass (Abatenh et al. 2017). Generally, hydrocarbons are considered as toxic substances that impact the environment negatively (Chen et al. 2015). The discovery of hydrocarbon degraders and their ability to utilize hydrocarbon in the form of a substrate is regarded as an extraordinary finding (Ławniczak et al. 2020).

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Heavy Metal Pollution in Water: Cause and Remediation Strategies

10

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Abstract

Contamination by heavy metals and metalloids in water resources, soil, and air is one of the most severe problems that compromise food and water safety and public health globally and locally. Water naturally contains heavy metals; however, its increase, although sometimes also determined by natural enrichment when passing through aquifers containing rocks with a high concentration of this material, is mostly linked to human activity, such as mining and industry, which generates waste such as lead, mercury, cadmium, arsenic, and chromium, which reach rivers and contaminate groundwater. The danger of heavy metals is greater since they are not chemically or biologically degradable. Once emitted, they can remain in the environment for hundreds of years. In addition, its concentration in living beings increases as they are ingested by others, so the ingestion of contaminated plants or animals can cause symptoms of poisoning. Today we know a great variety of methods and techniques that can be used for the removal of heavy metals from water, each of which shows advantages and disadvantages that must be analyzed. The search for new materials and alternatives for the disinfection of water contaminated with heavy metals, as well as the optimization of those that we know today, is a permanent task for the scientific community.

Keywords

Heavy metals · Water · Contamination · Remediation

10.1 Water Pollution

Water is essential in supporting human life and preserving biological diversity on earth. Water quality is vital as water sources are used for various purposes: domestic use, industrial activities, agricultural irrigation, and other economic activities. However, the problem of water contamination by heavy metals has greatly affected water quality due to rapid urbanization, population increase, and deficient water treatment systems.

Water pollution and its increasing scarcity mean that more and more people in different parts of the world have poor access to water, directly affecting their health and way of life. Therefore, it is essential to understand the causes and consequences of water pollution (Van der Perk 2014).

Humans and their intervention in nature are the main ones responsible for water pollution. Only 3% of the planet's water is freshwater (water that can be drunk) and, although there are drinking water-purification mechanisms, such as desalination, which help water consumption, the first and most urgent thing to do is to avoid its contamination (Chaudhry and Malik 2017).

The World Health Organization (WHO) defines contaminated water as "water whose composition has been modified so that it does not meet the conditions for the use that will allow it to reach its natural state." Oceans, rivers, canals, lakes, and reservoirs are all at the mercy of pollution (Moss 2008).

No form of life can survive without clean water, from humans to animals, plants, and organisms. In short, without clean water, there is no life. But how does water become polluted? The leading causes of water pollution are as follows:

- The dumping of industrial waste and garbage in rivers, canals, and seas. Above all, from companies that dump large quantities of polluting products derived from their industrial processes. Hydrocarbons, wastewater, detergents, plastics, and other solid waste end up in rivers and seas, where, in addition to their environmental impact, many of them end up being ingested by animals or small marine organisms (Dwivedi 2017).
- Rising temperatures. Global warming also causes the alteration of water by reducing oxygen in its composition (Chaudhry and Malik 2017).
- Agrochemicals. Fertilizers and pesticides, generally used by food companies, are absorbed by the soil, filtered by subway channels, and affect the water and the surrounding plants and can also reach drinking water reservoirs (Khanna and Gupta 2018).

Water pollution has devastating effects on protecting the environment and the planet's health. Some of the most important consequences of the different types of water pollution are the destruction of biodiversity, contamination of the food chain involving toxic transmission to food, and shortages of drinking water (Inyinbor Adejumoke et al. 2018).

According to UN data, two million tons of sewage flow daily into the world's waters. The most important source of pollution is the lack of adequate management and treatment of human, industrial, and agricultural waste. Freshwater animals are becoming extinct five times faster than land animals (Azevedo-Santos et al. 2021; Schwarzenbach et al. 2010).

If the mechanism of constant pollution in which we live is not stopped or changed, by 2050, there could be more plastics in the ocean than fish. Recycling, minimizing waste generation, consuming less, and caring for and valuing water consumption seem to be the possible solutions to a problem for which we are all responsible.

10.2 Heavy Metals

Contamination by heavy metals and metalloids in water resources, soil, and the air is one of the most severe problems that compromise food and water safety and public health globally and locally. Heavy metal is usually defined as a chemical element with metallic properties and high density, although some consider that it is necessary to refer to the atomic number or weight or to some of its chemical or toxicity properties to differentiate it from other metals (Malik et al. 2019).

Some heavy materials such as iron, cobalt, copper, manganese, molybdenum, and zinc are beneficial and necessary for species such as humans in small quantities. Metals such as mercury, lead, or chromium can become harmful in large concentrations or after transformations, as they increase in toxicity and bioaccumulation potential since they accumulate in the body and are not eliminated by either feces, urine, or sweat. For example, mercury is more toxic in the form of methylmercury or dimethylmercury, and Cr(VI) is highly dangerous; instead Cr(III) is an essential nutrient for humans (Yang et al. 2020).

Water naturally contains heavy metals; its increase may be due to natural enrichment when passing through aquifers containing rocks with a high concentration of this material or linked to human activity, such as mining and industry, which generates waste such as lead, mercury, cadmium, arsenic, and chromium, which reach rivers and contaminate groundwater (Bolisetty et al. 2019).

Industrial and mining activity releases toxic metals into the environment, which are very harmful to human health and almost all life forms. In addition, metals from anthropogenic emission sources, including the combustion of leaded gasoline, are found in the atmosphere as suspended material that we breathe (Rai et al. 2019).

For example, mercury, zinc, lead, copper, cadmium, chromium, and nickel are used in paint in the textile and printing industry and for electroplating metals, as well as for processing paper in the paper industry and as an additive in the fur industry, and arsenic is also used as an additive in the plastics industry (Briffa et al. 2020). Table 10.1 describes the main sources of heavy metals and their effect on human health.

Table 10.1 Origin of pollution and health effects of some heavy metals

			Maximum concentration level in the water ($\mu g\ L^{-1}$)		
Heavy metal	Source of pollution	Health effects	WHO	FAO	USA
Arsenic (As)	Mining of ores, arsenical pesticides, fossil fuels burning	Hyperkeratosis, cancer, brain damage, dizziness, and fatigue	10	100	10
Cadmium (Cd)	Fertilizers, electroplating, battery manufacturing metal-plating, rayon industries, mining	Lung and kidney damage, cancer, cardiovascular diseases	3	10	5
Copper (Cu)	Metal-plating, rayon industries, mining	Normocytic anemia, bone frailness, gastrointestinal disturbances	2000	200	1300
Chromium (Cr)	Leather industries, dye production, electroplating	Skin irritation, dermatitis, kidney and liver damage, hair loss	50	100	100
Lead (Pb)	Textile industries, automotive sector, petroleum refining	Nervous system damage, brain damage, fatigue	10	5000	15
Mercury (Hg)	Pesticides, batteries, paper industry	Arthritis, circulatory and nervous system damage, kidney damage	6	–	2
Nickell (Ni)	Alloy industries, pigment manufacturing sector, tannery industry wastewater	Asthma, dermatitis, cancer, and respiratory failure	70	200	–

Heavy metals are more dangerous since they are not chemically or biologically degradable. Once emitted, they can remain in the environment for hundreds of years. In addition, its concentration in living beings increases as others ingest them, so ingesting contaminated plants or animals can cause poisoning symptoms.

10.3 Bibliometric Analysis

Bibliometric indicators are instruments for measuring scientific productions and make it possible to analyze the impact caused by scientific work. They are statistical data deduced from scientific publications. Their use is based on the important role of publications in disseminating new knowledge, a role assumed at all levels of the scientific process. With these indicators, it is possible to determine the growth of any scientific area by considering the number of published works, the collaboration of authors, research centers, the impact of communications, countries, institutions, the production of scientists, considering the number of citations received, among others.

The topic of water pollution by heavy metals has been the subject of research for many decades. Over time, the knowledge generated on the theme has evolved. Only in the last decade, a large number of works have been generated that contribute to its development. To carry out an analysis of the subject in this decade, a search was done on the Web of Science (WoS) following the criterion that the sequence of words “Heavy metal pollution in water” appeared in the title or the abstract of the scientific publications.

The first result obtained reveals that 5388 scientific research papers have been published in WoS meeting the search requirements, which are distributed by years, as seen in Fig. 10.1. Since 2014 and up to 2021, the number of publications has steadily increased, which indicates the interest of the international scientific community in this topic. So far in 2022, the number of publications is also high, and this shows that it is still novel and that there are still niches of opportunity for the generation of new knowledge.

The analysis of the documents requires a statistical analysis of their origin, i.e., the key journals in the field. These journals address the main trends in research on the subject under analysis. Table 10.2 shows the journals with the highest number of articles published in this field, representing 22.4% of all the articles considered. The top three journals are *Environmental Science and Pollution Research* (285 articles, 5.3%), *Science of The Total Environment* (166 articles, 3.1%), and *Environmental Monitoring and Assessment* (138 articles, 2.6%). In terms of impact factors, most of the selected journals have an impact factor above 3.0, indicating their dominant academic influence.

Among the compiled information, the authors with the most publications are ordered from highest to lowest in Table 10.3. It can be seen that the author who has published the most papers on water pollution by heavy metals is Liu Y with 42, followed by Li Y and Zhang Y with 37 each, and Li J and Wang Y with 33 each.

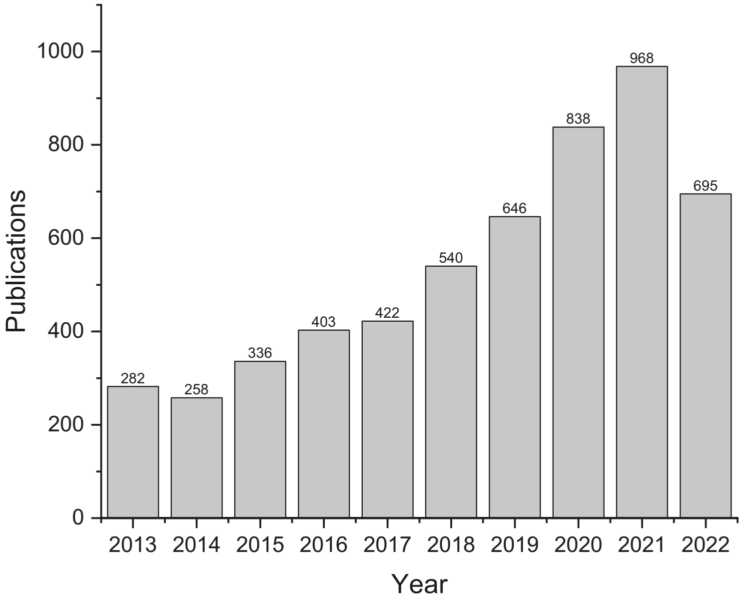

Fig. 10.1 Distribution of publications by year (2013–2022)

Table 10.2 Key journals for publications in the field of heavy metal water pollution

Order	Journals	Articles	%
1	Environmental Science and Pollution Research	285	5.3
2	Science of the Total Environment	166	3.1
3	Environmental Monitoring and Assessment	138	2.6
4	Chemosphere	120	2.2
5	Water	92	1.7
6	Environmental Earth Sciences	85	1.6
7	Desalination and Water Treatment	84	1.6
8	International Journal of Environmental Research and Public Health	84	1.6
9	Environmental Pollution	81	1.5
10	Fresenius Environmental Bulletin	73	1.3

The 5388 research papers developed in this decade are distributed according to the different research areas as shown in Fig. 10.2. Approximately 50% of the publications have been developed in the area of environmental sciences and ecology (ESE), followed in order by engineering, water resources (WR), and chemistry.

According to the WoS categories, publications are mainly grouped around environmental sciences, as shown in Fig. 10.3. This category accounts for about 50% of the works that have been developed. It is followed by water resources (WR), environmental engineering (EE), and chemical engineering (CE).

The Asia-Pacific and Middle East regions dominate the list of the ten countries that have published the most on this topic, as shown in Fig. 10.4a. The list (Fig. 10.4b)

Table 10.3 Order of authors with the highest number of publications

Order	Author	Publications
1	Liu Y.	42
2	Li Y.	37
3	Zhang Y.	37
4	Li J.	33
5	Wang Y.	33
6	Kumar A.	30
7	Wang J.	29
8	Kumar V.	26
9	Li H.	25
10	Wang L.	25

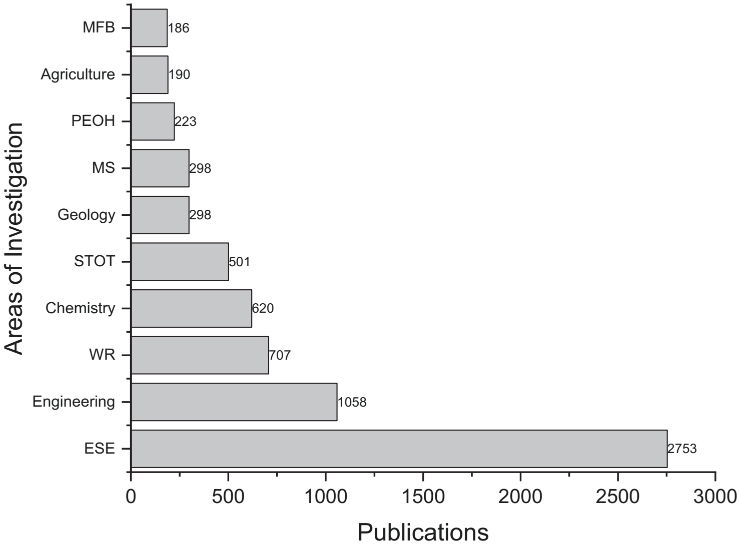

Fig. 10.2 Number of publications by area of knowledge. *ESE* environmental science and ecology, *WR* water resources, *STOT* science technology other topics, *MS* materials science, *PEOH* public environmental occupational health, *MFB* marine freshwater biology

is headed by the People's Republic of China, which accounts for about 44% of all publications in the last decade, followed by India with 17% and the United States of America with 7%.

Table 10.4 shows the ten articles with the highest citations in the last decade. "A review on the adsorption of heavy metals by clay minerals, with special focus on the past decade" by author Mohammad Kashif Uddin published in *Chemical Engineering Journal* in 2017, tops the list with 990 citations.

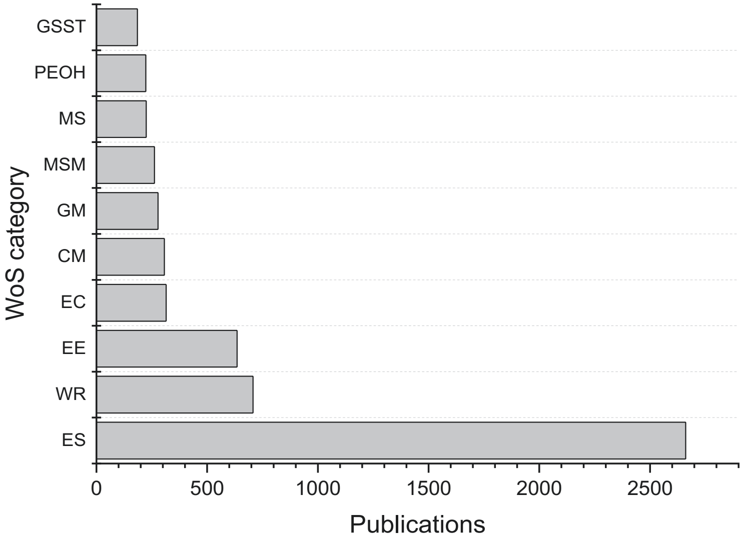

Fig. 10.3 Publications according to Web of Science categories

10.4 Remediation

10.4.1 Precipitation

Precipitation is the most common method for removing toxic heavy metals down to parts per million levels in water, because some metal salts are insoluble in water and precipitate when the appropriate anion is added (Charerntanyarak 1999). Although the process is cost-effective, its efficiency is affected by low pH values and the presence of other salts (ions). The process requires the addition of other chemicals, which eventually leads to the generation of a high-water content sludge, which is costly to dispose of.

Before selecting a chemical precipitation system for metal separation, several factors should be considered, including the demonstrated ability of the system to meet discharge limits, the capital expenditure required, the cost of operation and maintenance, the amount of pollutant and hydraulic quantity presently permitted, and those proposed for the future, the frequency, volume and characteristics of discharges, and the water conservation and recovery potential (BrbootI et al. 2011).

Chemical precipitation is most often carried out using sodium hydroxide, sulfate compounds (alum or ferric sulfate), or sulfides (sodium sulfide or iron sulfide) (Bhattacharyya et al. 1979). The addition of these compounds to metal-bearing wastewater forms metal hydroxides or metal sulfides, respectively, and the water solubility is limited. Precipitation is ineffective when the metal is in deficient concentrations since an excess of a precipitating agent is needed to form a precipitate. In many cases, the solid formed is not stable enough to separate from the solution. To overcome these difficulties, a co-precipitation treatment is often used, which

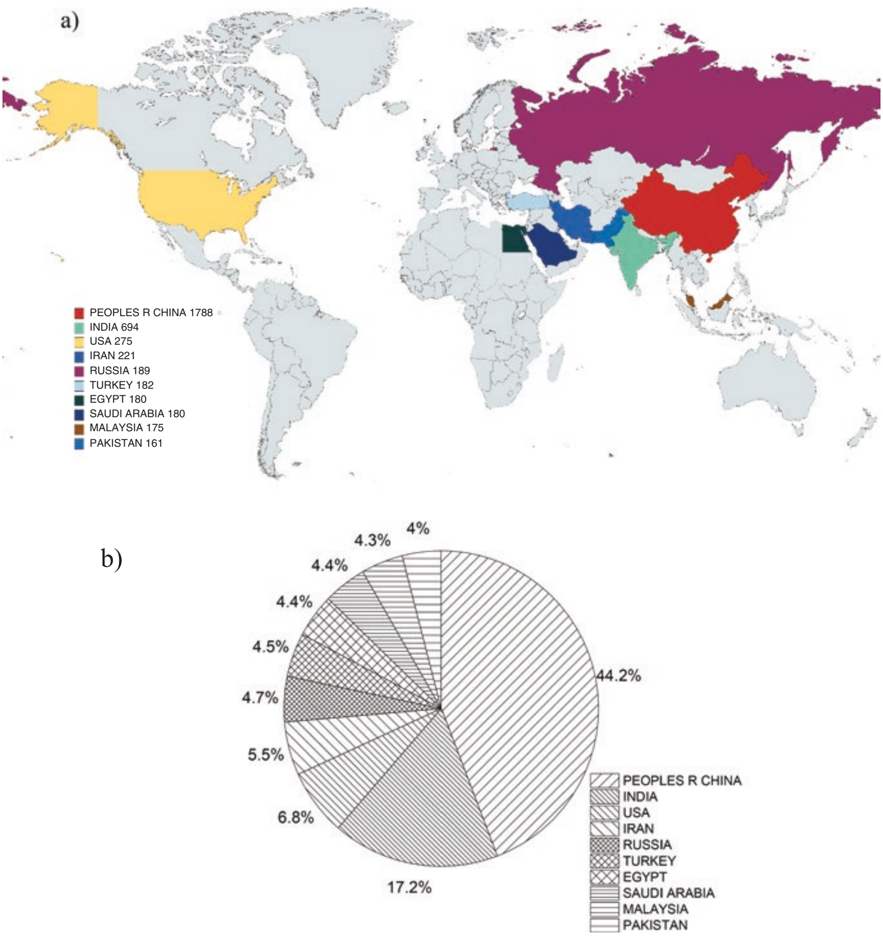

Fig. 10.4 Distribution by country of publications on water pollution by heavy metals: a) geographic location and b) percentage distribution

consists of adding iron hydroxide to the precipitating agent to form a precipitate. It consists of adding iron or aluminum hydroxide with the precipitating agent to act as a coagulant or to adsorb the metals that have not precipitated (Wang et al. 2005).

Hydroxide precipitation accompanied by coagulation-flocculation is widely used to reduce the concentration of metals such as lead, zinc, copper, and iron in wastewater. The precipitation process is highly dependent on the pH of the solution. When the pH ranges from 8.0 to 11.0, the removal can be greater than 99% for most heavy metals (Pang et al. 2009). Although this value is high, it is only achieved when the starting concentrations of the metals are in the order of parts per million and decreases drastically when working at lower concentration orders (Vardhan et al. 2019). Additionally, large amounts of solids are formed (Pohl 2020).

Table 10.4 Research papers with the highest number of citations

Title	Source title	Publication year	Total citations
A review on the adsorption of heavy metals by clay minerals, with special focus on the past decade	Chemical Engineering Journal	2017	990
Adsorption of heavy metals on conventional and nanostructured materials for wastewater treatment purposes: A review	Ecotoxicology and Environmental Safety	2018	764
A comparative review of biochar and hydrochar in terms of production, physico-chemical properties, and applications	Renewable and Sustainable Energy Reviews	2015	739
Heavy metal pollution in surface water and sediment: A preliminary assessment of an urban river in a developing country	Ecological Indicators	2015	727
Efficient techniques for the removal of toxic heavy metals from aquatic environment: A review	Journal of Environmental Chemical Engineering	2017	691
Adsorption of divalent metal ions from aqueous solutions using graphene oxide	Dalton Transactions	2013	592
Impacts of soil and water pollution on food safety and health risks in China	Environment International	2015	575
A review on modification methods to cellulose-based adsorbents to improve adsorption capacity	Water Research	2016	565
Sustainable technologies for water purification from heavy metals: review and analysis	Chemical Society Reviews	2019	552
Metal-organic frameworks for heavy metal removal from water	Coordination Chemistry Reviews	2018	512

The use of sulfide-containing or sulfide-derived compounds is characterized by lower solubility than when metal hydroxides are used, which allows higher percentages of metal ion removal to be achieved using shorter times. However, this process generates metal sulfides of very low solubility, is very sensitive to the added concentration of the precipitating agent, and can generate hydrogen sulfide as a byproduct, which has toxic effects on animals, humans, and the environment (Kumar et al. 2021). All these limitations make it necessary to search for novel precipitating agents such as potassium/sodium thiocarbonate and 2,4,6-trimercaptothiazine (Pohl 2020).

In addition, organic anions have been used to precipitate heavy metals in effluents from the mining and metallurgical industries. Such is the case of 1,3-benzenediamidoethanethiol dianion, which was developed to selectively and irreversibly bind soft heavy metals from an aqueous solution. The results for metals such as iron show that their concentration may be reduced from 194 to 0.009 ppm with this dianion at pH 4.5 (Matlock et al. 2002).

10.4.2 Electrochemical Methods

Electrochemical methods have been widely used for the removal of metals from pollutants in water. They present important advantages such as high separation efficiency; generally, no toxic substances are used, so they are safe and environmentally friendly. They are selective and controlled methods, and, under optimal electrochemical cell design, they are economical methods (Muddemann et al. 2019).

Figure 10.5 shows the main electrochemical methods for treating metals in water and their electrochemical basis. Most of them involve the electrolysis process, either directly or indirectly.

Electrodeposition is a direct electrolysis that involves the reduction of ions to the metallic state or an insoluble compound so that a solid deposition on the cathode occurs (Paunovic and Schlesinger 2006). In an electrodeposition process, many parameters must be controlled to achieve optimal results, for example, the material, shape, and position of the electrodes, voltage, current density, agitation, and the type of reactor (de Morais Nepel et al. 2020).

The electrodeposition process can be carried out at constant potential (potentiostatic) or constant current density (galvanostatic) (Tatiparti and Ebrahimi 2012; Vincent et al. 2020). Cathodes of the same electrodeposited metal, as well as inert stainless-steel electrodes have been used (Kuleyin and Uysal 2020). Advanced materials such as carbon nanotubes and graphene composites or graphene oxide electrodes have also been introduced (Stando et al. 2021). This method has been used for the removal of copper, cadmium, chromium, lead, uranium, zinc, antimony, nickel, and gold from wastewater (de Morais Nepel et al. 2020; Gu et al. 2020; Kuleyin and Uysal 2020; Vincent et al. 2020; Wang et al. 2020b, 2021; Wu et al. 2022).

Some treatment methods use electrolysis to separate the metal ions without their direct participation in the charge transfer reaction at the electrode. Electroflocculation is one of the most commonly used (Fig. 10.6). It consists of generating a metal hydroxide by taking advantage of the anodic oxidation of a sacrificial electrode, usually aluminum or iron, and the reduction reaction of water that generates OH− ions.

These hydroxides are colloidal, and in a first stage, destabilization and coagulation occurs, i.e., the aggregation of particles; these aggregates end up agglomerating

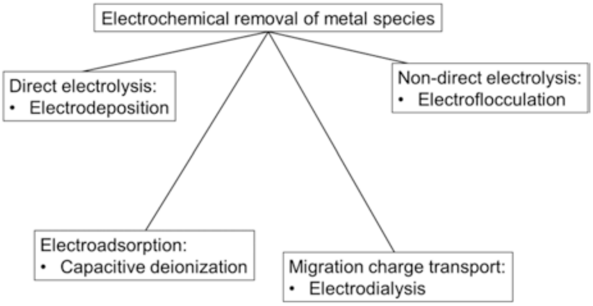

Fig. 10.5 Electrochemical methods for the removal of metals from water

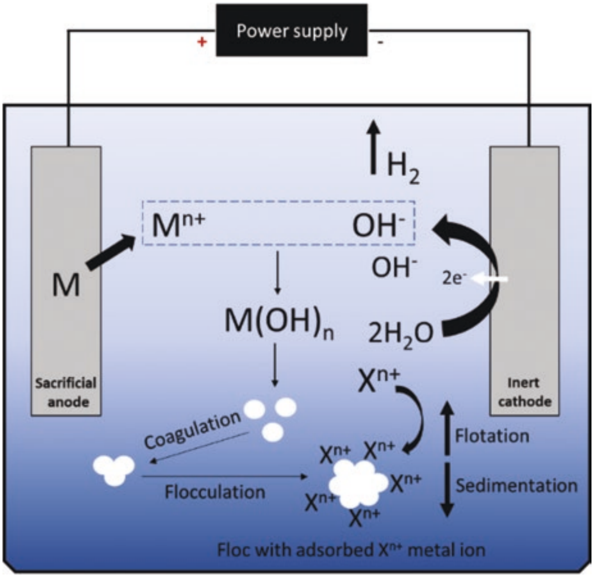

Fig. 10.6 Schematic of an electrochemical cell for removing X^{n+} metal cations by electroflocculation

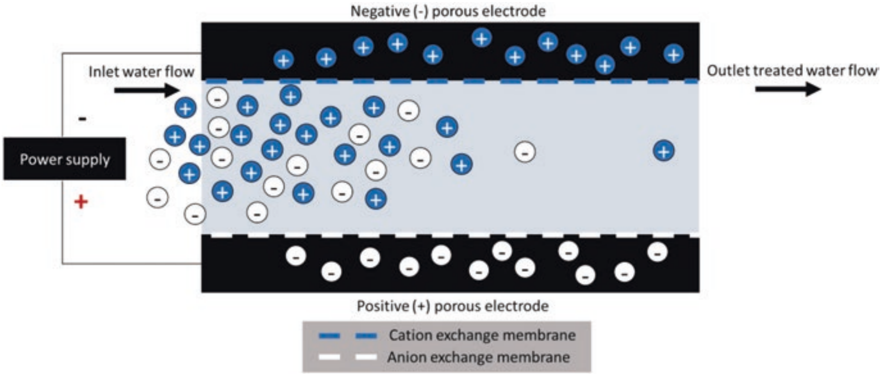

Fig. 10.7 Schematic of a capacitive deionization cell to remove cations and anions in solution

and forming flocs, which adsorb the contaminating metal ions on their surface. The flocs formed can be collected at the bottom of the tank when they sediment, or they can be floated on gas bubbles generated by the electrolysis of water or an auxiliary electrolyte. This separation of gas bubbles is known as electroflotation.

Capacitive deionization consists of the electroadsorption of ions by passing through a cell containing two porous electrodes polarized by a potential difference (Fig. 10.7). This method has been widely used for water desalination and removal of pollutant ions. The electric field between the electrodes causes the migration of anions towards the positive electrode and cations towards the negative electrode, establishing an electrical double layer in the pores of the material that stores the ions. This double layer behaves as a capacitor, allowing energy storage and recovery when the electrodes are depolarized (Shocron et al. 2022).

The main advantages of this technology are its high efficiency, the regeneration of the electrodes, and the recovery of part of the energy supplied (Cohen et al. 2013). The electrode material is of special importance since there must be a high porosity with sufficient size to adsorb the ions. Charcoal has been widely used; however, the development of new advanced materials with better textural and electrical properties can be an important improvement to the efficiency of this method because, for example, the more charge an electrode can store, the more effective the deionization process is (Zhao et al. 2020). In some cells, ion exchange membranes are used to increase the number of ions retained inside the electrodes, which significantly improves the deionization results (Wang and Lin 2019).

Different designs of capacitive deionization cells differ fundamentally in the use or not of ion exchange membranes, in the direction of the solution flow concerning the electric field between the electrodes, and in the type of electrodes. Some cells can combine capacitive and faradaic mechanisms (Tang et al. 2019). Flow electrode cells have recently appeared, where the anode and cathode are suspended particles (Zhang et al. 2021).

Capacitive deionization cells can operate at constant voltage and current (Wang and Lin 2018). The regeneration of the active sites of the electrodes can be done at an open circuit or by reverse polarization of the electrodes. The latter regenerates more active sites, increasing the capacity for a new deionization cycle. In the treatment of metal contaminants, capacitive deionization has been used primarily for the removal of arsenic, cadmium, chromium, copper, iron, lead, uranium, vanadium, zinc, iron, calcium, magnesium, and sodium (Chen et al. 2020; Kalfa et al. 2020).

Finally, electrodialysis consists of establishing a potential difference between two electrodes between which there are anion and cation exchange membranes (Fig. 10.8). The electric field causes the migration of the charged species towards the oppositely charged electrode and the selective membranes prevent the diffusion of the counterions. Electrodialysis technologies use cells with several anion and cation exchange membranes placed alternately, significantly improving separation (Ladole et al. 2021).

Water oxidation and reduction reactions usually occur at the cathode and anode, and when the concentration of chlorides is sufficient, their oxidation also occurs. These electrolysis reactions generate ions that maintain electrical neutrality. Electrolytes are commonly introduced into the anode and cathode half-cells (Hutten 2016).

10.4.3 Membrane-Based Methods

Heavy metals removal from water by membrane technology has been widely employed in practical applications and research since ultrafiltration (Bhattacharyya et al. 1978) and reverse osmosis (Sato et al. 1977) membranes were first demonstrated in the 1970s for the removal of Cu(II), Ni(II), and Zn(II). The separation of

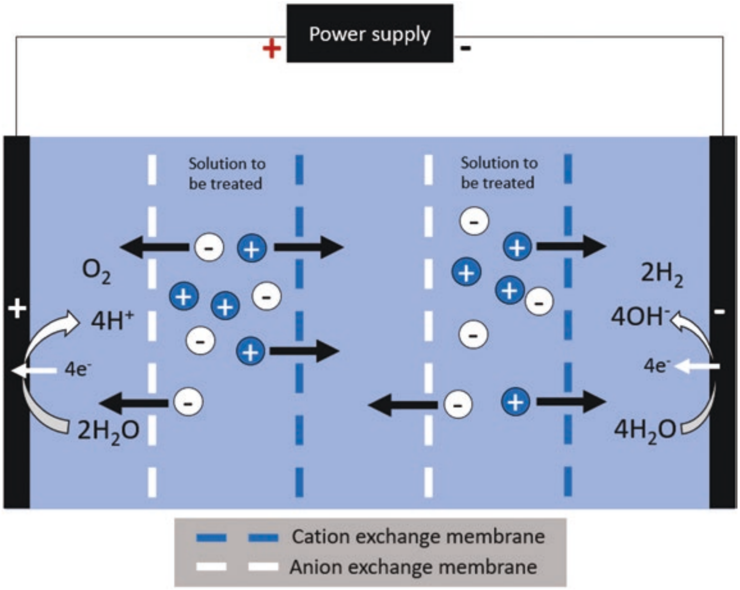

Fig. 10.8 Schematic of the electrodialysis process for removing cations and anions in solution

metal ions by membrane technologies is primarily based on size exclusion, Donna mechanism (charge repulsion), and adsorption capabilities of the membranes themselves. A membrane acts as a barrier, preventing certain compounds from passing through (Abdullah et al. 2019).

Usually, membrane performance is measured in terms of selectivity and flux rate (flow per unit area per unit of time). Permeate is the purified stream and retentate is the rejected stream (Ruhal and Choudhury 2012). Membranes can be classified according to their pore size, nature, and working principle. Several types of membranes are widely used in research, including microfiltration and ultrafiltration membranes (MF and UF), nanofiltration membranes (NF), reverse osmosis membranes (RO), liquid and supported liquid membranes (LM and SLM), as well as electrodialysis (ED).

10.4.3.1 Micro and Ultrafiltration Membranes

Due to their large pore sizes (0.1–10 μm), microfiltration is non-viable for heavy metal ions removal in water. In the case of ultrafiltration (0.01–0.1 μm), pores are still too large to be effective in rejecting metal ions. However, extensive work and research have been conducted on UF processes to make it a viable option in removing heavy metals.

There are two main ways to make UF processes workable: first, by manipulating the water matrix where the metal pollutants are present, increasing their size with complexation or micelle agents and thus being size excluded by the UF membrane (Huang and Feng 2019; Tortora et al. 2016; Xiang et al. 2022). Second, by manipulating the UF membrane's properties themselves, cladding functional groups or adsorbents to improve their rejection performances. This second option is derived from fabricating a subclass of UF membranes, called Mixed Matrix Membranes (Algieri et al. 2021; Mungray et al. 2012).

When talking about Mixed Matrix Membranes based on embedding adsorptive species in the membrane, their performance is only based on the nature of these species and the operational conditions (Abdullah et al. 2019). Wang et al. (2013) prepared a membrane by electrospinning with a very high charge density and large surface area based on polyacrylonitrile (PAN)/polyethylene terephthalate functionalized with cellulose nanofibers capable of removing bacteria and viruses by size extrusion and simultaneously adsorbing up to 100 and 260 mg of Cr(VI) and Pb(II), respectively, with a permeation rate of 1300 L m^{-2} h^{-1} psi^{-1}. By the application of a two-nozzle approach, electrospun polyvinyl alcohol (PVA)/PAN nanofibers were applied by Liu et al. (2020) for the removal of Cr(VI) and Cd(II) ions.

PAN fibers were modified by surface grafting and hydrolysis by crosslinking methods boosting their separation performances. Chelating action plus electrostatic interactions with metal ions were removal pathways achieved by a ring-opening reaction of the epoxy group with iminodiacetic acid, forming the functional PDF-g-PGMA/IDAA membrane. It can reach 100% rejection in heavy metal ions following $Pb^{2+} > Cu^{2+} > Cd^{2+}$. Ceramic microfilters were functionalized by Kim et al. (2021) with Mg being able to remove a wide range of heavy metals, including Fe, Cu, Ni, Zn, Cr, Pb, and As through precipitation, separation, and sorption. Life spam of the filter was considerably improved due to the fouling reduction after its functionalization with Mg.

10.4.3.2 Nanofiltration Membranes

Nanofiltration membranes have a pore size of 1–10 nm (Gao et al. 2014). Their transmembrane pressure (4.0–10.0 bar) is, therefore, lower than reverse osmosis membranes but higher than ultrafiltration membranes. Nanofiltration has become a widely used commercial process due to its pore size and loose selective thin film structure, favorable for metal ions exclusion in water. Most commercial nanomembranes are fabricated of aliphatic amine monomers, such as piperazine, having a negatively charged surface because of carboxylic and sulfonic species on the polyamide surface (Abdullah et al. 2019).

By grafting 0.6wt% Ethylenediamine carbon nanotubes on a polyether sulfone, a composite nanomembrane positively charged was fabricated by Peydayesh et al. (2020) with excellent heavy metals rejection following the order Zn (96.7%) > Mg (95.01%) > Cd (92.4%) > Cu (91.9%) > Ca (91.3%) > Ni (90.7%) > Pb (90.5%), due to Donnan exclusion mechanism. Another positively charged aliphatic polyamide nanomembrane synthesized by direct polymerization of polyethyleneimine on a polyether sulfone support was developed by Li et al. (2022). It was able to effectively reject Mn (98.78%) > Zn (98.32%) > Ni (97.74%) > Cu (95.67%) > Cd (90.49%) at concentrations of 1000 ppm.

Also, grafting polyethyleneimine to a nanomembrane using 2-chloro-1-methyl iodopyridine as an active agent derived in a film able to separate toxic heavy metals

($CuCl_2$ 96%, $NiCl_2$ 95.8%, $CrCl_3$ 98.0%), as well as dyes and divalent/monovalent salts (Qi et al. 2019). By surface coating and crosslinking method with glutaraldehyde, Ye et al. (2019) were able to cast polyelectrolyte complex nanoparticles with ammonium groups to develop novel complex nanomembranes effectively charged, providing a strong repulsion to metal cations, reaching up to 95% of Mg, Cu, and Zn rejection.

Al-Rashdi et al. (2013) studied the effect of pH on the retention performance of a commercial nanomembrane (NF270). They found that rejection improves when the pH is below the isoelectric point, indicating that the Donnan exclusion mechanism played a key role. At a concentration of 1000 ppm and pH = 1.5 and 4 bar, rejections of 99%, 89%, and 74% for Cd, Mn, and Pb, respectively, were found.

10.4.3.3 Reverse Osmosis Membranes

Compared to NF membranes, RO membranes are denser and have a pore size ranging from 0.1 to 1.0 nm. In terms of efficiency, it is one of the best alternatives for contaminants removal as it mostly allows water to pass through (Chon et al. 2014; Dialynas and Diamadopoulos 2009; Harharah et al. 2022; Verma et al. 2021). Its working principle is based on osmosis but works in the direction of water moving from high concentrated to low concentrated phase by applying an external pressure (Abdullah et al. 2019) (Fig. 10.9). More than 98% of Cu, Cr, and Ni removal was achieved by a commercial low-pressure RO membrane (ULPROM/ES 20) at metal concentrations of 10 mg L^{-1} (Ozaki et al. 2002).

A combination between UF and RO was applied by Petrinic et al. (2015), to remove suspended contaminants such as metal elements and organic/inorganic compounds, reaching 91.3–99.8% of removal efficiencies. Similar studies highlight the necessity of combining RO with other membrane filtration processes, as the study reported by Ricci et al. (2015), where a system comprised of sequential MF, UF, and RO units was implemented to recover noble metals above 95%.

Ni(II) was completely removed by merging UF and RO units (Petrinic et al. 2015), the pH playing a key role in the removal efficiency. Mnif et al. (2017), achieved a Cr(VI) rejection of 99.9% at a pH = 8, whilst if the pH dropped to 3, the efficiencies decreased to 91% (Çimen 2015).

Considering the operating pressures (20–30 bar) and energy needed (≈2.5 kWh m^{-3}) for RO processes (Aumesquet-Carreto et al. 2022), a prevailing

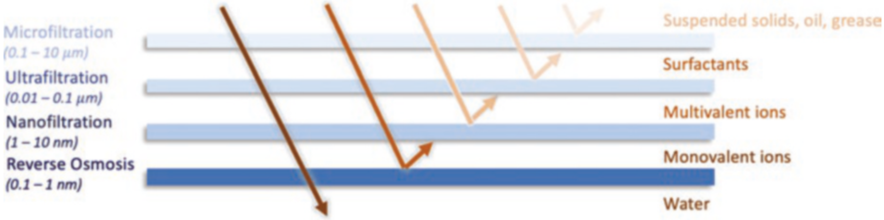

Fig. 10.9 Schematic representation of several filtration processes and rejected contaminants

need to combine RO with other filtration units arises to increase its feasibility and applications in heavy metals removal from water (Chung et al. 2014).

10.4.3.4 Supported Liquid Membranes

Liquid membranes are formed by a layer of immiscible liquid being the barrier to the feed solution contaminated with heavy metals (Abdullah et al. 2019). There are several types of liquid membranes described in the literature. The most widely used in heavy metal removal are polymer inclusion membranes (Zulkefeli et al. 2018) and supported liquid membranes (Yesil and Tugtas 2019). Emulsion liquid membranes (Ahmad et al. 2011) are found to be unstable for practical applications and bulk liquid membranes (Chang 2016) do not have enough surface area for the current application.

An emerging class of supported liquid membranes is supported ionic liquid membranes (Abdullah et al. 2019). Ionic liquids offer the advantages of versatility and easy tunability of their physicochemical properties through a proper selection of the anion-cation pairs that conform to them (Ramos et al. 2020b). A plethora of different anion-cation combinations can be done (around 10^8) (Ramos et al. 2020a), offering a vast research area to be studied in removing heavy metals from water bodies.

Common supports for ionic liquids are polypropylene, polyvinyl chloride, polyethylene terephthalate, among other polymeric materials. In contrast, the most used ionic liquids are imidazolium and phosphonium based cations such as trihexyltetradecyl phosphonium chloride (Regel-Rosocka et al. 2015) and [OMIM][BF4] (Abebe et al. 2017) that work as supported liquid membranes for effective remediation (>85%) of heavy metals as Zn, Pb, Cd, and As (Imdad and Dohare 2022).

A recent work developed by Zheng et al. (2022) demonstrates the viability of supported liquid membranes for heavy metals removal. They grafted the ionic liquid 1-vinyl-3-butyl imidazolium tetrafluoroborate on polyether sulfone by radiation and electrospinning processes, obtaining nanofibrous membranes able to adsorb up to 187.3 mg g^{-1} of Cd(II) with good recyclability over cycles, and also removing dyes and possessing antimicrobial properties.

10.4.3.5 Electrodialysis

Electrodialysis consists of several ion exchange membranes (anionic and cationic) stacked together under the action of an electric field, concentrating the electrolytes in a concentrated stream (Min et al. 2019) (Fig. 10.10). Cations can pass through the anionic membranes but are retained in the cationic layers, in a similar way as anions with the cationic membranes. This process can generate hydrogen gas and oxygen/chlorine in the anode (Abdullah et al. 2019).

Compared to MF, UF, NF, and RO, electrodialysis reduces the use of chemicals and causes less secondary environmental pollution (Babilas and Dydo 2018; Gurreri et al. 2020; Min et al. 2021). In ED, operational parameters play an important role in heavy metal separation. The most determinant variables are the applied current and voltage, temperature, pH, and membrane properties (Juve et al. 2022). Increasing temperatures lead to higher removal efficiency of the ions, enhancing their mobility

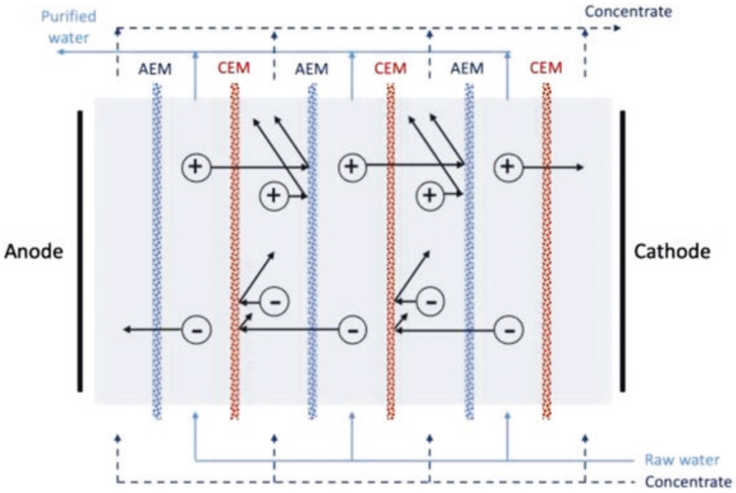

Fig. 10.10 Schematic of electrodialysis working principle for heavy metals removal in water. *AEM* anion exchange membrane, *CEM* cation exchange membrane

towards the concentrate (Benneker et al. 2018) but increasing energy costs and making the process less viable.

The surface charge of ED membranes and morphologies has an important impact on heavy metal separations. It can be enhanced by the presence of specific functional groups that alter the hydrophobic/hydrophilic properties of the membrane to increment selectivity (Irfan et al. 2019). Most ED membranes are based on a polymeric matrix with attached acid or quaternary ammonium groups (Juve et al. 2022; Ran et al. 2017).

The selection of appropriate membrane technology for removing heavy metals from water bodies requires consideration of several factors. Other pollutants present in water may affect the efficiency of the membrane. For instance, when adsorptive membranes reach their saturation point, they must be regenerated, a process that may not be feasible for practical use. For heavy metals removal to be viable, membrane technologies with energy implications, such as RO and ED, must be optimized. Additionally, it is important to carefully select the chemicals introduced into water bodies (complexation/micelle agents) and consider their impact on the environment. It is also necessary to consider the generation of byproducts of remediation as a determinant parameter in selecting a membrane technology.

Table 10.5 summarizes the advantages and disadvantages of each membrane technology covered in this section.

10.4.4 Phytoremediation and Microbiological Interactions

Phytoremediation is a green technology also called agroremediation, botanoremediation, or green remediation; it is a sustainable and green approach to remediate

Table 10.5 Summary of membrane processes for heavy metals removal

Membrane process	Advantages	Disadvantages	References
Ultrafiltration	Cost-effective and low transmembrane pressures. Effective for macro-metal ions	Generation of byproducts. High maintenance costs and fouling risks	Xiang et al. (2022)
Nanofiltration	Better metal ions rejection compared to UF and lower operational costs	Lower water permeation and high energy consumption compared to UF. Fouling control	Xiang et al. (2022)
Reverse osmosis	Performance in heavy metal rejection. Easy operation and no added chemicals needed	Energy requirements and water permeability. Membrane fouling and durability	Imdad and Dohare (2022)
Supported liquid membranes	Low cost and high efficiency. Good stability and selectivity. High water permeability.	Risk of leakage. Need of regeneration after saturation point. High material costs	Imdad and Dohare (2022)
Electrodialysis	Excellent metal separation and reusability.	High energy and operational costs. Membrane clogging due to macromolecules. Fouling	Imdad and Dohare (2022)

soil (Alonso-Bravo et al. 2018), water (Viramontes-Acosta et al. 2020), and even air (Ortiz Cáceres 2020). This technique successfully uses cosmopolitan plant species that can remove and store organic and inorganic contaminants in high concentrations in their roots and plant tissues (Ashraf et al. 2019).

Several technologies are available to remediate soils contaminated by heavy metals. However, physicochemicals are more expensive (e.g., excavation of contaminants, material, and chemical/physical treatment) or fail to achieve a long-term solution or aesthetics. For this reason, phytoremediation can provide a cost-effective, simple, feasible, durable, aesthetically pleasing, and publicly accepted solution for the remediation of contaminated sites.

Implementing phytoremediation is straightforward because it does not require expert personnel or expensive high-quality equipment. Phytoremediation is a decontamination process that is mediated by plants, and their biomass. It includes a wide variety of plants such as grasses, shrubs, and trees that, in symbiosis with microorganisms, favor the restoration of the environment (soil, water, and air), through the degradation, accumulation, and stabilization of pollutants. Phytoremediation is considered a green technology with the potential to reduce the generation of secondary waste and remove contaminants (organic pollutants, heavy metals, etc.) from the soil (Shah and Daverey 2020).

Phytoremediation plants must comply with the following characteristics (González-Gómez 2010; González-Chávez 2017; López et al. 2004):

- The plant must be tolerant to high concentrations of heavy metals and carry out its development in the presence of this.
- Being an accumulator of heavy metals, remediation must be carried out since this is the important purpose of phytoremediation.
- It must have an immediate increase and high productivity rate since this enables good pollutant removal rates and optimizes phytoremediation processes. The plant must maintain its reproductive capacity in the presence of disturbances through succession.
- Plants must be easy to harvest.
- They must have stress resistance since it is fundamental that the plant can resist stress situations generated by chemical, physical, biological, or climatic conditions.
- Being local species, representative of the natural society, it is advisable to use native plants to alter the local ecosystem as little as possible.
- Among the main factors affecting phytoremediation are biomass, pH, microorganism, speciation, and chelators in the soil (Marrero-Coto et al. 2012).

10.4.4.1 Phytoremediation Mechanisms

Plants can develop several mechanisms to remove contaminants (Fig. 10.11) from the rhizosphere to the aerial tissues, among which phytostabilization, which consists of stabilizing them in the rhizosphere; phytodegradation, which consists of changing the oxidation state of the elements to eliminate them at the root level; phytostimulation, which is assisted by microorganisms, phytodegradation; and phytovolatilization, which consists of removing the contaminants at the leaf level once

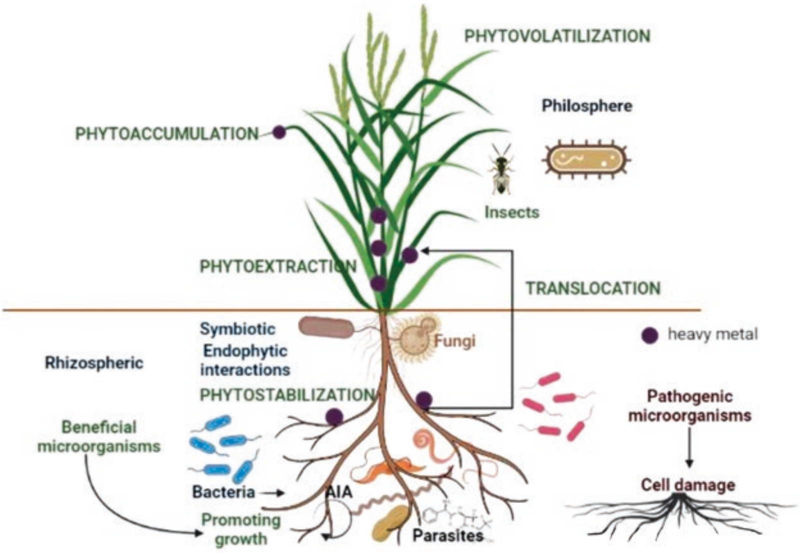

Fig. 10.11 Phytoremediation mechanisms and biological interactions. (Created in BioRender.com)

the plants have transformed them, are the most important mechanisms to be able to apply phytoremediation as a decontamination technology.

However, the most important mechanism to apply phytoremediation as a decontamination technology is phytoextraction, which consists of extracting contaminants from the rhizosphere and transporting them to aerial tissues. Among the factors that affect this mechanism are biomass, temperature, pH, light, ion exchange capacity, cation exchange capacity, salinity, the type of element if it is organic or inorganic, as well as microorganisms since these can change the pH due to the substances they secrete, provide nutrients and phytohormones (Zhang et al. 2018).

The mechanisms of soil and water phytoremediation can be divided into four subsets, as described in Fig. 10.11. These soil and water toxic metal remediation technology are: (1) phytoextraction, also called phytoaccumulation, is the process that takes place in plants found in soil contaminated by heavy metals; (2) phytovolatilization, which is the evaporation of certain metals from the aerial tissues of plants; (3) phytostabilization, which is the use of plants to be able to remove the bioavailability of toxic metals in contaminated soils; and (4) phytostimulation, the use of plant roots to remove toxic metals (Kushwaha et al. 2015).

The biological changes that occur mainly in nature are called natural depletion and are assisted by plant-microorganism interaction. A phytoremediation plant executes any of the above mechanisms in three stages: absorption or uptake, excretion, and detoxification of pollutants. The uptake of contaminants is done through root tissue and leaves through the stomata and cuticle of the epidermis. This uptake happens in the rhizome dermis of the adolescent roots, which can uptake compounds by osmosis defined by external factors such as temperature and soil pH.

Other relevant components that influence the uptake of contaminants are their molecular weight and hydrophobicity, which determine that these molecules cross plant cell membranes. After crossing the membrane, contaminants are distributed throughout the plant. Contaminants absorbed by plant roots will be excreted by the leaves (phytovolatilization). Once the concentration of contaminants is high, only small fractions (less than 5%) are removed without changing their chemical composition.

10.4.4.2 Interactions of Plants with Microorganisms

Phytoremediation plants can grow naturally in contaminated sites and tolerate high concentrations of heavy metals because they have developed mechanisms to do so or have achieved symbiosis with the microbiota of the environment in which they develop. Roots are the main tissue involved in the absorption of metals through association and interaction with soil microorganisms.

Among these associations are those established with bacteria and endophytic and/or mycorrhizal fungi (EMF), a symbiotic interaction between soil microorganisms and roots. These associations give plants stress resistance, improve plant biomass, their antioxidant system, and their potential to accumulate metals.

Therefore, the success of phytoremediation also depends on beneficial associations between microorganisms and plants (Vigliotta et al. 2016), because beneficial microorganisms promote the growth of roots and air tissues, but pathogenic

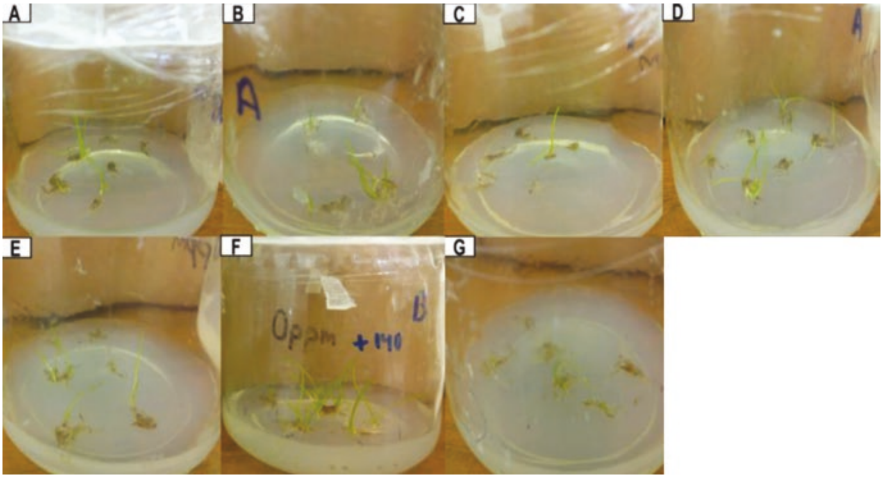

Fig. 10.12 Growth stimulation assay of *T. latifolia* in vitro in the presence of lead. (**a**) 0 ppm Pb; (**b**) 5 ppm Pb; (**c**) 10 ppm Pb; (**d**) 25 ppm Pb; (**e**) 50 ppm Pb; (**f**) 25 ppm Pb plus GRC140; (**g**) 50 ppm plus GRC140

microorganisms damage plant tissue (Fig. 10.12). Rajkumar et al. (2009), reported that inoculation of soils with *Psudomona aureginosa* significantly increased the bioavailability of Cr and Pb compared to uninoculated controls. In addition, they also observed that *P. aureginosa* significantly enhanced Cr and Pb accumulation in maize tissues.

Rajkumar et al. (2009), mentioned that in this case, the enhancement of heavy metal uptake may be due to the production of siderophores, particularly pyoverdine (dihydroquinoline type chromophore, with a peptide chain of 6–12 amino acids) and phytochelatins (cysteine-rich protein); siderophore production by bacteria (SPB) resistant to metals may increase the efficiency of phytoextraction directly, improving the accumulation of metals in plant tissues.

Siderophore production by rhizospheric bacteria solubilizes unavailable forms of heavy metal-containing minerals through complexation. Plants can take up metals from the siderophore-metal complex, possibly mediated by root processes, such as chelate degradation and metal release, direct uptake of the siderophore-metal complex, or by a ligand exchange reaction.

Recent studies with hyperaccumulator plants have revealed that inoculation of soils/seeds with metal-resistant endophytic bacteria enhances plant growth and accelerates the phytoremediation process naturally or artificially in metal-contaminated soils, improving nutrient acquisition, cell elongation, metal accumulation or stabilization, and metal stress relief in plants. Likewise, these microorganisms can passively activate or promote plant growth through various mechanisms such as nitrogen fixation, phosphate solubilization, siderophore production, phytohormones, and ACC deaminase (Ma et al. 2011).

Rolón-Cárdenas et al. (2021) obtained four bacterial isolates from the rhizosphere of *Typha latifolia*, showing that they have biochemical activity for the promotion of seedling growth exposed to various concentrations of cadmium. The bacterial isolates characterized in this study are of the genus *Pseudomona*. In addition to exhibiting high tolerance to Cd, these bacteria probably present high tolerance to other heavy metals because the gene that confers tolerance to Cd also confers tolerance to other heavy metals. On the other hand, Ma et al. (2016), found that *Pseudomonas libanensis* TR1 and *Pseudomonas reactans* Ph3R3 are resistant to multiple metals such as Cd, Cr, Cu, Ni, Pb, and Zn.

In recent studies, the effect of *Pseudomonas* sp. on the promotion of plant growth of roots of *T. latifolia* plants tolerant to high concentrations of Cd (500–750 ppm) and Pb (5–50 ppm) was evaluated (Fig. 10.12), demonstrating that bacterial isolates (*Pseudomonas rhodesiae*) can increase up to 50% Cd removal compared to plants without bacterial inoculation (Moctezuma Granados 2017), as well as that *P. rhodesiae* increases Cd translocation to shoots (Rolón-Cárdenas et al. 2021). Recently, the effect of endophytic and/or mycorrhizal fungi (EMF) associated with *T. latifolia*, the interactions of this plant species with EMF, and the mechanisms of EMF in the promotion of plant growth and phytoextraction of heavy metals are being evaluated.

10.4.5 Treatment Wetlands

Treatment wetlands (TW), also called artificial or constructed wetlands, are nature-based systems that can be used for the treatment of wastewater of very varied composition and are ideal for operation in small communities or socio-economic objectives and even for isolated users. Through physical, chemical, and biological processes, as well as their multiple combinations, they effectively remove many types of contaminants.

Microorganisms present in TW play an important role in the removal processes of different contaminants, including metals (Wang et al. 2022a). Although domestic and municipal waters do not contain significant amounts of metals and metalloids such as boron, selenium, and arsenic (Březinová and Vymazal 2015), industrial waters such as the metal-mechanical, mining, and textile industries, among others, have these pollutants in concentrations that represent a threat to human health and ecosystems, also considering that in many places these wastewaters are mixed in municipal drainage systems without any prior treatment.

Since conventional treatment systems and some advanced treatments generally imply higher costs in their installation, operation, and maintenance (Sylwan and Thorin 2021), it is necessary, then, to evaluate the effectiveness of TW and the way to improve it since it is a green technology with collateral benefits for human communities and ecosystems, in the removal of toxic metals and metalloids, an aspect that has been recently reviewed (Nisa et al. 2022; Singh et al. 2022).

Toxic metals and metalloids are removed in TW through a combination of biotic and abiotic mechanisms, including filtration processes, sedimentation, precipitation and coprecipitation, sorption and ion exchange, biosorption, redox processes, including those mediated by microorganisms present in the system and phytoremediation processes carried out by the present macrophytes such as rhizofiltration, phytostabilization, phytoaccumulation, and phytovolatilization (Yu et al. 2021).

Figure 10.13 schematically presents these processes. In general, the predominant role in the removal of metals is played by the processes that occur in the substrate and sediments, with the role of macrophytes being of less importance (Ventura et al. 2021). The greatest accumulation of metals is observed in sediments and by being confined in them, the possible toxic effect on aquatic biota is reduced, as has been demonstrated in the evaluation of the removal of Cu and other divalent metal ions in a wetland system for more than 20 years (Knox et al. 2021).

Although some of these elements are essential for the metabolic processes of macrophytes and microorganisms in treatment wetlands, their presence above certain levels can affect the biota of these systems and, consequently their removal. Likewise, the different species of macrophytes have different metal removal capacities, depending on the biomass ratio in their roots, stems, and leaves (Schück and Greger 2020). This explains the different results reported in the literature when evaluating the removal percentages (Batool and Saleh 2020).

Due to the above, more and more attention is currently paid to the processes of improved removal of metals and metalloids in treatment wetlands, which includes, among others, the use of substrates with properties that intensify physical processes such as ionic exchange or precipitation, a more careful selection of macrophytes,

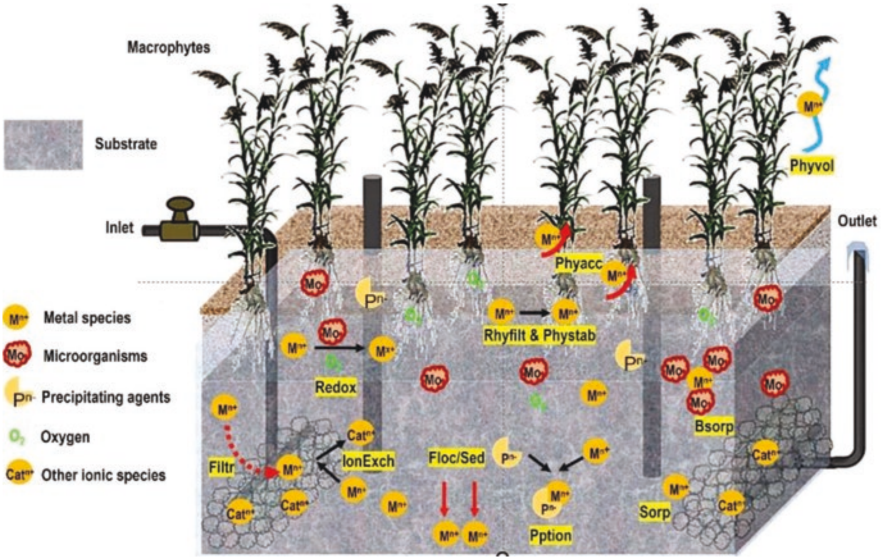

Fig. 10.13 Abiotic and biotic removal processes in TW. *Filtr* filtration, *IonExch* ion exchange, *Floc/Sed* flocculation/sedimentation, *Pption* precipitation, *Sorp* sorption, Bsorp biosorption, *Redox* oxidation/reduction processes, *Rhyfilt & Phystab* rhyzofiltration and phytostabilization, *Phyacc* phytoaccumulation, *Phyvol* phytovolatilization

including polycultures, bioaugmentation with suitable microorganisms, the use of hybrid systems, the use of external additives, and treatment intensification mechanisms such as artificial aeration and recirculation, among others (Yu et al. 2022).

Some examples of improved metal removal in treatment wetlands are illustrative of this. Regarding the selection of the substrate, the use of biochar and organic waste has shown to be effective, improving the removal of metals in mining waste, improving the binding capacity of the substrate, and intensifying the biotic removal mechanisms (Wang et al. 2023). In systems with intermittent aeration, filled with biochar, the volatilization and assimilation processes of Hg(II) by plants are increased, which contribute to better removal of this toxic metal (Chang et al. 2022).

Likewise, using zeolites combined with organic fillers is effective for removing Cu in the treatment of leachate from sanitary landfills (Wdowczyk et al. 2022). Inoculation with arbuscular mycorrhizal fungi and the use of aeration in vertical wetlands improve the removal of Pb, Zn, Cu, and Cd (Xu et al. 2022). The coupling of TW with microbial fuel cells, together with an adequate selection of the substrate and macrophyte, has been shown to improve the removal of Zn and Ni in sludge (Wang et al. 2022b).

10.4.6 Adsorption

For solutions with a high concentration of heavy metals, some of the technologies mentioned above can be used; however, when the concentrations are low (less than 100 mg L^{-1}), more economical and efficient methods (such as adsorption) are required (Volesky 2003).

Adsorption is a separation process by which certain components of a fluid phase (liquid or gas) are transferred to a solid substrate, becoming physically or chemically bound on the surface of the adsorbent (Ruthven 1984). Adsorption is an effective method of removal at low levels of metal ions. However, the economic viability of this process depends on an effective means of regenerating the solid once its adsorption capacity is exhausted (Suzuki and Suzuki 1990).

The adsorbent is characterized by its high porosity, with extremely small pore sizes resulting in its internal surface area much larger than the external surface area. Differences in molecular weight or polarity cause some molecules to be retained more strongly than others, which makes the adsorption process selective (Yang 2003).

Physical adsorption is caused mainly by Van der Waals and electrostatic forces, which occur between the molecules of the adsorbate and the atoms that make up the surface of the adsorbent. These adsorbents are mainly characterized by surface properties such as surface area and polarity. The ion is adsorbed by the solid depending on the relative charge between the two. This process can be slow or fast, depending on the composition of the adsorbent, the adsorbate, and the temperature (Bruch et al. 2007).

Chemical adsorption is due to forces of a chemical nature and is a process that depends on the temperature, the chemical nature of the solid, and the concentration of the species to be adsorbed (Webb 2003). The two types of adsorption do not necessarily occur independently; thus, in natural systems, it is common for both to occur on the same solid surface (Wang et al. 2015b).

Common materials that have been used for contaminant adsorption include activated carbon, silica gel, activated alumina, and graphene. These commercially available adsorbent materials are highly efficient for removing heavy metals thanks to their high specific area and abundant functional groups that are present on the surface and that allow the adsorption process to exist (Table 10.6).

The most commonly used adsorbents with high adsorption capacity are chitosan, zeolite, lignin, and activated carbon (AC) (Yang 2003); however, some of these adsorbents, such as activated carbon, have a high cost in the adsorption process, which limits their use in wastewater treatment. AC, because of its non-polar surface and low cost, is the adsorbent of choice for removing a wide range of pollutants; however, as it is not selective, it can also adsorb harmless components that are in higher proportions than the more dangerous pollutants such as heavy metals (Mariana et al. 2021), and for this reason, various solid materials are recently being developed that improve, in certain applications, the properties of AC (Abdulrasheed et al. 2018).

Table 10.6 Most common adsorbents applied in adsorption remediation

Adsorbent	Example/raw materials	Advantages	Disadvantages
Activated carbon	Wood, peat, coconut shells, coals	Pore size distribution, surface chemistry, mineral matter content, chemical nature which can be easily modified, chemical treatment available, cost-effective technology	Commercial activated carbon is quite expensive Secondary pollution generated by spent activated carbons Rapid saturation High cost of reactivation
Polymeric materials	Alginate, silk, lignin, chitosan, cellulose, cyclodextrin	Suitable and cost effective, biodegradable, and biocompatible, chemical stability, tailorable structure, high adsorption, and rate capacity	Denaturation by extreme temperature, performance depends on pH, not suitable for column step
Agricultural residues	Fruit peels Bagasse, coir pith, maize cob, sawdust, bark	Large quantities available, low or no cost, strong affinity and high selectivity towards heavy metals due to the abundant availability of binding groups on the surface area, easy acquiring	Effectiveness depends on pH and temperature, not suitable for industrial scale yet
Industrial wastes	Fly ash, red mud, sludge, metal hydroxide sludge	Higher area and porosity, low or no cost, large quantities available	Additional cost for processing, not suitable for industrial scale yet
Magnetic adsorbents	Hematite, magnetite, spinel ferrite, ilmenite	Small size, large surface area, large number of active sites, high operation efficiency, low cost	Additional cost for processing, not suitable for industrial scale yet

The high adsorption capacity of AC is due to their high internal surface area, and porosity and pore size distribution plays an important role. In general, micropores (smaller than 2 nm) provide the increased area and retention capacity. In comparison, mesopores (between 2 and 50 nm) and macropores (larger than 50 nm) are necessary to retain large molecules, such as dyes or colloids, and favor access and rapid diffusion of the molecules to the internal surface of the solid (Rodriguez-Reinoso 1997).

The structure of ACs is important because, on its surface, different functional groups from chemical activation make it chemically reactive (Bandosz et al. 1992). This is due to the interaction of the free radicals on the carbon surface with atoms such as oxygen and nitrogen from the activation method. Figure 10.14 shows the main functional groups that can occur on the surface of an AC.

Rossner et al. (2009), used an AC, a carbonaceous resin, and two high silica zeolites to evaluate the removal of a mixture of contaminants from lake water. Adsorption isotherm experiments were conducted with a mixture of 28 emerging contaminants at environmentally relevant concentrations (approximately 200–900 ng L^{-1}). Among the adsorbents tested, AC was the most effective, and the doses of activated carbon typically used to control taste and odor in drinking water (<10 mg L^{-1}) were sufficient to achieve efficient removal for most of the emerging contaminants tested.

Table 10.7 shows the adsorption capacity of different AC for heavy metals and their dependence on the conditions under which adsorption occurs.

Recent studies have established alternative methodologies for the adsorption of contaminants, such as heavy metals, using materials of biological origin, such as bacteria, algae and fungi, industrial, agricultural, and urban wastes, due to their high feasibility, low cost, and high removal efficiency. One of the techniques used for these processes is biosorption, which consists of the selective transfer of one or more solutes from a liquid phase to a batch of solid particles of biological material and involves the participation of various physical and chemical mechanisms depending on different factors such as pH or temperature (Abdi and Kazemi 2015).

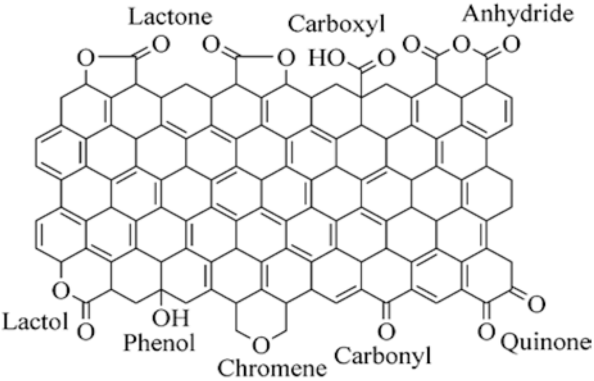

Fig. 10.14 Main functional groups in activated carbons

Table 10.7 Adsorption capacity of different AC for heavy metals

AC	Metal	pH	q (mg g^{-1}) or R (%)	References
AC from *Sargassum* spp.	Cr(VI)	2.0	91.92%	Esmaeili and Ghasemi (2012)
	Cu(II)	4.0	97.0%	Esmaeili et al. (2010)
AC from apricot stone	Ni(II)	6.0	98.51%	Kobya et al. (2005)
	Co(II)	6.0	99.11%	
	Cd(II)	6.0	99.68%	
	Cu(II)	6.0	97.48%	
	Pb(II)	6.0	99.93%	
	Cr(III)	6.0	98.99%	
	Cr(VI)	1.0	99.99%	
AC from pulverized waste tires	Pb(II)	NC	322.5 mg g^{-1}	Shahrokhi-Shahraki et al. (2021)
	Cu(II)		185.2 mg g^{-1}	
	Zn(II)		71.9 mg g^{-1}	
Commercial AC	Pb(II)		42.5 mg g^{-1}	
	Cu(II)		15.0 mg g^{-1}	
	Zn(II)		14.0 mg g^{-1}	
AC from coconut shell	Pb(II)	6.0	92.72 mg g^{-1}	Anirudhan and Sreekumari (2011)
	Cu(II)	6.0	73.60 mg g^{-1}	

NC no control of pH

Due to the natural origin of the substrates and the elimination of residual sludge during the removal process, this alternative is a system that not only removes the polluting metal, reducing the environmental impact generated on the environment in which it is discharged but also allows it to be recovered and integrated into a new productive cycle (Volesky 2003).

10.4.7 Bioremediation

Human beings, with our daily activities, generate enormous amounts of waste, which affect the environment (soil, water, and air, mainly). In short, environmental pollution threatens our well-being (Van der Perk 2014). Fortunately, the scientific community has developed techniques to try to restore environments damaged by pollution. One of these techniques is bioremediation, which is defined as a biotechnological process that uses organisms to recover a polluted environment, whether it is a terrestrial or an aquatic environment (Vidali 2001).

Ecosystems can naturally attenuate pollution, i.e., they purify and regenerate soil or water in the face of pollution of anthropogenic origin. Millions of microorganisms, including yeasts, non-pathogenic bacteria, and fungi are capable of degrading toxic substances, especially heavy metals, reducing their toxic character or even rendering them harmless to the environment and human health. It is a process that happens every day in a prolonged way. Bioremediation replicates this capacity of nature but accelerates the process (Sales da Silva et al. 2020).

Fig. 10.15 The most common organisms used in bioremediation processes: (**a**) bacteria, (**b**) fungi, and (**c**) plants

Bioremediation uses living organisms; however, not all organisms can be used in the bioremediation of environments. In fact, organisms are chosen according to their qualities to immobilize, mineralize, or degrade pollutant compounds and special attention is paid to their enzymes. In general, the organisms most commonly used in bioremediation processes are bacteria, fungi, and plants (Fig. 10.15a–c, respectively). Sometimes, organisms are genetically modified, so their qualities are closer to those required for bioremediation (Iwamoto and Nasu 2001).

Bioremediation processes commonly involve oxidation-reduction reactions where reduced contaminants are oxidized, and oxidized contaminants are reduced (Ihsanullah et al. 2020). Many different types of contaminants can be removed with this technique: polycyclic aromatic hydrocarbons, petroleum, pesticides, chlorophenols, heavy metals, dyes, sulfates, etc. (Kour et al. 2021; Lellis et al. 2019; Verma and Kuila 2019). Bioremediation is so complex that it can be classified into multiple types depending on the criteria chosen. Here are two types of bioremediation classification.

According to the bioremediation strategy:

Biostimulation This type of bioremediation strategy takes advantage of the particularities of the organisms already in the soil or water body to be treated and seeks to adapt the environmental conditions to enhance their development and the consequent degradation of pollutants. In short, biostimulation consists of incorporating nutrients or modifying environmental variables such as soil or water pH (Adams et al. 2015).

Bioaugmentation This other bioremediation strategy involves the incorporation of organisms, which can degrade compounds into a contaminated environment. In this way, the aim is to optimize the remediation process (Adams et al. 2015).

Depending on the organisms used for bioremediation:

Enzymatic degradation This technique refers to the exclusive use of enzymes to remediate a contaminated environment.

Microbial bioremediation In this case, it refers to using bacteria and fungi to remediate the contaminated site. Species that are capable of metabolizing contaminating compounds are sought.

Phytoremediation Bioremediation is carried out exclusively by plants. There are several types of phytoremediation depending on the qualities of the plants: some are capable of degrading the compounds, others of immobilizing them in their leaves, etc.

Bioremediation is generally used to remediate environments that hydrocarbons, such as petroleum, pesticides, heavy metals, etc. have contaminated. Plants can extract heavy metals from substrates by adsorbing them (Verma and Kuila 2019). Examples of plant species used for the remediation of environments contaminated with heavy metals include *Thlaspi caerulescens* (Fig. 10.16a), which adsorbs cadmium (Luo and Zhang 2021), and *Chrysopogon zizanioides* (Fig. 10.16b), which adsorbs zinc and lead (Punamiya et al. 2010).

The fungus *Pycnoporus sanguineus* also has high efficiency in adsorbing heavy metals in aqueous solution (Yahaya and Don 2014), particularly lead, cadmium, and copper. In addition, this fungal species could be used for soil bioremediation, specifically for soils contaminated with oil spills, since it can grow on this compound and tolerate high temperatures (Sales da Silva et al. 2020).

Fig. 10.16 Plants used for heavy metals adsorption: (**a**) *Thlaspi caerulescens* and (**b**) *Chrysopogon zizanioides*

10.4.8 Novel/Recent Methods

The study of mechanisms and methodologies for the removal of heavy metals in aqueous matrices has had a considerable boom in the last decade, so much so that numerous studies have been developed with novel and promising methods to reduce the concentration of heavy metals in water to levels that are safe for use in different daily activities.

10.4.8.1 Biofiltration

Biofiltration uses microorganisms attached to a porous medium to break down contaminants in wastewater streams. These microorganisms can grow in a biofilm on the surface of the medium or be suspended in the aqueous phase around the particles that make it up. Some parameters like O_2 content, temperature, pH, initial concentration of the pollutant, etc. regulate the removal efficiency of biofilters. The removal efficiency can be improved by chemical or genetic modification of the filter media or microorganisms, respectively.

The possibility of an effective application of biofilters to remove toxic heavy metals from contaminated water is high, especially on a large scale. Microbial cloning can improve the efficiency of the elimination and, therefore, the reduction of the cost of the treatment as reported by Srivastava and Majumder (2008). This technique is capable of removing heavy metals up to levels of the order of parts per billion (ppb), which makes it a highly applicable technique, as well as being relatively cheap. Its scope of application extends to wastewater from industries such as fertilizers, chemicals, dyes, paper and pulp, textiles, pharmaceuticals, pigments, etc. In short, biofilters are a beneficial emerging technology for treating wastewater contaminated with heavy metals.

10.4.8.2 Forward Osmosis

Forward osmosis is a membrane separation technology that is considered an economical method for wastewater treatment due to its low pollution potential and energy-saving characteristics. Currently, osmosis is thin-film composite (TFC) membranes manufactured by creating a dense layer of polyamide by interfacial polymerization on a support layer. However, the dense and hydrophobic polyamide layer leads to lower separation efficiency. Therefore, there is an urgent need to improve the separation performance of TFC membranes in wastewater treatment (He et al. 2022).

10.4.8.3 Novel Nanomaterials

In the last 5–10 years, developing new environmentally friendly nanomaterials with excellent sorption capabilities, performance, and stability has enabled massive advances in heavy metal ion capture.

Fang et al. (2018), synthesized a ZnS nanocrystal adsorbent to remove Hg^{2+}, Cu^{2+}, Pb^{2+}, and Cd^{2+} from wastewater. This adsorbent showed a high adsorption capacity based on the ion exchange reaction. In addition, it was shown that the adsorption capacity towards metallic species increases when the K_{sp} of its sulfide has an increasingly low value. Additionally, it was shown that the adsorbed heavy metal could be replaced by another heavy metal when the sulfide of the latter is more stable than that of the former. This mutual substitution character of the metal sulfides was used to separate multiple heavy metals. According to the results, all other heavy metals could be adsorbed and separated from the wastewater one by one. The results showed that this cation exchange-based sequential adsorption and separation method is promising for removing and recovering multiple heavy metals from wastewater.

Metalorganic frameworks (MOFs) are formed by the coordination of a metal ion such as Zr(IV), Mg(II), Fe(III), Ca(II), Al(III), Zn(II), Cu(II), Cd(II), Co(II), Ln(III), or Ti(III) and an organic ligand such as amines, benzoic acid, sulfonates, phosphates, carboxylates, etc. (Li et al. 2018; Wu et al. 2018). MOFs are attractive materials for adsorption and have gained popularity compared to other traditional materials such as activated carbons, silica gel, or activated alumina (Ricco et al. 2015; Yang and Yin 2017). Some MOFs have shown an excellent adsorption capacity for metals like As(V) (Wang et al. 2015a), Cu(II) (Zhang et al. 2015), Hg(II) (Liang et al. 2016), and Cr(VI) (Yang et al. 2016).

Carbon materials are widely used in the removal of heavy metals. Recently, carbon nitride materials have attracted a revival activity. This material is synthesized as thin two-dimensional sheets, giving it a large surface area, large pore volumes, and large exposed sites on the surface, thus possessing excellent characteristics for photocatalysis and has gained significant attention for heterogeneous catalysis and adsorption applications (Lin et al. 2015).

Among all the graphitic carbon nitriles, g-C_3N_4 has been reported as the most stable allotrope, which exhibits a controllable structure, products that are not harmful to environmental health, high chemical stability, and resistance to temperature changes in addition to other physical and chemical properties that have allowed their introduction in the field of adsorption (Shen et al. 2007; Wang et al. 2010).

Metal oxides are promising adsorbent materials due to their high adsorption capacity, susceptibility to modification and easy synthesis, high economic value, and the fact that they can be widely produced. Nanometer metal oxides, in particular, show great potential to remove most trace metal contaminants, making them beneficial materials.

For example, it has been reported that they generally show adsorption capacities between 10 and 200 times greater than conventional adsorbents such as activated carbon. This is because it has a highly reactive surface, which benefits the adsorption of contaminants (Wang et al. 2020a). Nanoparticles tend to aggregate easily, but the surface modification of nanometric metal oxides can improve the adsorption performance. Chai et al. (2021), reported that the introduction of amine groups in Fe_3O_4 increases the adsorption capacity of Cr(VI), for example.

Nanometric metal oxides have a large number of active sites on their surface that allow them to interact with various contaminants. Additionally, smaller particles exhibit faster adsorption kinetics as they have a lower resistance to diffusion. Reduction or oxidation has been suggested to occur between metal oxides and some specific multivalent heavy metal ions, for example, Cr(III), Cr(VI), As(III), As(V), Sb(III), Sb(V), etc. as they have a low affinity towards adsorption sites. Metal oxides involved in redox reactions include Fe_3O_4, MnO_2, TiO_2, and CeO_2 (Chai et al. 2021).

10.5 Challenges in Heavy Metal Pollution in Water

One of the biggest problems humanity will face in the coming decades will be the scarcity of drinking water due to climate change, which will increase periods of drought, as well as the high rate of pollution that many of the main drinking water sources have. From large pieces of trash to invisible chemicals, a wide range of pollutants ends up in our planet's lakes, rivers, streams, groundwater, and eventually, our oceans.

Water pollution, along with drought, inefficiency, and population growth, has contributed to a freshwater crisis that threatens the sources we depend on for drinking water and other vital needs (Mekonnen and Hoekstra 2016). Water pollution can cause human health problems, wildlife poisoning, and long-term damage to the ecosystem.

As is evident, the only way to solve the water contamination problem comes from two sides: not contaminate it and clean what is already contaminated. In this way, pollution that ends up destroying both aquifers and other types of water reserves can be avoided and minimized, so it is a battle that must be fought simultaneously on all fronts. As time goes by, new and diverse forms of treatment for contaminated water are emerging, which allows a greater range of possibilities in the field of water resources decontamination processes.

The main challenge is to change the remediation approach that we currently have and convert it into a systematic approach to prevention. Strategies must be directed towards preventing metallic contaminants from ending up in the water, especially the water used in the usual tasks of human beings.

While the reduction of the discharges of heavy metals in the water is achieved, the technologies used for their removal must be made more and more friendly to the environment, minimizing the use of new chemical species and the generation of residuals. All of the above must be achieved while maintaining high energy efficiency and economic profitability.

10.6 Conclusions

Environmental pollution is positioned as one of the most important problems affecting society in the twenty-first century. The loss of air quality, water resources, and soils available for agricultural activities has increased exponentially. A growing problem of contamination by heavy metals is identified at a global and local level, which severely compromises health, food safety, and the environment. Due to its high toxicity, the impact on health caused by prolonged exposure or bioaccumulation of heavy metals is alarming. Depending on the type of metal or metalloid, conditions ranging from damage to vital organs to carcinogenic developments occur (Rai et al. 2019).

It is currently widely accepted that the distribution, mobility, biological availability, and toxicity of chemical elements are not a function of their total concentration but rather depend on the chemical form in which they are found. It is necessary to know the chemical species of the elements to understand the chemical and biochemical reactions in which they intervene and, therefore, obtain information regarding the essential and toxic nature of the chemical elements and the remediation strategies to apply in order to reduce or minimize the effects of the contaminant in human and environmental health. Speciation analysis will become an essential tool for risk assessment in the environment, allowing more effective trace element diagnoses and controls to be performed.

Today we know a great variety of methods and techniques that can be used to remove heavy metals from aqueous matrices, each of which shows advantages and disadvantages that must be analyzed. The search for new materials and alternatives for the disinfection of water contaminated with heavy metals, as well as the optimization of those that we know today, is a permanent task for the scientific community.

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11

Responses of Marine Fungi to Heavy Metal Contamination

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Abstract

The marine ecosystem, which makes up around two-thirds of the planet's surface, suffers from aggressive heavy metal contamination, especially in the last few years. This pollution causes shifting in many marine life forms threatening our fisheries and human health. The natural sources of marine heavy metal pollution are rock erosion, and volcanic dust, while the artificial sources are related to human attitudes like sewage, ship accidents, industrial wastes, and agricultural wastes. Because they are so common in nature, microorganisms also predominate in locations where heavy metals have been contaminated. Heavy metals are easily changed by them into harmless forms. Microorganisms have two responding mechanisms of heavy metal contamination that comprise enzymatic breakdown of the target contaminants and/or heavy metal resistance. In this chapter we will discuss marine heavy metal contamination sources, their risks to the marine life forms, and different responses of marine fungi to heavy metal contamination.

Keywords

Heavy metal · Marine · Pollution · Ecosystem · Fungi

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11.1 Introduction

Heavy metals (HMs) are a chemical group of elements comprising metallic properties like lanthanides, transition of metals, metalloids, and actinides. Some of them have identified as essential enzymes cofactors. The first group of these metals includes cobalt, copper, iron, manganese, molybdenum, selenium, and zinc, which are represented as the trace elements and regulated inside the viable cells through interactions with binding cell proteins which are huge risk to cell function (Ibrahim et al. 2020; González Henao and Ghneim-Herrera 2021). The second category consists of non-essential metals such as cadmium, plutonium, lead, tungsten, mercury, arsenic, and vanadium, which are strong poisons that can infiltrate cells and tissues due to their ionic charge (Pande et al. 2022). The latter group has high attention as they are not essential for living organisms, and high toxic even with low levels. According to the Environmental Protection Agency, lead, arsenic, cadmium, and mercury are categorized as the highest toxic metals to the environment (Goyer 2004; González Henao and Ghneim-Herrera 2021).

Marine sediments contaminated with HMs are critical environmental issue, especially in the coastal areas with high anthropogenic activities like dumping, sewage discharge, industrial practices, and excessive agricultural treatments as chemical fertilizers (Mahmoud and Bagy 2018; Zhang and Wang 2020). Heavy metals are introduced to marine ecology naturally through weathering, rock erosion, and volcanic dust. It's essential for life in small quantities and can become hazardous if present in excess, including Na, Ca, Fe, Mn, Cu, K, Zn, Mg, and Co (Sarkar et al. 2017). While metals like Cs, Cd, Hg, Pb, and Al are not known to have any significant metabolic roles in living things, they can still be harmful if they build up in the environment. It was found that Cr, Zn, Pb, Cd, and Cu are present in many different locations of the aquatic ecosystem. Heavy metals have a very high level of environmental toxicity because they are typically linked to particles that remain in solutions for long period, when they are evolved into the marine ecosystem; they can last for a longer time affecting the whole aquatic ecosystem (Gadd 2010; Pande et al. 2022).

Using of microorganisms in heavy metal remediation needs knowledge of genomes, proteomics, transcriptomics, synthetic biology, and signaling systems (Hemmat-Jou et al. 2018; Mahmoud 2021). Fungi may be more efficient in HMs bioleaching than acidophilic bacteria (auto/heterotrophic), and as a result, their usage may be a promising and practical method for the bioremediation of heavily contaminated marine sediments (Dell'Anno et al. 2022). Marine fungi are characterized with adaptation to stress factors such as high salinity and extreme pHs compared to the terrestrial ones. Their mycelia protect them against the invasion of insoluble materials; also they produce degradative enzymes extracellular to degrade the diverse HM substances. These degradative enzymes mostly are non-specific and can act upon many substrates. The filamentous mycelia growth form provides a mechanical adjunct. Because of their large surface-to-cell ratio it provides high contact with the substrate, and high fungal enzymes that prevent the insoluble substrates to cross the fungal cell membrane rather than bacteria. Besides, the fungal cells also play significant roles in pollutants detoxification by involving in the microbial biofilm-forming communities (Vala et al. 2018; Sayqal and Ahmed 2021).

The binding forces between HMs and the viable cells could be covalent bonding, extracellular precipitation, van der Waals forces, redox potential, and electrostatic or combination between more than one type. This binding occurs by adsorbing the metal cations with negatively charged hydroxyl, carboxyl, and phosphoryl groups already present on the fungal cell walls (Njoku et al. 2020). In comparison to organic pollutants like petroleum hydrocarbons and insecticides, heavy metals are more persistent in sediments. Additionally, they move about in sediments where their speciation and pH are changing. Consequently, a portion of the entire mass may seep into an aquifer or become bioavailable to living things (Santona et al. 2006). HMs affect the microbes' growth, activity, and even their survival. They have many detoxifying mechanisms of HMs depending on alteration of the physical and/or the chemical status of the metals to convert them to non-toxic stages (Dixit et al. 2015). HM bioremediation strategies at contaminated sites depend on the interaction type of metals and microbes. The interaction could occur as binding, volatilizing, oxidizing, reducing, immobilizing, and transformation of HMs (Njoku et al. 2020).

11.2 Marine Heavy Metal Contamination Sources

Pollutants like HMs, hydrocarbons, halogenated compounds, and metalloids are released into our environment via the industrial activities, waste disposal wrong practices, incomplete organic matter combustion (fertilizers), as shown in Fig. 11.1. Dell'Anno et al. (2020) reported that HMs affect the biological cell DNA, amino acids, activity, organelles, membranes, and their metabolic enzymes (Dell'Anno et al. 2020). It also generates modulation in the cell cycle, apoptosis, and carcinogenesis (Kim et al. 2015a). Releasing pollutants from the human activities are the main threat to the ecosystem's composition, health, and transition (Buah-Kwofie et al. 2018; Mahmoud et al. 2020a). According to Briffa et al. (2020) the accumulation of HMs affects highly on the microbial types which will reflect on the ecosystem's biodiversity, functioning, and provisioning with cascading effects on the human life.

Several industries related to mining, surface finishing, metallurgy, electro-osmosis, electroplating, electrolysis, tanneries, photography, varnishes, distilleries, energy, fertilizer, iron, steel production, and paints release large amount of HMs during their operation into the environment (Alvarez et al. 2017). Mining and metallurgical process caused direct HM contamination through the extraction process, and raw materials processing, while other industries cause indirect HM contamination through burning of fossil fuels (Li et al. 2015). Remarkably, textile and tanneries processes cause high lead, chromium, arsenic, and cadmium pollution especially to the water environment (Bhuiyan et al. 2010; Ibrahim et al. 2022).

Agricultural practices also contribute to the HM pollution through the uncontrolled using of fertilizers, fungicides, herbicides, pesticides, irrigation with wastewater, and soil amendments (Giri et al. 2017). Phosphate fertilizers contain nickel,

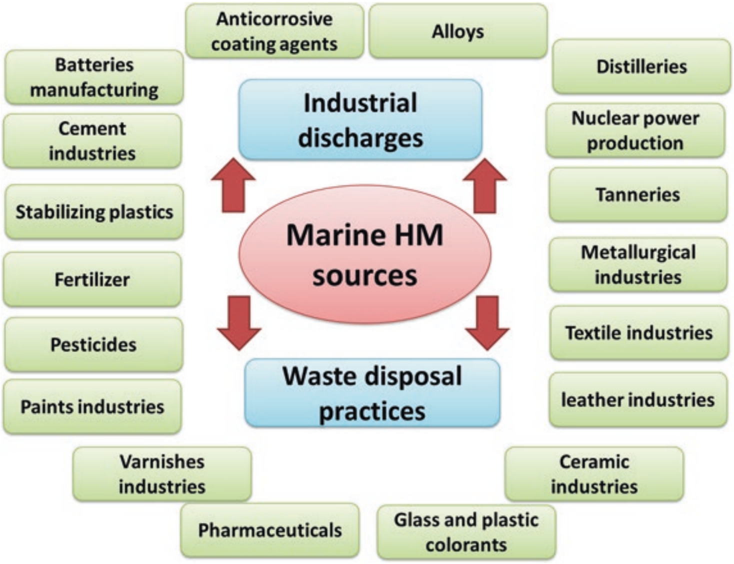

Fig. 11.1 Different pollution sources of marine environment

lead, zinc, and cadmium; pesticides also contain active ingredients like mercury, copper, lead, and arsenic (Paranjape et al. 2014; Ibrahim et al. 2021). Urbanization could generate HMs like Fe, Mn, and Cr in the solid waste, sewage, wastewater, and sludge (Kothe et al. 2010). Natural activities also cause HM contaminations including volcanic eruptions, rocks, oceanic evaporation, mineral erosion, and the pedogenic activities as reported by Alvarez et al. (2017).

The sources of chromium releasing through the environment are the industries of electroplating, paints, dyeing, textile, ink, and printing; tanning of leather; ceramic industry; fuel production; and anticorrosive agents (Malaviya and Singh 2016). While cadmium-releasing industrial sources are mining, coatings, electroplating, plastic, batteries, pigments, phosphate fertilizers, fossil fuel, and cement industries (Khan et al. 2016; Mahmoud et al. 2020a). Lead originates from the geochemical processes, industrial processes like pesticides, batteries, fuel additives, ceramics, pigments, photographic materials, coating, automotive, and the industrial incinerators (Pepi et al. 2016). Natural geologic activities like volcanoes, sedimentary rocks of marine environment, weathering, fossil fuels, and mineralization processes, and also anthropogenic activities like mining, pesticides, herbicides, fertilizers, coal, and medical products are the arsenic major environmental sources (Altabbaa et al. 2022).

11.3 Risks of Heavy Metals to the Marine Life Forms

High HM concentrations in fresh and marine sediments could easily be transferred to humans through the food chain causing serious consequences on the natural ecosystems and eventually to our health (Priyadarshanee and Das 2021). HMs generate oxidative stress on the living cells which will interfere with the protein folding, physiological process causing several cellular disorders in all the marine life forms (Kalaimurugan et al. 2020; Abdu et al. 2017) as shown in Fig. 11.2. Heavy metals in the aquatic environment get absorbed by living organisms and through the food chain, and the human population also is likely to accumulate such metals (Dash et al. 2021). Unlike most contaminants, heavy metals are non-degradable. Exposure to such heavy metals results in several health implications ranging from headache to allergy to respiratory tract disorder to various cancers depending on the type and concentration of the heavy metal (Vala and Dave 2017; Mahmoud et al. 2020b). By attaching to thiol and other groups on protein molecules, which could take the role of naturally occurring metals in the prosthetic groups of enzymes, HM may affect the structure and function of the enzyme. Deoxyribonucleic acid (DNA) has been found to bind with and be disrupted by metals (Jadoon and Malik 2017). The bioaccumulation of certain metals is a significant factor in their toxicity, which may cause the development of symptoms after repeated exposure. Their accumulation may also cause their mobilization across food chains, which may have an impact on higher organisms (Mahmoud 2021). There is strong evidence that certain metals, like chromium and nickel, can lead to cancer, as well as circumstantial evidence for many others (Shah 2017). Due to their high lipid solubility, dimethylmercury and tetraethyl lead are particularly hazardous because they can enter the body quickly and remain there which exacerbate their toxicity (Bjørklund et al. 2017). Because they can enter the body through the lungs and organomercurials can travel through

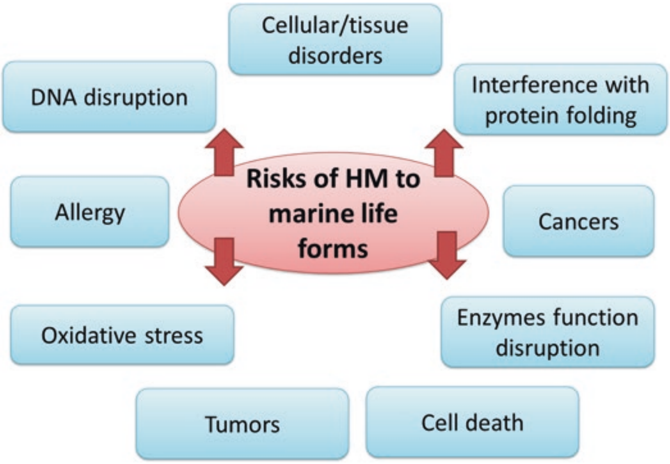

Fig. 11.2 Risks of heavy metals to the marine life forms

the placenta, volatile metals and their compounds may also be harmful. In a study on the marine copepod *Acartia tonsa*, the organism is capable of storing mercury and methylmercury (Lee and Fisher 2017). Plankton can transmit trophic levels and absorb methylmercury (Schartup et al. 2017). Heavy metal accumulation was visible in marine mussels *Mya arenaria* and *Astarte borealis* found in the Norwegian and Baltic seas (Pempkowiak et al. 1999). *Cancer pagurus* (edible crab) from Scottish shore showed heavy metals present in the edible parts (Maulvault et al. 2012). *Epinephelusareolatus*, *Sparussarba*, and *Lutjanusrusselli*, from Hong Kong showed high Zn, Pb, and Cu levels (Wong et al. 2001).

11.4 Biological Removal of Heavy Metals by Marine Fungi

The three significant ex-situ processes for HMs removal are biological, physio-chemical, and thermal treatments (Haynes and Zhou 2021; Suyal and Soni 2022). The physio-chemical approach is used to activate the metal solubilization in sediments through the application of aqueous solutions of chelating agents via electrochemical processes. Also, this method reduces the metals' mobility via complexation process with stabilizing agents as recorded by Dell'Anno et al. (2022). Thermal treatments of HM-contaminated sediments include two ways: HM desorption from HM-contaminated sediments and/or inducing their immobilization properties (Haynes and Zhou 2021). Although the two processes are effective in the site treatments, there are limited using of these approaches for their high costs, low specificity, and produces toxic wastes with high amounts (Fashola et al. 2020). Within the last few years biological treatment strategies have gained more attention for their low economic costs and being environmental friendly (Magan et al. 2022). Biological treatment of HM-polluted sediments using microorganisms includes mobilization (extrication) and immobilization (stabilizing). However scientists preferred immobilization (stabilizing) as they effectively decreased HM concentrations in contaminated sediments as stated by Peng et al. (2018).

Generally degradation of HMs and organic pollutants in the polluted water sites involves aerobic or anaerobic microbial respiration and fermentative metabolism, but HM transformation or sequestration that does not involve degradation will be based on the bioleaching, bioaccumulation, and biotransformation activities (Kumar et al. 2019). The occurrence of metal in the natural environment and anthropogenic activities are the reasons for their encounter with microbes. Substantial variations in heavy metal concentrations are observed in the marine subsurface. The composition and concentration of heavy metals play an important role in shaping microbial community structure (Pachiadaki et al. 2016). Due to relatively low oxygen concentration and higher salt concentrations in seawater, removal of metal contamination using organisms from non-marine sources could be difficult, for applying microorganisms to the marine environment they have to be able to tolerate high salt concentration and low oxygen (Kim et al. 2015b). Microorganisms including bacteria and fungi play an important role in the bioremediation of heavy metal contamination. Microbial remediation of heavy metal contamination is environment-friendly, efficient, cost-effective, and recycles bio-products. Involvement of microbial processes epitomizes logical and long-term solutions for remediation. Vidal and Vidal (1980) examined arsenic metabolism (As(V)) in marine yeast *Rhodotorula rubra* and based on qualitative analysis of metabolism products, reported generation of As(III), methylarsonic acid, dimethylarsinic acid, and volatile alkylarsines. Similarly, marine yeast *Rhodotorula rubra* has been harnessed for arsenic metabolism by Maher and Butler (1988). Abe et al. (2001) stated isolated copper tolerated *Cryptococcus* sp. from deep-sea sediment could remove 30–90% of the supplied heavy metals. Strains of *Yarrowia lipolytica* have been reported to be potential hexavalent chromium remediators (Imandi et al. 2014).

Vala et al. (2004) tested two seaweed-associated fungi *Aspergillus flavus* and *A. niger* for their hexavalent chromium tolerance potential. Taboski et al. (2005) assessed the toxicity of Cd and Pb to *Corollospora lacera* and *Monodictys pelagica* from the marine environment and found that *M. Pelagica* bioaccumulated over 60 mg/g Cd and over 6 mg/g Pb, while *C. lacera o*ver 7 mg/g Cd and up to 250 mg/g Pb. Khambhaty et al. (2008) observed that *Aspergillus niger* as the most efficient Hg(II) marine biosorbent and dead biomass exhibited 40.53 mg/g Hg(II). Examination of arsenic tolerance and accumulation potential of *Aspergillus* sp. isolated from coastal waters of west coast of India revealed the fungus tolerated 100 mg/L As(III) or As(V) (Vala and Upadhyay 2008). *Aspergillus niger*, *A. wentii*, and *A. terreus* isolated from Gujarat coast were efficient hexavalent chromium removals (Khambhaty et al. 2009a). Cr(VI) sorption capacity by marine *A. niger* was 117.33 mg/g with efficiency 100% (Khambhaty et al. 2009b). Based on fourier-transform infrared spectroscopy (FTIR) analysis, amino, -CH_2, hydroxyl and phosphorous groups were found to be involved in binding of Cr(VI) to fungal biomass of *Trichoderma viride* isolated from the Mediterranean Sea (El-Kassas and El-Taher 2009).

Vala (2010) investigated tolerance to and removal of arsenic by *Aspergillus candidus* isolated from coastal waters of West coast of India. The fungus exhibited tolerance to trivalent and pentavalent forms of arsenic (25 and 50 mg/L). Two marine fungal strains of *Dendryphiella salina* were observed to absorb 80–92% Hg^{2+} (Mendoza et al. 2010). *Aspergillus flavus* and *A. niger* marine fungi exhibited arsenic tolerance as reported by Vala et al. (2010) and Vala et al. (2011), respectively. Three species of Thraustochydrids viz. *Aplanochytrium* sp., *Thraustochytrium* sp. and *Schizochytrium* sp. were used for chromium removal (Gomathi et al. 2012). *Aplanochytrium* sp. could remove 69.4% chromium. Vala and Sutariya (2012) explored the degree of arsenic tolerance and removal efficiency of two facultative marine fungi *A. flavus* and *Rhizopus* sp. Upon exposure to 25 and 50 mg/L sodium arsenite (As (III)), both the fungi exhibited As tolerance and arsenic accumulation. Slightly better accumulation was observed by *Rhizopus* sp. Cd tolerance showed by *A. versicolor*, *A. fumigatus*, and *Paecilomyces* sp. (Mohammadian Fazli et al. 2015). *Aspergillus niger* was recorded as chromium biosorbent by Acosta-Rodríguez et al. (2018). *Aspergillus niger*, *and A. nidulans* were tolerant to Hg, and As (Rodríguez et al. 2018). Rose and Devi (2018) also recoded the Cu and Ni tolerance by *A. awamori and A. flavus*. Vala (2018) also reported volatalization of 15.75%

supplied trivalent arsenic from the culture medium by *Aspergillus sydowii.* Pb, cd, and U were removed by *Penicillium chrysogenum* (Alothman et al. 2020).

11.5 Heavy Metals Removal Strategies by Fungi

In heavy metal-contaminated soil, microbes predominate, and they employ a two-pronged defense strategy that includes the creation of enzymes that breakdown the target pollutants and resistance to the appropriate heavy metals (Dixit et al. 2015). Biosorption, biomineralization, bioleaching, metal-microbe interactions, biotransformations, and bioaccumulation are some of the different types of bioremediation. The bioremediation strategy is used in accordance with the mechanism through which metals interact with microorganisms at the contaminated site. The metal can be dissolved by microbes, and they can also oxidize or decrease transition metals. By oxidizing, binding, volatilizing, reducing, immobilizing, and otherwise altering heavy metals, microorganisms and metals interact (Sarkar et al. 2017; Pande et al. 2022).

Metal adsorption: metals that have been bound to extracellular substances become immobile and are unable to enter cells. Both economically and ecologically, it is vital for metals to bind with microbial cells. Cell surface binding plays a significant role in the dispersion of metals ecologically, especially in aquatic environments (Mahmoud 2021). Electrostatic contacts, covalent bonds, extracellular precipitation, redox interactions, or a combination of all these processes are the forces that allow metal ions to bind to the surface of cells. The chemistry underlying this interaction is that the negatively charged hydroxyl, carboxyl, and phosphoryl groups on the microbial cell walls adsorb the metal cations. The metal nucleation then confines these. Metals that bind to extracellular surfaces become immobile, blocking them from entering cells. In the outer membrane of microbes, phosphoryl groups and phospholipids interact strongly with cationic metals (Njoku et al. 2020). By living and dead *Aspergillus* fungal biomass, element chromium has been adsorbing, followed by sequestration of the aforementioned functional groups. In addition, non-specific sulfate transporters present inside the cell, such as glutathione, facilitate the absorption mechanism and are crucial to the intracellular reduction of heavy metals (García-Hernández et al. 2017). It has been discovered that when the metal ion comes into contact with the cell wall of fungus, the metal can be deposited either on the surface or within the structure of the cell wall before interacting with cytoplasmic components. Chelation, precipitation, and cell wall adhesion are all aspects of extracellular mechanism (Bellion et al. 2006). The internal mechanism used by fungi, known as bioaccumulation, involves attaching to substances including organic acids, peptides, and polyphosphates and transporting them into specific intracellular compartments. Here, cell wall ruptures that are preceded by enhanced permeability that causes the subsequent exposure of metal-binding sites cause intracellular uptake to occur. Additionally, fungi produce antioxidant molecules to help their own cellular detoxification system (Goutam et al. 2021).

Metal immobilization: by altering the physical and chemical properties of the metals, this approach reduces metal mobilization. To repair polluted soil, there are two methods that can be used: ex situ immobilization and in situ immobilization. Ex situ approach involves removing contaminated soil from its original location; however doing so poses significant risks. Immobilization using the biosorption technique primarily aims to convert the initial soil metals into more geochemically stable phases. With the exception of alkali metals, which are cations like K^+ and Na^+ and which can be a crucial passive approach, any microbiological material can operate as an effective biosorbent for the metals in dead and living organisms (Gadd 2010). Sorption affects bioavailability, which makes it beneficial in the interactions between microbes, metals, and minerals, and so plays a significant part in bioremediation techniques. Microbes can convert heavy metals to a lower redox state, which lowers the mobility and toxicity of certain elements. Gold Au(0) and elemental silver Ag are produced as a result of the microbial reduction of gold species and ionic silver(0) (Southam et al. 2009). Microbes produce a variety of organic and inorganic biominerals, such as phosphates, oxalates, oxides, sulfides, and carbonates, which cause the metal to get immobilized. In rocks, sediments, and soils, iron-containing minerals are worn partially by chemical reaction and partially by microbial and fungal activity. Precipitation of Fe^{3+} may result from the removal of ferric iron. In an aquatic environment, hydrous iron oxides produced by bacteria can accumulate metals by co-precipitation or adsorption. The adsorbed metals may get remobilized as a result of acidification or the reduction of iron oxides (Ehrlich and Newman 2009). To remove heavy metals (Cu, Pb, and Cd) from an aqueous solution with a low concentration, researchers immobilized the live conidia of the heavy metal-resistant *Penicillium janthinellum* strain GXCR using polyvinyl alcohol (PVA)-sodium alginate (SA) beads (Cai et al. 2016; Pande et al. 2022).

Metal biosorption: a biosorbent with a higher affinity for the sorbate (metal ions) is used in the process of biosorption, which is continued until equilibrium is reached between the two elements. One of the key steps in the bioremediation of heavy metals is thought to be biosorption. Both living and dead cells participated in the biosorption process because the dead biomass of fungi is more able to remove heavy metals than the living biomass since it first involves physical processes and then physiological processes (Dixit et al. 2015). On the basis of their dependence on the cell's metabolism, biosorption mechanisms can be separated into two groups: metabolism-dependent and non-metabolism-dependent. When metal is transferred across the cell membrane, intracellular buildup results, it leads to metabolism-dependent biosorption. When the uptake of the metal results through the physicochemical interaction between the metal and the functional group present on the surface of the microbe, it is known as non-metabolism-dependent biosorption (Goutam et al. 2021).

Metal mobilization: protonolysis, siderophores, redox processes, methylation, and indirect Fe^{3+} assault are all methods for mobilizing metals (Gadd 2010). Microbes have the ability to assemble metals and, via redox reactions, can spread onto mineral surfaces (Lloyd and Lovley 2001). Some metals and metalloids produce methylated derivatives as a result of methylation, which improves the mobility of these elements. The aerobic state of *Alternaria* and *Penicillium* spp. can mediate the methylation of the metalloids Te, Se, and As, as well as Pb, Hg, and Sn (Gadd 2010). The volatility, toxicity, and solubility of certain elements' methylated derivatives vary. The volatile methylated chemicals frequently disappear from the soil. However, some heavy metals may not be removed from the soil by methylation. For instance, fungi has the ability to methylate the deadly chemical methyl mercury from the mercury ion (Barkay and Wagner-Dobler 2005; Pande et al. 2022).

11.6 Responses of Fungi to Heavy Metal Contamination

Due to their phenomenal development and exceptional binding abilities, fungi become a superior option for bioremediating heavy metals. Because of their quick rate of multiplication and ease of morphological and genetic modification, they may be quickly and affordably cultured in large numbers. First, Paul Stamets came up with the word "mycoremediation," which means "removal of harmful chemicals by fungi." Minerals and metals found in both manmade and natural environments are used by microbes through a variety of processes. The transformation of heavy metals into non-toxic forms is aided by changes in their physical and chemical states (Goutam et al. 2021; Mahmoud 2021; Abeed et al. 2022). Four phenomena have been commonly involved in the responses to metal.

Exopolymer binding: exopolymers, also known as extracellular polymeric substances, are frequently found in nature and provide protection against phagocytosis, desiccation, and parasitism. Polysaccharides, carbohydrates, and occasionally fatty acids, nucleic acids, and fatty acids are examples of exopolymers that are used for extracellular binding (Schiewer and Volesky 2000). Many microorganisms create exopolysaccharides (EPS), which have a strong binding to the metals. By mobilizing or immobilizing the hazardous metals, EPSs restrict their entry into the cell and are hence essential for metal cycling (Gomathy and Sabarinathan 2010). Lead, cadmium, and uranium can all be effectively bound by these interactions. Metal binding is facilitated by the presence of negatively charged groups on exopolymers like amine, amide, succinyl, phosphate, hydroxyl, and uronic acids (as metals are positively charged). Metals become immobilized as a result, and their entry into the cell is so prevented (Goutam et al. 2021).

Siderophores: these compounds, which are among the most well-known and large, can join and shuttle Fe. They function as much specialized Fe^{3+} ligands. Their primary duty is to raise the concentration of iron in places with extremely low iron levels before transferring it into the cell. The siderophore mediates entry of Fe^{2+} and Fe^{3+} into the cell. Siderophores interact with metals that share a chemical structure with iron, such as aluminum, gallium, and chromium, for example, and produce trivalent ions that are similar in size to irons (Goutam et al. 2021). When a siderophore attaches to a metal, the metal's bioavailability is decreased, which lowers the metal's toxicity. The organisms have evolved specialized strategies to make sure that the requirement for Fe is met, such as attaching to solid iron minerals like Fe oxides or producing species-specific siderophores (Gomathy and Sabarinathan 2010; Goutam et al. 2021).

Precipitation: it results in the immobilization of metals or heavy metals, making naturally soluble metals insoluble. It could be cellular metabolism-dependent or cellular metabolism-independent. When dependent precipitation occurs, the metal that is taken from the solution is a part of the microorganisms' dynamic defense mechanism. When metal and the cell surface interact chemically, independent precipitation is produced (Goutam et al. 2021).

Biosurfactant complexation: microorganisms create some substances that are spat away. This group of substances is known as a biosurfactant. They are capable of forming complexes with metals like lead and cadmium. It raises the solubility by increasing the mobility of the resulting complex. The cells are not harmed by these complexes. Numerous studies have found that metal-contaminated areas serve as superior isolation sites than uncontaminated sites for microorganisms that produce biosurfactants (Gomathy and Sabarinathan 2010).

11.7 Conclusion

Marine environment suffer from irresponsible human practices and the industrial wastes. Huge amounts of heavy metals enter the marine environments daily. Marine microorganisms control this contaminant through their heavy metal removing strategies and responding mechanisms. But due to unequal entrance to these metals to the marine environment many marine life forms suffer every day. Decreasing and controlling the industrial wastes and the wrong human practices represent the main solution of heavy metal pollution of marine environment.

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Microplastic Pollution in Marine Ecosystem and Its Remediation

12

Harshal S. Jadhav, Abhay B. Fulke, Marissa R. Jablonski, and Manisha D. Giripunje

Abstract

Since the initiation of the plastic industrial manufacturing, its production rate has been continuously increasing and attaining high records. However, along with the increase in its production, generation of the huge plastic waste also begins. Plastic polymers are extremely difficult to degrade and might accumulate within the environment for years. Also, poor plastic waste handling or management issues are responsible for the huge plastic pollution, most of which have also reached the marine environment. Eventual degradation of the plastics in the marine environment from long time generates microplastic pollutants there. Presence of microplastics in the marine environment would be responsible for evolution of the potential plastic-consuming organisms. Microorganisms attached to plastic surface make biofilm by electromagnetic interaction forces. Further, extracellular and intracellular microbial catalysts or enzymes metabolize plastics eventually. Although remediation of such microplastics in the marine environment is a difficult task, potential strategies could be implemented for the removal of such pollutants. Based on the technique of ocean currents, these microplastic pollutants would have transferred from their high concentration in ocean to low concentration at coastal regions and eventually settled there in

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coastal sediments. Potential microplastic remediation techniques could be applied on the different coastal region such as coastal sediment circulation, implementation of controlled natural reactors in intertidal regions, and use of specialized membrane. This would establish a continuous transport of microplastic pollutants from ocean to coastal region to maintain microplastic particulate equilibrium, thereby evacuating microplastics from the marine environment.

Keywords
Microplastic · Bioremediation · Marine ecosystem · Biofilm

12.1 Introduction

Microplastic in marine environment is pervasive. Marine environment consists of five oceans—Pacific, Atlantic, Indian, Arctic, and Southern. Seas have divided different oceans along the terrestrial borders. During festivals, national programs, or cultural and societal celebrations plastic pollution increases. In general, plastic waste generated must be recycled by proper protocol or dumped in dumping grounds, as per town or municipal waste managements. However uncontrolled dumping of plastic waste should not be recommended (Ma et al. 2016). Composting of soil by chemically and biologically treated plastic waste would otherwise be an appropriate option.

Most of plastic wastes have reached oceans by rivers. While they move with the flow of rivers with high speed, these plastic pollutants may encounter large and small rocks that come in the path of river flow. Shearing and tearing of such plastic pieces occur to generate microplastics. Other possible sources of plastic waste to ocean might be through large and small vessels or ships, coastal visitors, or tourists and by action of heavy rain flows or massive winds. Rotation of earth generates air which is responsible for wave formation in ocean waters. Circulation in the bottom ocean water occurs by means of atmospheric changes. Continuous circulation and wave action in ocean water cause mixing of plastic waste and break it into microplastics. Plastic waste floats on the ocean surface initially and hence comes in contact with relatively cold water and continuous contact with sunlight might increase brittleness to disintegrate into microplastics. Plastic would sink or fall to the ocean bottom due to change in surrounding situations or due to biofilm.

Almost all sewage discharge points, or other waterways should drain at different rivers or directly into the oceans. Such wastewaters are believed to comprise all pollutants including microplastics. Plastics are difficult in degradation and have multifaceted health risks. Extremophilic organisms are more capable in degradation of such materials. Extremophilic organisms are the ones that can survive in extreme conditions like temperature, pH, salinity, nutrient concentrations, dissolved oxygen, pressure, and pollutants. Presence of microplastics in sewage to some extent also increases production of methane gas (Wei et al. 2019). Although methane gas can be utilized as a source of fuel, uncontrolled production of methane would otherwise be hazardous as it is a potent greenhouse gas which might alter the atmospheric conditions by interfering ozone.

In this chapter, we have discussed how marine ecology is disturbed due to the presence of huge amount of plastic waste and the continuous transport of the plastic waste and microplastics in oceans. Also, plastic degradation pathway has been conferred along with microplastic remediation strategies and associated challenges that possibly arise during the implementation of remediation strategies. Future prospects in the microplastic remediation research also have been stated thoroughly.

12.2 Microplastic Pollution in Marine Ecosystem and Its Impact

Marine ecosystem is one of the complex structures whearas most of its parts have surrounded by water. Study of communication within biological and nonliving matters occur in ecosystem. Nature of this communication must be properly understood in order to remove microplastic pollution from oceans.

Continuous mixing of water in marine environment must encounter various different living and nonliving matters. Among biological component of ecosystem mass and energy transfer from primary producers or autotrophs to primary consumer or herbivores to secondary consumer or carnivores. There is inverted pyramid of biomass for marine ecosystem as combined biomass of marine carnivores fishes is greater than herbivores marine animals and marine phototrophs or algae biomass are least as mentioned in Fig. 12.1. However huge number of marine microorganisms play significant role in balancing environment. The number of microbes in

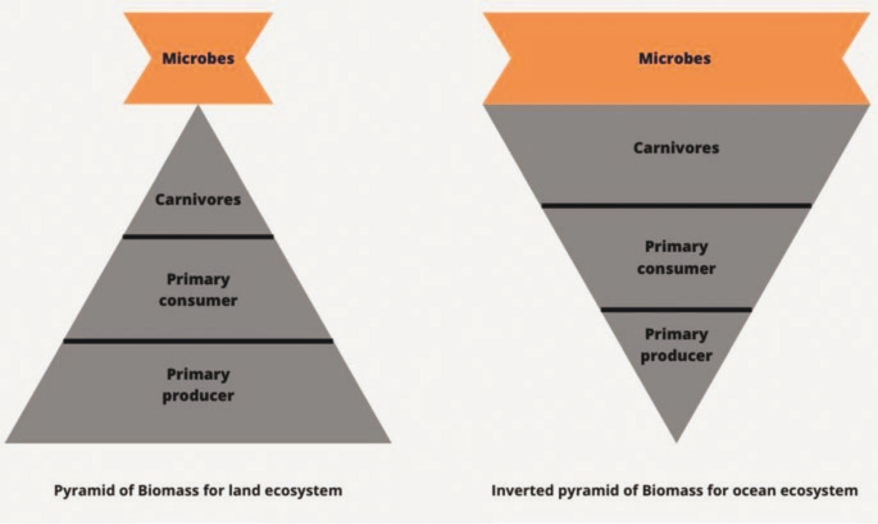

Fig. 12.1 Comparison of ocean and land ecosystem with respect to biomass pyramid

ocean exceeds 10^{29} cells and they may contribute 90% of biomass in the ocean (McIntyre 2010). The overall biomass of microorganisms in ocean would be similar to overall biomass of terrestrial plants (Ray and McCormick-Ray 2013); and there is normal pyramid of biomass for land ecosystem in which primary producers, that is plants represent the base of the pyramid have large in biomass. Among marine microorganisms, viruses are the most abundant of all (Danovaro et al. 2008). These microorganisms are known to make biofilm on the surface of plastic in order to consume it as nutrient. While microbes make biofilm, it increases the weight of the plastic in the ocean thereby making it fall toward the bottom. Once these microplastics have reached the bottom of the ocean, they interfere with the normal processes' benthic organisms, or they may alter the processes. For example, some microphytobenthos are involved in interaction with microplastics; however, while doing so they decrease the normal growth rate of microphytobenthos while increasing the amount of cyanobacteria form (Hope et al. 2020). Normally, microorganisms including cyanobacteria make symbiotic relation with other organisms mutually helping them in various different processes. Since decreasing marine organisms' growth rate due to interference by microplastics would eventually lead to increase in number of free microbes in marine environment, such processes would disbalance the marine ecosystem. Marine environment is very dynamic in nature which continues to be in contact with different stress conditions. These stress conditions include extreme temperature, pressure, salinity, pH, oxygen level, nutrient bloom, and various pollutants including plastics. Such stress conditions would favor the evolution among organisms in order to survive in extreme circumstances. Mutation occurs easily in microorganisms that transmit effortlessly to their offsprings. Such changed microbial species which may develop a gene for plastic degradation enzymes would now be able to degrade plastic or other pollutant.

Marine microorganisms are associated with every organ or cell of marine animals or plants. Although most of the marine organisms are not able to distinguish plastics; however, some of them might develop biochemical mechanism to consume plastic as nutrient, specifically hydrocarbon source. These organisms have evolved mainly after 1930s, because industrial production of today's four major plastics had begun during 1930–1940 (Jadhav et al. 2022). Microorganisms are easy to mutate and if such microbes in some instances transfer mutated gene by horizontal gene transfer to germline or gonad cell of other living being then such organisms also evolve likewise (So et al. 2022). In such a manner, symbiotically associated microorganisms play significant role in marine ecosystem balance thereby facilitating appropriate functioning of marine ecosystem. Chances of getting plastic-consuming marine animals would be higher at plastic contaminated marine environments such as North Pacific Ocean, Indian ocean, North Atlantic Ocean, South Pacific and South Atlantic oceans since plastic pollutant stress condition is high in these regions. Possible reason behind getting most of the plastic pollutants in these regions is due to formation of ocean gyres. These are circulatory movements of the ocean water as a result of formation of air flow movement in the ocean on account of continuous motion of the earth due to the resultant magnetic field of the earth, which imparted formation of magnetic dipole around axis of the earth, thereby rotation of the earth around its axis occurs. Motion of the earth is also responsible for continuous wave action in oceans. At daytime, low-pressure zones are formed over the ocean surface due to differences in heat capacities among different states of matter as solids have high heat capacity than water and water has high heat capacity than air. Hence air moves from coastal area to sea to open ocean at daytime while reverse in direction at nighttime, that is from open ocean to coastal area.

Solute particles in the ocean have tendency to move continuously from a region of higher concentration to a region of lower concentration to maintain their equilibrium. These movements established ocean currents. A huge number of different particles form continuously within the ocean in different locations. Excessive diversity among ocean particles generates complex pattern of ocean currents in all directions. Likewise, microplastic particles are present ubiquitously in the world oceans (Zhang et al. 2017). Microplastic particles also have to sense electric force or current pattern in ocean as their movement from higher concentration to lower concentration. Marine animals pass through huge amount of water in the ocean. Simultaneously the water that enters the body is filtered out to retain necessary food that is present within the water. In this way microplastics with biofilm on its surface would enter and are retained in the body in huge amount. Since not all marine animals have mechanism to degrade the plastics, these microplastics might accumulate within the organism and transfer from one organism to another in the trophic level. Therefore, bioaccumulation of microplastics takes place and also as it passes to higher trophic level, it magnifies there; since large marine animals consume huge number of small fishes and along with them accumulated microplastics also passes to large animals, hence biomagnification of microplastics happens there (Akhbarizadeh et al. 2019). Bioaccumulated plastics also decrease the reproduction capability of marine and freshwater fishes (Assas et al. 2020). Reproduction fitness is very necessary for an organism along with survival fitness to sustain in the given ecosystem. Biofilm on the accumulated microplastics may interfere the necessary microorganisms or microbiota available within the body of marine fishes. This biofilm would also contain pathogens as it continuously passes from different locations within the ocean. Such pathogens could be able to transfer from one location to other or even one country to another by the transport of plastics in the ocean. Such new pathogens would be harmful to marine animals at the new location. Hence interaction of plastic-associated biofilm with marine animals would be harmful. Also, those marine organisms not able to consume plastic particles are thrown out of body by any of the means.

Each and every planet in the solar system possesses their own orbit around the sun in a specific and well-defined manner where they continuously revolve as mentioned in Fig. 12.2. Position of the planets and their corresponding gravitational field and its impact on other planets are responsible for their movement around the sun along with the centripetal forces exerted on the planet toward sun due to the gravitational field of sun. Formation of magnetic dipole is the reason for the generation of gravitational field of that planet. North pole attracts south pole and vice versa in magnetism, a phenomenon which is responsible for rotation of earth around its own axis. Revolution axis of the earth around the sun has been fixed in such a way

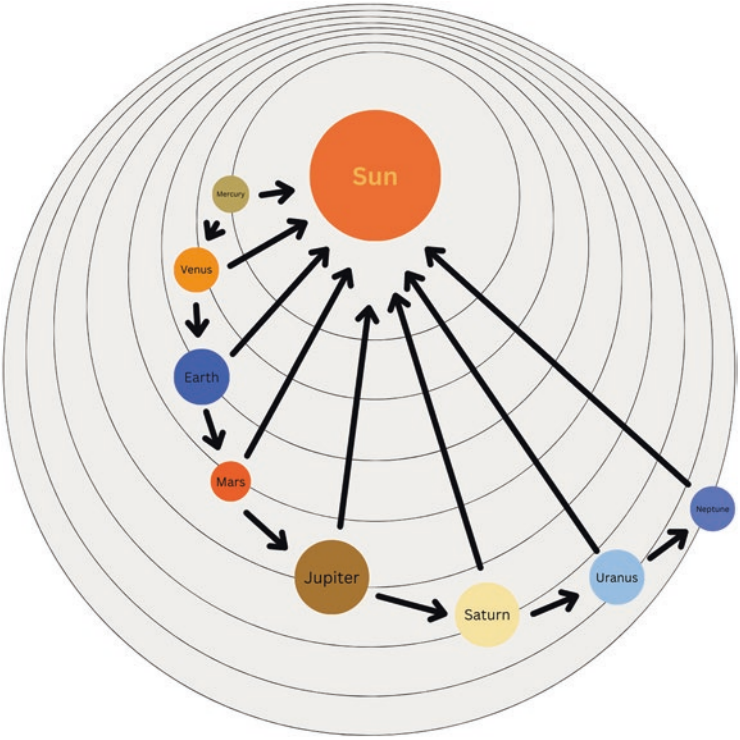

Fig. 12.2 Revolution axis of planets around the sun in the solar system

that different regions on the earth experience different climate conditions which also depend upon rotation of the earth. Some part of this revolution axis must be slightly near to the sun (due to eliptical shape of revolution axis of earth around sun) hence responsible for summer season, while other part away from the sun and could be responssible for winter season. These differences in climate must be responsible for the generation of variation in air flow pattern and circulatory movements in the ocean. Also, these climatic variations would create a vast diversity among ocean ecosystems. Due to the spherical shape of earth, tropical region experiences most heat from the sun followed by temperate and the polar region gets the least heat. A large evaporation of the ocean water occurs in the tropical region, especially in the summertime of year. This evaporation of water generates most of the clouds in the tropical region responsible for creation of low-pressure zones toward temperate and polar regions with respect to clouds; therefore continuous movement of clouds from tropic to temperate to polar occurs for maintaining equilibrium. Rain starts occurring in a particular region once these clouds become cooled. Due to rain, ocean water becomes more diluted in temperate region whereas evaporation decreases the amount of water and increases particle concentration in the tropical region. Therefore, particle movement starts from their high concentration to low concentration, that is from tropical region to polar region. Also, presence of sunlight itself is responsible for the creation of exceptionally large diversity in the tropical region which further contributes to a great amount of particulate matter. Marine algae and photosynthetic microorganisms' response to sunlight is the photoautotrophic process of production of their own nutrient of food. However, presence of microplastics would interfere the process of photosynthesis among photosynthetic bacteria such as *Prochlorococcus* (Tetu et al. 2019). *Prochlorococcus* is the most abundant photosynthetic bacteria which contributes most of the oxygen that we breathe. Plastic additives or the substances that are utilized during production of plastics are mainly known to inhibit the photosynthesis process.

As mentioned before in this section that the oceanic gyres and thermally influenced oceanic currents because of particle or solute transport from its higher to lower concentration are major reasons behind the transport of plastic pollutants along with other particulate matter from tropical region to temperate and polar regions. This movement especially occurs at the upper surface of the oceanic water. Temperate and polar regions are relatively colder than tropical regions. Therefore, particle movement is faster at tropical regions which are continuously in contact with higher density electromagnetic radiations from the sun. However, particle movement gets slower to temperate and polar regions, respectively; regions which are relatively away from direct solar radiations therefore comparatively colder than tropical regions. Due to the less availability of sunlight, primary productivity is already lower in temperate and tropical regions at upper surface water and in that the presence of plastic pollutants would also interfere with the photoautotrophic process or photosynthesis. This also lowers the primary productivity in colder regions which would significantly affect the ecosystem there. Eventually, particulate matters along with plastic pollutants start sinking to the bottom in the temperate and polar regions as a result of decreasing particle movement and ultimately increasing particle density due to biofilm formation by microorganisms. On the other hand, in the tropical region, due to the continuous availability of solar radiations, upper surface water evaporates and upwelling of bottom water takes place. To fill this gap in the bottom region of tropics, movement of water from adjacent bottom regions, that is bottom water of the temperate and polar regions, occurs. Due to this, a continuous water circulation loop is formed in which surface water along with all the particles moves from tropics to temperate and to polar, matter sinks to the bottom there and bottom water from these colder regions with all matter along with plastic pollutants moves toward the tropical region. Further, this matter eventually starts settling toward the benthic region in tropical region. Hence benthic zone would significantly be affected by plastic pollutants along with other particulate matter in the tropical region. All such matter including plastic pollutants which have not been consumed by any of the organisms or degraded by any of the biological, chemical, or physical means would transfer into the ocean floor geology. Such circulatory movements and settling of large proportion of matter at the tropical bottoms is the key reason while getting most of the petrochemical substances especially in the Arabian countries which is located at the upper tropical region.

12.3 Mechanisms of Plastic Degradation by Microbes

Mechanism of plastic degradation is initiated by interaction between microbes and plastic. Continuous ionic interactions mainly take part in such interactions. In this, flow of electrons occurs from high to low concentration in order to establish equilibrium. This sets a continuous loop of interaction between microbial membrane lipids and plastics in either direction depending upon the concentration of either substrate, that is membrane lipid, or microplastic. Microorganisms start eating plastics to increase their number. As plastic amount decreases due to microbial consumption, competition among different species in biofilm increases for plastic as nutrient. This competition would result in the decline phase among bacteria. This process would be responsible for maintaining equilibrium with respect to microorganisms and microplastics nutrients. Continuous circulation of ocean water and arbitrary mixing of particulate matter including plastic waste and microplastics result in different microorganisms being present within the ocean water column with microplastic particles. This applies the force of interaction among microplastics and microorganisms. These microorganisms form biofilm on the surface of plastic. Magnetic forces of interactions mainly take part in interaction among the microorganisms while they make biofilm. Microbial membrane is equipped with macronutrient such as magnesium and micronutrient such as manganese which are known to have magnetic and paramagnetic characteristics. Microorganisms are also found to secrete siderophores. Siderophores are high affinity iron chelating molecules or proteins that carry iron within the microbial cell. After their transport within the cell, iron molecules make wall for interaction in adjacent to inner microbial membrane within the microbial cell. Therefore, magnesium or manganese ions present on outer membrane lipids or membrane proteins of one microbial cell make strong magnetic interactions with the iron molecules present within the cell of other microbial cells. In this way, complex structure of microbial biofilm is developed on the surface of the plastic (Satlewal et al. 2008; Debbarma et al. 2017). Microbial biofilm may produce interaction among intraspecific, that is same species microbes, or interspecific, that is different species microbes. Once these microbes make biofilm by interacting among themselves and their interaction with substratum, in this case microplastics, they combinedly regulate the expression of genes involved in plastic degradation and believed to behave like eukaryote altogether although they are prokaryote when present in free form and can survive independently. Such type of cooperative phenomenon is necessary among the microorganisms specially to carry out the extremely difficult work. In this case, plastic molecules are very difficult to be degraded or consumed by single microorganism. Therefore, by forming biofilm microorganisms would make this wok easy by combinedly regulating expression of genes required for plastic degradation among every possible microbial cell involved in plastic degradation. Already discussed that many of the microorganisms that are able to mitigate plastics have obtained their nutrients from plastic sources, especially carbon and hydrogen which are the major macronutrients required by any of the organisms, that is why they mitigate plastic. However, other macronutrients like oxygen, nitrogen, phosphorus, sulfur, etc. and micronutrients like iron, zinc, manganese, etc. are also required in sufficient amount for an organism to carry out plastic-mitigation process. However, depending on the nature of an organism which varies by changing environmental parameters, amount of macronutrient and micronutrient required also varies. The natural environmental location, in this case ocean, where microplastic mitigation by microorganisms occurs need not necessarily contain all the required parameters for mitigation of microplastic pollutants. There are microorganisms within the biofilm which may not be able to degrade microplastics directly; however they are consuming plastics degraded by actual plastic degrading microorganisms, and in return they must be supplying other necessary requirement, especially depleted nutrients to actual plastic degrading microbial species within the biofilm. Such interdependent microorganisms from a plastic-associated biofilm are responsible for making plastic-associated microbial ecology very much active. The structure of such biofilms would be continuously changing by changing environmental situations.

Once the microbial species get attached on the surface of plastics and make biofilm by electromagnetic force of interactions, the next task is to degrade plastics or cut it into small pieces. Microbial cells are so tiny in size that large plastic particles cannot be engulfed by it. In order to pass plastic particles within the microbial cell, it should be small enough in size so as to pass by microbial pores or cell surface transporter proteins. For this purpose, plastic degrading microorganisms from the plastic-associated biofilm secrete the extracellular catalytic factors. These catalytic factors might consist of plastic degrading enzymes and plastic solubilizing ions and molecules. Enzymes such as oxidoreductases are well known to be involved in extracellular degradation of the plastics. These enzymes are known to perform the oxidation or photooxidation on the surface of plastics for shearing and tearing of the otherwise compact plastic molecules. Plastic molecules consist of a large chain of hydrocarbons, which are generally made from the chemical treatment of the petroleum hydrocarbons. These plastic polymers are compact and complex in characteristics so that it is very much difficult to degrade by any of the physical, chemical, or biological processes. Oxidoreductases are well known to loosen these stronger covalent interactions within complex plastic polymers to make it simple. Due to such oxidation process, chemical bonds within plastic molecules become weakened and the compact hydrocarbon chains are now open which can easily interact with the other set of enzymes which are similar to PETase (enzyme that degrade polyethylene terephthalate or PET), those explained by Yoshida et al. (2016). These enzymes should be distinct from the oxidoreductases in a way that they are performing plastic degradation process in a specific manner. However, plastic degradation performed by oxidoreductases is nonspecific oxidative plastic degradation. Such set of enzymes involved in the specific degradation of polymer chains must be similar in mechanism to those of DNA degrading nucleases or DNA endonucleases or DNA exonucleases. Endonucleases are the enzymes that degrade the interconnected chemical bond within the polymer, while exonucleases are the enzymes that degrade the chemical bond from the end of the polymer chains. In order to perform their functions by such nucleases enzymes, especially exonucleases, they require at least one open end of the polymer strand where they can bind and cut polymer specifically. The reason while using these specific plastic degrading enzymes by microorganisms is because microorganisms need very short and soluble pieces of plastics so as to pass them by microbial membrane protein transporter, otherwise large uncut plastic pieces cannot be transported within the microbial cell and also, microorganisms do not have any mechanism to engulf particles. A hydrolysis reaction is basically carried out by such plastic degrading enzymes which could be able to generate short stretches of plastic polymers, either three carbon long or more than that depending upon the nature of the enzyme. Once plastic polymers have specifically degraded by these specific plastic degrading enzymes, then short stretches of degraded plastic molecules react with other ions or molecules secreted by plastic degrading microorganisms for the plastic-associated biofilm. The reason of this reaction is making short plastic stretches soluble in nature so that it can be easily transported across the microbial membrane and thereby metabolize further within the microorganisms present in either the plastic-associated microbial biofilm or single species of microorganism or pure microbial culture which is degrading plastics in a controlled laboratory condition. After their passage across the microbial membrane, these soluble plastic pieces are catabolized other set of intracellular microbial mechanism. This mechanism should involve either transport of the soluble plastic molecules into the microbial tricarboxylic acid cycle or TCA cycle or citric acid cycle or Krebs cycle for simultaneously removing carbon in the form of carbon dioxide thereby completely metabolizing plastic polymer and generating other essential intermediate metabolites or involvement of other potential plastic degrading enzyme decarboxylase as mentioned in Table 12.1. The energy dissipated by such enzymatic reaction would be stored by microorganisms in an energy storage currency molecule such as Nicotinamide adenine dinucleotide (NADH), Flavin adenine dinucleotide (FADH), or Adenosine triphosphate (ATP) which can be further utilized by microbial synthesis or constructive metabolism. Intermediate metabolites in the central tricarboxylic acid cycle are continuously generated in the

Table 12.1 List of potential plastic degrading enzymes and their probable functions

Serial number	Name of the plastic degrading enzyme	Function of enzyme
1.	Extracellular oxidoreductase	Nonspecific oxidation or photooxidation of polymer surface
2.	Plastic degrading site-specific enzymes For example, PETase	Site-specific degradation of the polymer chain by an enzyme-catalyzed hydrolysis reaction
3.	Decarboxylase	Sequential removal of the carbon dioxide from the hydrocarbon chain of plastic polymer and energy obtained from this reaction should be stored in NADH, FADH, etc
4.	Dehydrogenase	Largely required in the generation of energy currency molecules from plastic catabolism such as NADH, $FADH_2$, etc
5.	Synthase	Group of enzymes essential for the synthesis of biomolecules from plastic such as carbohydrates, lipids, nucleic acids, vitamins, cellular skeleton, etc. by constructive metabolism

process and are well known for their role in the other microbial biomolecular synthesis processes. The major reason for consuming plastics as nutrients by any of the microorganisms from the biofilm is for the growth and the multiplication of the same microorganisms. Major biomolecules required for the multiplication of microorganisms are carbohydrates, proteins, lipids, nucleic acids, vitamins, and cellular skeleton. These biomolecules should be acquired from the plastic consumption process by any of the microorganisms in the plastic biofilm. Enzymes such as dehydrogenases are primarily involved in the generation of NADH or $FADH_2$ energy currency molecules, while large group of synthase enzymes are required for the generation of various different microbial biomolecules required for the multiplication of microbial cells. Therefore, the major role of plastics as microbial nutrient source is to established microbial growth and multiplication.

12.4 Microplastic Remediation Strategies

Marine environment in the coastal area represents tidal action due to the differences in the position of the earth with respect to the moon and their corresponding gravitational forces. In 24 h of time, two low tides and two high tides are experienced in an alternate manner and consecutively by all the marine coastal areas on the earth. All the marine pollutants including plastic waste have been thrown out of ocean to the coastal area as a result of ocean current action; therefore by only seeing the coastline one can state how much polluted that marine region is. It happens like this because as plastic waste enters the ocean by various different routes as explained earlier, it would cut into small pieces by various different chemical, physical, and biological actions. For example, some chemicals secreted by an organism would degrade or cut plastics in small pieces; different physical actions like photocatalysis by solar radiations including oxidation of plastic particles, also continuous circulation in ocean water exerts shearing and tearing of plastic materials; and some biological organisms like Antarctic krill could consume the plastics as nutrient source and can also cut them into small micro or nano pieces (Dawson et al. 2018). Such small pieces of plastic particles such as microplastics and nanoplastics are ubiquitously distributed all over the ocean in the world along with other particulate organic matter particles. Particulate organic matter can be utilized in the ecosystem by next higher-level organisms in the trophic level thereby distributing their biomass and energy. However, microplastics are the pollutants which could be present in the environment for years, even thousands of years as such and might harm various different biological organisms which have not developed a mechanism to consume them as a source of nutrient. Also, remnants of the plastic debris which have not been degraded completely by any of the physical, chemical, or biological means would gather in the environment, including oceans, and interfere with normal environmental processes.

Microplastic particles are in a continuous loop of movement in order to establish an equilibrium as a result of the ocean currents and thermally influence ocean water circulations, as most of them settle at the bottom as sediments in the ocean water. In their movement in ocean waters, microplastic particles would interact with many of the marine microbes which form a biofilm on it. Due to the formation of biofilm, density of microplastics alters, either increases or decreases, depending on the number of microorganisms attached or amount of microplastics consumed. Likewise, because of tidal actions, during high tides in ocean waters, most part of the coastline experiences sea water or ocean water coverage. Also, coastal regions are exceptionally low in depth. Therefore, almost all microplastics in the high tide water would settle at the coastal sediments. As ocean waters with extremely low amount or concentration of microplastics have returning back to the open ocean during low tide, it again concentrated with microplastics from open ocean to attain microplastic homogeneousness and evenly distributing microplastics in ocean water. Now, the major trick is to remove microplastics from coastal sand from all over the world for making it ready to be filled with huge amount of microplastics again from the open ocean. Since after removal of microplastics from coastal sand, it would have extremely low concentration of microplastics as compared to open ocean water. Therefore, microplastics in open ocean would move again from their high concentration to low concentration coastal sand. However, it is very much necessary for periodically removing or mitigating microplastics from coastal sand once it attains homogeneous microplastic concentration all over the coastal sand areas and ocean waters.

In this way microplastic pollutants should be effectively and quickly removed from the oceans around the world, mainly during the low tide period. Coastal marine sediments with microplastic pollutants can be effectively used for construction work. Therefore, after removal of upper sediment layer along the coastline, the sediment layer below that has comparatively very less microplastic pollutant would now be exposed to ocean water during high tide. This sediment layer would again be filled with microplastic pollutants coming from open ocean due to ocean current action in correspondence with microplastic particles. Once microplastics spread ubiquitously and equally along open ocean and coastal sediments, the amount of which can be checked by any of the available methods discussed by Prata et al. 2019. Again, whenever homogeneousness has been achieved among coastal sediments and open ocean water, upper sediment layers of coastal areas all around the world need to be removed and can be remediated into construction work suitably or in an environment-friendly way.

Another potential strategy for the mitigation of microplastics from the oceans is by the implementation of soil-based and water-based reactors as shown in Fig. 12.3, in the similar way mentioned by Jadhav et al. (2022), in the review article on plastic-mitigation strategies. However, external plastic source as a nourishment source (as mentioned by Jadhav et al., 2022) need not require to provide in such reactors, because it must implement for the purpose to mitigate oceanic microplastics. These soil-based and water-based reactors must be installed in a controlled manner so that different organisms required in such system should disturbed or thrown out the system by external forces exerted by sea water. As already discussed, the sediments in the intertidal regions would contain lots of microplastic pollutants due to the action of ocean currents. If such soil-based and water-based reactor systems are operated

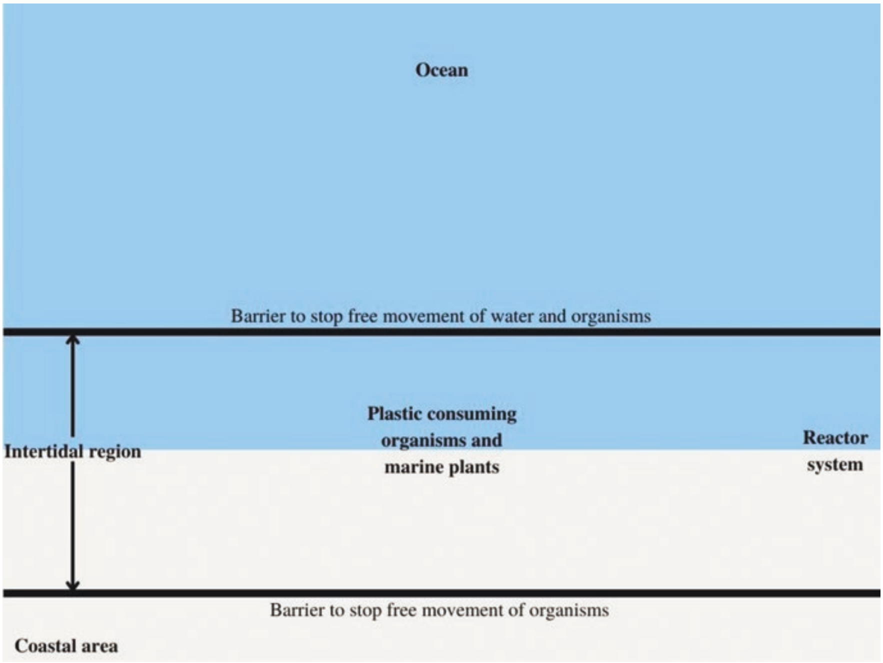

Fig. 12.3 Illustration for natural reactor system in the remediation of microplastic pollutants from the intertidal regions of coastal area

in the intertidal regions of the selected marine coastal areas which experience continuous flow of microplastics from all the possible locations of the oceans, then this task of oceanic microplastic remediation would be easy. Biological organisms like marine worms or invertebrates which can consume plastics as a nutrient source have to be utilized in the initial stage in this reactor system. Such organisms would utilize microplastics from intertidal regions of the selected coastal regions where implementation of such reactors are planned, thereby lowering microplastic amount or concentration in those regions. This would lead to the transfer of microplastics from oceans toward such coastal regions where these natural reactors are operating because of oceanic currents, from high microplastic concentration in ocean to relatively low microplastic concentration in such coastal regions due to the continuous consumption of microplastics there by selected marine worms. This process would set the continuous flow of microplastics toward reactor regions, and which would eventually remove the microplastic concentration from the ocean. For this purpose, marine worm species could be probably isolated from the benthic regions of tropical oceans. Because most of the plastic pollutants from the oceans of the world have settled down toward the benthic regions of the tropical zones as discussed in the earlier section, that is, “Microplastic Pollution in Marine Ecosystem and Its Impacts,” of this chapter. Thermally influenced ocean water circulation due to the particulate movement which is a result of oceanic currents is responsible for the settlement of most of the plastic pollutants including other particulate matter at the benthic regions of the tropical areas. Also, many of the coastal regions at temperate and polar oceanic areas would experience large plastic pollutants including other particulate matter since the surface water movement has continuously occurred from tropical to temperate and polar regions. These colder regions especially temperate regions would contain wide variety of diversity in different organisms including marine plants, animals, and microorganisms due to the availability of suitable atmospheric conditions for the growth of such biological organisms. The availability of large plastic pollutants at the surface water near coastal regions of such temperate areas would help in the acquirement of the plastic degrading capability among many of the diverse species at the temperate regions. Due to this, selected marine invertebrates, those which can degrade or consume plastic as nutrient with faster rate would have isolated from benthic regions of tropical marine environment and coastal or estuarine regions of temperate environment. Along with invertebrates, some large marine plants, including marine algae would also possess the ability to consume plastic directly or in association with microorganisms or other invertebrates symbiotically. Therefore, in the initial phase of implementation of soil-based reactors at selected coastal regions which experience most of the microplastic pollutants, isolated plastic degrading marine invertebrates are required to be provided with such reactors. Such marine invertebrates would consume the microplastic pollutants continuously within such soil-based rectors and in turn their secretion as a part of metabolism of plastic would increase the fertility of the soil at those regions. Further, essential marine plants isolated from plastic contaminated zone could probably grow at those regions where these soil-based reactors are implemented in order to utilize the fertility of the soil, which was increased by selected invertebrate species. In this way, a continuous flow of microplastics should occur from the open ocean to coastal areas where soil-based reactors have been implemented and eventually the biomass of such microplastic pollutants would transfer and be utilized in the growth of large marine plants, thereby generating large and spectacular marine forests within such regions where reactors have been installed.

Finally, microplastic remediation strategy might include the implementation of selectively semi-permeable membranes at coastal regions where most of the microplastic pollutant transfer would occur from open oceans. Such microplastic pollutants would be trapped within such membranes which can be removed further and recycled by any of the suitable methods. Such membranes should made by the material which can possess the strong and appropriate force of ionic interaction with microplastic pollutants only, however leaving other matter along with water without disturbing. Thus microplastic pollutants would be quickly removed by this strategy, in which after the interaction of microplastics with membranes, water of the ocean is left with negligible microplastic concentration which would again be filled with microplastic pollutants for attaining equilibrium corresponding to microplastic pollutants. Once again when ocean water passes by the membranes during tidal action, it leaves microplastic pollutants there to be trapped within the membranes and therefore eventually, however with quicker rate, plastic pollutants can be removed from the oceans.

12.5 Challenges in Developing Microplastic Bioremediation Strategies

The major challenges in developing three different microplastic bioremediation strategies mentioned in the earlier section include the following:

1. Decreasing sand level along the coastline due to continuous removal of sand from the coastline containing microplastic pollutants. Marine sand mainly consists of salts and marine pollutants. Soluble salts can dissolve in marine water, however insoluble salts precipitate. This process occurs by chemical reaction of two or more inorganic compounds. Such inorganic minerals are continuously supplied by river waters that meet the oceans. During their continuous flowing from mountains and plains, rivers carry large amount of minerals from the weathering of rocks and soils that come in the path of the river flow. Along with these minerals supplied by river water, marine environment also contains their own particulate organic as well as inorganic matter. This matter is either eventually transported toward the coastal region and gets settled in the sediments or gets dissolved in the ocean water and distributed all over the marine environment. Large amount of such inorganic compounds or elements can be supplied by the precipitation of dissolved compounds near the coastal areas from where the sand has to be removed. Also, by precipitating any compound or pollutant, toxicity of the marine water could be altered. Finally, by this way, such coastal sediments with microplastics could be removed and utilized for the construction work in the same manner that has been discussed earlier. This again decreasing coastal sediment level can be maintain by increasing sediment levels by precipitating dissolved molecules or elements.
2. In the second oceanic microplastic remediation strategy mentioned in the earlier section, it would be difficult to have control on the different organisms or marine worms or invertebrates supplied to the soil-based reactor, due their tendency to flow toward the ocean water. Also, adaptation and survival of such organisms at the new location is also a challenging task. To overcome this, a well-controlled environment including dam system or barrier system needs to be implement at selected coastal regions where soil-based and water-based rector system must be installed, and large marine forests must be developed. Height of this barrier system should be maintained at such a level that it will allow only flow of water from the ocean to the coastal area once the level or height of ocean water increases, especially during high tide. Once the opposite site of the barrier, that is the coastal region, is filled with water, it should not exceed the height of the barrier or dam, otherwise plastic-consuming invertebrate species would be flown out of the reactor toward the ocean. In the meantime, continuous monitoring of the presence of microplastic pollutants in the water of the reactor system is required. Once microplastic pollutants become negligible or below the required limit, then this water in the reactor system must be replaced by water from the ocean. Leaving of the microplastic depleted water toward the ocean should be during the low tide period. This can simply be done by opening the gate of the

dam or barrier. While doing this, all the plastic-consuming invertebrates within the reactor should gather at a particular location so that it could leave the reactor. This reactor can also be turned into invertebrate aquaculture system. Adaptation and survival of species within the reactor also is a challenging task. However, isolation of such plastic-consuming species from the nearby location of the region where this reactor system must be installed should be helpful.

3. While developing selectively semi-permeable membranes, one should know the exact interaction forces among plastics and microbes. Based on the plastic-microbial interaction knowledge, a suitable material can be prepared which can specifically interact with microplastics from the ocean leaving other particles as such. However, implementing such nets along the selected intertidal areas which experience gathering of large number of microplastic pollutants from all dictions in the ocean is itself a costly process and their continuous monitoring and maintenance are also required. Also, removal of the attached microplastic pollutants from the membrane is also challenging. This removal of microplastics from the membrane can be done by applying liquid having higher ionic concentration than membrane, although more research is required in developing this membrane-based technology of microplastic remediation from ocean.

12.6 Conclusion and Future Prospects

This chapter mainly focuses on the microplastic-mitigation techniques. Industrial production of the plastics started in the early twentieth century, since then revolution in the field of plastic has begun. Due to user friendly properties, rate of production of the plastics has been continuously increasing. However, poor plastic-waste-handling practices responsible for the generation of the huge plastic pollution have spread all over the planet from terrestrial to marine environment. Presence of plastic pollution may interfere with the normal environmental processes and has resulted in many of the health-related issues among natural flora and fauna, including microbes. These plastic particles are exceedingly difficult to degrade and may transfer from one organism to another in the trophic level. Due to the normal physical, chemical, and biological processes in the marine environment, shearing and tearing of plastic pollutants generate microplastics in the marine environment, which is difficult to mitigate. Presence of microplastics in the marine environment is also found to be interfering with the process of photosynthesis thereby decreasing oxygen-production capacity among some of the major photosynthetic cyanobacteria and algae. Also, accumulation of the plastic pollutants in the natural marine environment might be responsible for the generation of new plastic degrading organisms by probable evolution among the marine species. Due to the presence of the ocean currents in marine water, particulate matter including plastic or microplastic pollutants are transported from their high concentration at ocean to low concentration at coastal sediments. This process would transfer large microplastic pollutants from open ocean to coastal regions and would settle there at coastal sediments. Such microplastic pollutants could be removed by any of the strategies, for example, coastal sediment removal or establishment of natural reactor at intertidal regions consisting of plastic degrading organisms or plants or installing plastic collecting filters at intertidal regions. Removal of microplastic pollutants from coastal regions creates large concentration gap in microplastics; therefore its continuous transport occurs from ocean to coasts which can be eventually removed and due to this, microplastic concentration becomes lesser in the ocean. Removing and utilizing coastal sediments containing microplastics for construction would decrease the sediment height at coastal regions; however continuously precipitating dissolved molecules or elements from coastal regions also maintains the level of sediments and simultaneously decreases the microplastic pollutants from the ocean. Also, precipitation of particular compound or pollutant would be able to alter or maintain the toxicity of the marine water. On the other hand, while installing natural reactors at intertidal regions for the establishment of marine forests, it would be better to have control on plastic-consuming organisms which would otherwise leave the region, and therefore suitable barrier systems need to be implemented. Marine microbes also play a significant role in the degradation of plastics. Due to continuous circulation and mixing of the ocean water, it encounters various microorganisms with microplastics. Microorganisms interact with microplastics by electrostatic force of interactions and microorganisms make biofilm by interacting among themselves by magnetic forces of interactions. Microbial secretions consisting of plastic degrading enzymes, ions, and elements are responsible for extracellular degradation of plastics and making them soluble. Soluble plastic mix would be easily transported across the microbial membrane which metabolize further within the microbial cells. For decreasing the generation of plastic pollution, awareness in the people against the utilization of plastics should increase. Generation of plastic pollution has to stop completely. While implementing potential microplastics remediation strategies, continuous monitoring of the level of microplastic pollutants should be done from the coastal and open ocean sediments and water samples from different regions of the world until the extent of the microplastic pollutants have decreased from the marine ecosystem. Due to this, we will come to know how much of microplastics have been mitigated in the natural ecosystem after implementing the microplastics remediation strategies.

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13

Microplastic Pollution in Aquatic Environment: Ecotoxicological Effects and Bioremediation Prospects

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Abstract

Microplastic pollution, or commonly known as "White Pollution," has been drawing great interest as a new omnipresent environmental pollution. Plastic is potentially used in all kinds of industries, and their irresponsible waste disposal is the reason behind both aquatic and terrestrial pollution. The degradation period of plastic is a long-term process and occurs due to solar radiation, thermal oxidation, thermolysis, photo-oxidation, etc. The end result is tiny particles known as "microplastics" (diameter less than 5 mm) and "nanoplastics" (diameter less than 100 nm). MPs and NPs act as carriers for various chemical and biological toxins and are ingested by zooplankton, bivalves, echinoderms—these move through the food web and damage the internal digestive system of living organisms. To eliminate the ecological threats, a bioremediation system is urgently required. Microorganisms like *Ideonella sakaiensis* are known to have enzymes that degrade plastic and can be useful as remediators. This chapter discusses the sources and transports routes of microplastics in the aquatic environment and their ecotoxicological effects on living organisms. Moreover, it highlights the bioremediation of environmental microplastics, its mechanism, and some necessary environmental policies to prevent it.

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Microplastics · Aquatic environment · Ecotoxicological effects · Bioremediation · Environmental policies

13.1 Introduction

Plastic (from Greek "Plastikos") means malleable or moldable. It is considered an integral material at present due to its durability as a key attribute (Sharma and Chatterjee 2017). It is a versatile material due to its strength, low price, lightweight, and resistance to corrosion. They are known to have higher electrical and thermal insulation values. Currently, the most popular synthetic plastic materials are polypropylene (PP), high- and low-density polyethylene (PE), polystyrene (PS), polyvinyl chloride (PVC), and polyethylene terephthalate (PET)—all together, they contribute to 90% of total production (Do Sul and Costa 2014).

Since the middle of the nineteenth century, several tonnes of plastics have been manufactured. Fast-forwarding to this day, plastic has gained a prominent place in the consumer market and is a ubiquitous component of modern life. According to the current trends, 25 billion of global plastic production has been predicted by 2050 (Yang et al. 2015). Tons of discarded plastics reach the environment through recycling, landfilling, or incineration and accumulate in the ecosystem. Eventually, plastic loses its mechanical integrity through biotic and abiotic pathways, and "microplastic" (MP) is introduced into the environment (Xu et al. 2020).

Microplastic is a predominant pollutant in both terrestrial and aquatic environments. This contaminant ends up in the environment and suffers fragmentation and degradation. Microplastic (MP) is defined by size ranging from 100 nm to 5 mm, and the size of nanoplastic (NP) is below 100 nm (Tang et al. 2021). Evidence of microplastic ingestion by marine organisms and subsequent transfer through trophic levels has been reported (Setälä et al. 2016; Pirsaheb et al. 2020). Nanoplastics are more hazardous in comparison as they are permeable to biological membranes.

This chapter will summarize the multidisciplinary overview of microplastic pollution in the aquatic environment. Though available literature relevant to this topic is vast, we will focus on some particular aspects such as classification and sources of microplastic and its toxicity in the environment.

13.2 Classification of Microplastics

13.2.1 Based on Size

Although Thompson et al. (2004) were the first to report microplastics, they did not specify the size based on which microplastic could be identified. However, several authors have set limits to distinguish between microplastic and macroplastic. Macroplastics are discarded plastic chunks visible to the naked eye among other sand and gravel (Malankowska et al. 2021). This criterion is of importance, because it helps differentiate between small macroplastic (one that can be detected using simple techniques) and microplastics (due to their much smaller size, it requires detection through optical instruments) (Costa et al. 2010). Collection, estimation of prevalence in the environment, and characterization of microplastics have been challenging due to their minute dimensions.

According to IUPAC (International Union for Pure and Applied Chemistry), the dimension of microparticles ranges between 0.1 and 100 μm (Vert et al. 2012). Nevertheless, in recent years, researchers have agreed that plastic particles having the longest dimension below 5 mm will be considered microplastic. Subsequently, countries of the Asia-pacific region, the European Union, the European Chemical Industry, and the EPA (United Stated Environment Protection Agency) collectively identify this criterion to be specific for microplastic based on various scientific reports (Wright et al. 2013). Along with microplastics, which are extensively explored as ocean contaminants, Andrady (2011) introduced a new concept, namely “nanoplastic,” in 2011, which is identified as a product of the fragmentation of microplastic. After a few years, the upper limit for the size of nanoparticles was set to be 100 nm (Jambeck et al. 2015). This particular restriction is significant as plastic particles below this limit could have the potential to disrupt the cell membrane, which is not possible in the case of microplastics (Nguyen et al. 2019). With time and more investigation, many scientific communities have agreed on this size limit for nanoplastics. However, due to the scarcity of reliable literature, it is quite challenging to establish a perfect size limit and the hazardous effects of nanoplastics on living organisms.

13.2.2 Classification According to Origin

13.2.2.1 Primary Microplastics

Primary microplastics are defined as those which were originally fabricated as microplastics (Boucher and Friot 2017). These particles are added to various products such as air blasting technology, cosmetics, and scrubbers for facial cleansing (Derraik 2002). Other products like plastic capsules and vectors for medicines are primary microplastics themselves (Lindeque et al. 2020). Auta et al. (2017) gave other examples of products containing microplastic like cleanser, peelings, shower gels, eye shadow, foundation, mascara, blush powder, nail polish, hair colors, deodorants, shaving creams, bath lotions, baby products, sunscreen, mosquito repellants, etc. This depicts that personal hygiene and beauty-care industry, followed by the detergent industry, are the prominent manufacturers of products with added microplastic. According to a report, polyurethane is one of the common polymers that is manufactured as microplastic and it contributes to almost half of the total microliter coming from the cosmetic industry (Lei et al. 2017). These products are used by the population at large and lead to ultimate release into the environment. Other than this, microplastics are released into the environment during fabrication and maintenance of larger plastic products, that is, tires, synthetic textiles, paint, etc. (Shahnawaz et al. 2019).

13.2.2.2 Secondary Microplastics

Small plastic particles generated due to the fragmentation of discarded microplastic through biological, chemical, and mechanical processes are defined as secondary microplastics (Shahnawaz et al. 2019). The degradation process involved are biodegradation, thermo-oxidative degradation, photodegradation, mechanical weathering, etc. (Avio et al. 2017) (discussed in this chapter in another section). The breakdown of macroplastics increases their amount in the aquatic environment and becomes detrimental to aquatic organisms. Due to mass reduction in size, secondary microplastics get easily ingested in living organisms, making them susceptible to hazardous consequences (Law and Thompson 2014).

13.3 Distribution of Microplastic in Aquatic Environment

Microplastic pollution has heavily affected the aquatic environment. Reports of its presence in rivers, lakes, lagoons, coastal areas, and estuaries are available in the literature (Jiang et al. 2019; He et al. 2021; Sighicelli et al. 2018; Free et al. 2014; Quesadas-Rojas et al. 2021; Edo et al. 2020; Hantoro et al. 2019; Zhao et al. 2015). Microplastics are transported through the actions of rivers, wind, and ocean currents to beaches, shorelines, subsurface waters, and deep-sea sediments in remote locations (Eerkes-Medrano et al. 2015). The concentration of microplastics varies as per ocean currents distribution, depending on particle density and location of the sources. The consistent and buoyant nature of the particles helps them get dispersed widely through hydrodynamic processes (de Carvalho and Neto 2016). According to Cózar et al. (2014), the concentration was higher in the convergence zone of ocean surfaces. The sources and impacts of microplastics in aquatic environment are depicted in Fig. 13.1.

The abundance of microplastics in the aquatic ecosystem has been depicted through various surveys, including the polar region (Barnes et al. 2009), midocean islands (Martins et al. 2020), and deep-sea (Claessens et al. 2013). The omnipresent pollution by this contaminant in the Northeast Atlantic Ocean was demonstrated by Lusher et al. (2014). The average concentration of plastic was 2.46 particles m^{-3}. According to Desforges et al. (2014), the subsurface water layers of the northeastern Pacific Ocean contained microplastics with an average concentration of 4600 particle m^{-3}. According to their study, off-shore Pacific waters had microplastics in density ranging from 8 to 9200 particles m^{-2}, which was found to be increased by sixfold on West Coast Vancouver Island. The average abundance of the contaminating particles were in the range of 0–1.31 particles m^{-3} and 0–11.5 particles m^{-3} in the pelagic water layer of the Arctic Sea and south of Norway, respectively (Lusher et al. 2015a, b). The analysis of the particle composition depicted that they might be end- products from macroplastic degradation or wastewater effluent. Isobe et al. (2015) recorded a total particle density of about 1.72 million pieces km^{-2} in seawaters around Japan and East Asia (10 times and 27 times greater than the North Pacific and world oceans, respectively).

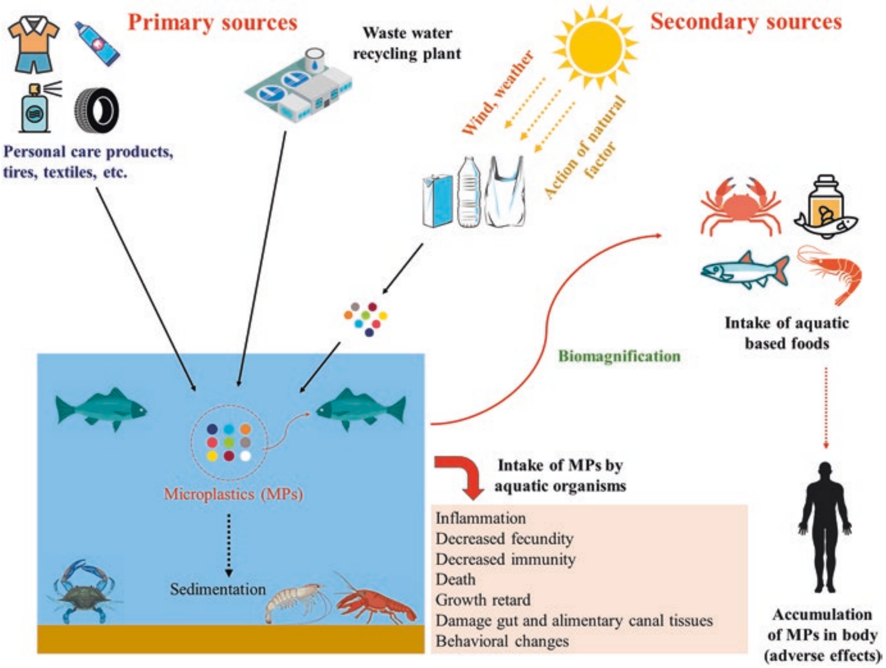

Fig. 13.1 Sources and implications of microplastics in aquatic environment

From the polluted subsurface waters, these particles accumulate in the sediments due to sinking. Various literatures support this theory and have reported high concentrations of plastic particles (in the range of 770–3300 plastic items kg^{-1}) in the sediments of the Rhine estuary and Wadden Sea (Cole et al. 2011). Microplastics concentration was denser in beach sediments (340.7–4757 particles m^{-2}) in comparison to the water column (204.5 and 1491.7 particles m^{-3}) on the south-east coast of South Africa (Nel and Froneman 2015). Fauziah et al. (2015) conducted a study at the sand beaches in peninsular Malaysia to quantify microplastic debris and found 2542 pieces (265.30 g^{-2}), which is an alarming number from a total of six beaches. Estuarine sediments from Vembanad lake of India (which is also a Ramsar site) were investigated to quantify microplastic particles by Sruthy and Ramasamy (2017). The range was 96–496 particles m^{-2} and the dominant polymer component for the pollution was low-density polyethylene.

In comparison to the marine environment, information regarding microplastic pollution in the freshwater environment is quite scarce. The source and transportation routes of this pollutant in this environment are diverse. Terrestrial, glacial, lacustrine, and marine environments are connected through the fluvial system, comprised of running water bodies. Rivers and streams play a highly dynamic role in accumulation and transportation of microplastics. They are connected with various pathways and release routes for the contaminant and are responsible for subsequent pollution. Jiang et al. (2019) investigated the pollution level in five rivers on the Tibet plateau. The surface water had a higher concentration of microplastics

(483–967 items m^{-3}) than the sediment samples (50–195 items kg^{-1}). Similar reports have been found in Indian rivers like Nethravati and Alakananda stretch, where sediments had less concentration of microplastics (Amrutha and Warrier 2020; Chauhan et al. 2021). On the other hand, Li et al. (2020) have depicted that the flowing water in the river might have less concentration of microplastic than the sediments; furthermore, sediments act as a temporary storage space for the contaminants. For example, during a study in Pearl river, China, it was found that sediments contained a higher density of microplastics, that is, 685 items kg^{-1}, in comparison to the column water (Fan et al. 2019). Hence, it is quite difficult to correlate the microplastic concentration in flowing water and static sediment. Lakes are another important component of the freshwater system. They receive microplastics through industrial effluent, surface runoffs, wastewater plants, etc., and rivers are the main carrier route of it. Lakes are complex systems that can potentially transport, disperse, or accumulate contaminants. Su et al. (2016) reported that the concentration of microplastics in Taihu lake, China, was 3.4–25.8 items L^{-1} and 11.0–234.6 items L^{-1} in surface water and sediments, respectively. Reports of heavily contaminated Lake Victoria, Africa, have been published (Egessa et al. 2020). The major pollutant resulted from microplastics' fragmentation, and the prime contributor was polyethylene (60%). Several studies have depicted a higher level of pollution closer to the population centers and have pointed out surface currents as the reason behind the dispersion of microplastics in the surface waters (Eriksen et al. 2013; Fischer et al. 2016; Dusaucy et al. 2021).

13.4 Degradation of Plastic in Environment

One of the prime features responsible for the versatility of a few synthetic polymers is resistance to environmental processes. However, this particular fact is the reason behind the very slow degradation process and longer residual periods for plastics in the environment. Plastic has hundreds or even more years of longevity depending on its properties and surrounding environmental factors (Anderson et al. 2016). Degradation is a physical or chemical transformation resulting in reduced molecular weight of polymer under environmental factors such as heat, light, moisture, or biological activity (Shah et al. 2008). The physical integrity of a polymer is affected by its molecular weight, and significant degradation of a polymer can weaken the material due to bond scission. Extensive degradation of plastic can even fragment it into powder form upon handling. Even these fragments can undergo further degradations through biological processes and result in "complete mineralization" (Urbanek et al. 2018). The degradation process is categorized into abiotic and biotic processes based on the nature of the causative agents.

13.4.1 Abiotic Degradation of Plastic

Abiotic degradation refers to changes in physical or chemical functionality of plastic due to the action of abiotic parameters such as air, water, light, heat, and mechanical processes. Usually, abiotic degradation occurs prior to biodegradation due to the scarce bioavailability of macroplastics (Andrady 2011).

13.4.1.1 Photo-oxidation of Plastic

Photo-oxidation is defined as the process of degradation of polymer by the activity of light. It is one of the primary factors that cause damage to the polymer in ambient conditions. Many synthetic polymers are susceptible to decomposition initiated by visible light and UV rays. Mainly, UV-A rays having medium energy (wavelength: 315–400 nm) and UV-B rays having high energy (wavelength: 290–315 nm) determine the lifespan of plastic in the outdoor environment (Jensen and Kops 1980). Most synthetic polymers are prone to absorption of high-energy radiation coming from the UV section of the spectrum, leading to activation of electrons and resulting in cleavage, oxidation, and decomposition. Degradation of microplastic occurs in the ether portions of soft fragments, and products with ester, formate, aldehyde, and propyl groups are formed (Singh and Sharma 2008). Photodegradation can alter plastic's physical, optical, and visual (yellowing) properties (Martin et al. 2003).

Ultraviolet radiation can successfully cause the cleavage of C–C bonds. The most damaging wavelength of UV radiation for a particular polymer depends upon its bond structure, and therefore, the value varies for different polymers. The required wavelengths for degrading polyethylene (PE) and polypropylene (PP) are 300 nm and 370 nm, respectively (Fernando et al. 2007). PE and PP absorb radiation, generating free radicals, which form hydroperoxides, the formation of which leads to the breakage of double bonds present in the backbone chain and the production of smaller degradation end-products (Yang et al. 2018). Phenyl rings present in polystyrene (PS) get excited upon absorption of UV radiation and form a triple-state, making it susceptible to decomposition. Alternating subunits of terephthalate and ethylene glycolate present in polyethylene terephthalate (PET) are linked through ester bonds. Upon photo-oxidation, the ester bonds cleave to form carbon dioxide, carbon monoxide, terephthalic acids, carboxylic acids, anhydrides, and esters (Fairbrother et al. 2019).

Decomposition initiated by UV irradiation is more effective in plastic exposed to air or the surface of a beach in comparison to plastic floating in the water. The reason behind the retarded degradation of microplastic in aquatic environment is the reduced temperature and oxygen concentration. Furthermore, the process is hindered due to fouling effects. Floating plastics develop surface fouling quickly; layers consist of biofilms of debris, algal mat, and colonies of invertebrates, respectively (Muthukumar et al. 2011).

13.4.1.2 Thermal Degradation of Macroplastics

Thermal degradation is the breakdown process of macroplastics by the action of energy that is generated from elevated temperatures. At high temperatures, plastic can undergo thermo-oxidative degradation. Long and complex polymer chains break when polymers absorb sufficient energy to surmount the energy barrier, generating reactive free radicals (Pirsaheb et al. 2020). They react with available oxygen and subsequently, hydroperoxides are formed, which in turn produce alkoxy radicals and hydroxyl free radicals in a process quite similar to the photodegradation of macroplastics. It is a self-propagating reaction that keeps on repeating unless the source of energy is cut off or inert end-products are produced by the collision of radicals. A vital difference between the photodegradation and thermo-degradation of plastics lies in the steps leading to the auto-oxidation cycle; another difference is that the former process occurs only on the surface of the polymer, whereas the latter process occurs throughout its structure (Tyler 2004).

The precise temperature required for this process varies among different polymers and is affected by the thermal characteristics (melting point and glass transition temperature) of plastic and the availability of oxygen in the environment (Crawford and Quinn 2016). The occurrence of exothermic oxidation in the environment is unlikely as the key requirement is high temperature. However, slowly progressing thermal oxidation of synthetic polymers may occur along with photodegradation in areas that are directly exposed to the sunlight. Kamweru et al. (2011) stated that temperature and ultraviolet radiation act synergistically on plastic decomposition. In another study, Kotoyori (1972) found that increasing humidity lowered the activation energy bar for the thermal decomposition of plastics.

13.4.1.3 Chemical Degradation of Macroplastics

Pollutants present in the atmosphere, such as ozone (O_3), nitrogen dioxide (NO_2), sulfur dioxide (SO_2), and volatile organic compounds (VOCs), either directly cleave the chemical bonds within the structure of polymers or act as catalyzing agents in the formation of radicals by photochemical processes leading to degradation (Cooper and Corcoran 2010).

Ozone (O_3) is produced from oxygen (O_2) under the activity of ultraviolet rays and lightning. Its concentration is low in the atmosphere compared to the ground, where its concentration increases due to pollution by SO_2, NO_2, and VOCs (Placet et al. 2000). Even at lower concentrations, O_3 actively attacks the unsaturated double bonds present in the polymer structure, causing bond scission in the chain. In contrast to unsaturated polymers, O_3 reacts quite slowly with saturated polymers (Mohan et al. 2019). Sulfur dioxide (SO_2) can be excited upon absorbing UV irradiation, producing a singlet or triplet state that either readily reacts with unsaturated C–C bonds or produces O_3 from oxygen (O_2) through photochemical reactions. Nitrogen dioxide (NO_2) is also very reactive due to the presence of odd electrons in the molecular structure; hence, it reacts with unsaturated C–C double bonds in the polymer chain. Similar to SO_2, NO_2 is capable of producing O_3 from O_2 during the photochemical reaction (Min et al. 2020).

pH and salinity are the two most influential chemical parameters affecting the decomposition of macroplastics in the aquatic environment. A denser concentration of H^+ (responsible for lower pH) or OH^- (responsible for higher pH) can catalyze the hydrolysis of some particular synthetic polymers such as polyamides (PAs) (Wadsö and Karlsson 2013). These two parameters can also alter the surface properties of other macro- and microplastics and regulate their behavior in an aquatic environment and toward other pollutants present in water (Liu et al. 2019).

13.4.1.4 Mechanical Degradation of Macroplastics

The decomposition of plastics due to the activity of external forces is known as mechanical degradation. Examples of external forces are abrasion and collision of plastics with rocks or sands due to the activity of winds or waves. Freezing-thawing cycle of plastics in water can lead to mechanical degradation (Pastorelli et al. 2014). The mechanical properties of plastic determine the effect of outdoor forces. Among them, an important feature is "elongation at break," which is defined by the ability of plastic to resist changes of shape without any tear or formation of crack (Krasilnikov et al. 2005). Plastics with lower elongation values at break are more prone to fragment under external tensile forces. Constant application of stress on plastics ultimately results in the breakage of chains in polymers (Sugimoto et al. 2020).

This kind of degradation is important for synthetic polymers. One of the major contributors of microplastics has been domestic washing due to the abrasion, shear, and stress on fibers during laundry (Cesa et al. 2020). Another noticeable source is abrasion of tire against road, which leads to scratching or microcutting of the tire and creating microplastics (Corcoran 2021).

Bond scissions and breakage of chains during decomposition by light, temperature, and chemical components affect the mechanical characteristics of macroplastics and precisely their tensile strength (Andrady 2017). O'Brine and Thompson (2010) found that environmental degradation processes reduced elongation values at the break of plastics, which subsequently decreased the need for external forces to fragment the plastics and enable the mechanical decomposition of macroplastics.

13.4.2 Biotic Degradation of Macroplastics

Biodegradation is a biochemical process that transforms compounds through complete mineralization by microbes. The end-products of the process are water and carbon dioxide under aerobic conditions and methane and carbon dioxide under anaerobic conditions. Microorganisms such as bacteria, fungi, insects, etc., are the prime agents for the biological decomposition of plastics (Crawford and Quinn 2016). Abiotic degradation processes, producing low- molecular- weight compounds, aid in eventual biotic degradation, as macromolecules could not be taken up and metabolized directly by microbes (Chen et al. 2019). Metabolites produced in this process are nontoxic to the ecosystem and are redistributed via nitrogen, carbon, and sulfur cycles (Cau et al. 2020). Though the source of biological degradation is microorganisms, nature is chemical; these chemicals are enzymes aiding in catalysis. The success of microbial action on polymers depends upon the availability of enzymes, the existence of a substrate site in the polymer for the enzymes to act upon, the specificity of the enzyme, and the availability of coenzyme if required (Reich and Stivala 1971).

Macroplastics are categorized into hydrolyzable and nonhydrolyzable based on the absence or presence of amide and ester functional groups, which can be attacked by extracellular hydrolases. Polymers like PE, PP, and PVC are identified as non-hydrolyzable, and the action of extracellular enzymes on them is complex. Santo et al. (2013) found the enzyme laccase released by actinomycete *Rhodococcus ruber* to be able to degrade PE. Hydroquinone peroxidase secreted by *Azotobacter beijerinckii* could degrade PS (Nakamiya et al. 1997). Hydrolyzable polymers like PET, PA, and polyurethane (PUR) are susceptible to biodegradation as extracellular hydrolases can act upon them (Chen et al. 2019). According to De Sá et al. (2018), PETase found in *Ideonella sakiensis* could hydrolyze PET in the environment. Enzymes like cutinase, serine esterase, lipase, and nitro-benzyl esterase are capable of hydrolyzing PET; on the other hand, cutinase, hydrolase, and amidase are involved in PA hydrolysis (Guebitz and Cavaco-Paulo 2008).

Oxidative and hydrolytic decomposition of plastics by different extracellular enzymes leads to chain breakage, generating polymers and fragments of short-chains (e.g., monomers, dimers, and oligomers). When the molecular weight of these fragments is small enough, they are taken up by microorganisms, assimilated, and subjected to subsequent intracellular metabolism (Wilkes and Aristilde 2017). Eventually, after both extracellular and intracellular pathways, mineralization results in the formation of carbon dioxide and water under aerobic conditions and in the formation of carbon dioxide, methane, water, organic acids, and ammonia under anaerobic conditions. Nonetheless, anaerobic biodegradation requires more time for complete mineralization than aerobic biodegradation (Gu 2003).

13.5 Sources of Microplastics in Aquatic Ecosystem and Transport Route

"Freshwater" indicates streams, rivers, lakes, and ponds, all of which have distinct features. The transport and retention of microplastics in the freshwater environment is a complex system. The freshwater environment acts as a conduit for microplastics entering into the system from the terrestrial region, acts as a hotspot for the production of microplastics through the degradation of macroplastics, and also acts as a basin retaining the plastic particles in sediments (Horton and Dixon 2018).

Disposal of waste in an inadequate manner leads to the release of macroplastics into the freshwater environment. The sources are general littering, wastes from landfills, or transportation from land via surface runoff. Apart from macroplastics, microplastics are significantly released into freshwater systems directly. Runoff and drainage from agricultural soils render ponds, lakes, and rivers contaminated with agricultural plastics, fibers, and microbeads (Steinmetz et al. 2016). Untreated and unfiltered urban runoff and storm drainage, containing road paint, particles of tires (generated from the collision between roads and tires), etc., pollutes the freshwater bodies (Treilles et al. 2021). Although wastewater treatment plants are used to remove microplastics, direct input of effluent-containing plastics into the water bodies can cause contamination (Murphy et al. 2016). During high flow, combined sewage overflows (CSOs) channel the untreated wastewater into nearby rivers to reduce the overpressure on drainage systems, inducing microplastic pollution in rivers.

Studies suggest that due to the drainage systems, water bodies act as more of a hotspot for pollution than the surrounding urban areas; thus, storm drainage, surface runoffs, inputs from CSOs, etc. should be controlled, and proper care has to be taken for their disposal (Horton et al. 2017). Along with rivers, other freshwater bodies like lakes and ponds receive wind-blown debris and land runoffs as inputs, and it gets accumulated over time due to their standing nature leading to burial and preservation within the sediments for a long time (Vaughan et al. 2017).

After entering the rivers, the particle of plastics is subjected to the same transportation system as the other sediments like sand and silts for mobilization. The speed of the water flowing in the river gives it energy, leading to the transportation of a greater portion of the particles (Knighton 2014). In the sections of the rivers where the energy drops and the flowing speed reduces, it is more likely for the plastic particles to settle down into the sediments. Additionally, the sedimentation helps in the burial of microplastics (Corcoran et al. 2015). Thus, the sediments would retain the microplastics throughout their movement through the freshwater systems (Nizzetto et al. 2016).

For all the microplastic wastes released from the freshwater and terrestrial environment (horizontal transportation), the oceans represent an ultimate sink (Lechner et al. 2014). After it reaches the oceans, they are dispersed widely and rapidly. Additionally, microplastics move through a vertical transport system via biofouling, incorporation in the marine snow, and excretion through fecal pellets (Rummel et al. 2017). This is considered vertical transportation. Apart from receiving inputs from rivers, oceans are contaminated with plastic by mismanaged fishing, which involves accidental cargo loss, illegal dumping, abandoned fishing nets, sinking of crafts, etc. (Xue et al. 2020). The major pollutant in these cases would be macroplastics, which will transform to microplastics and accumulate in the sediments over the years.

13.6 Ecotoxicological Impacts of Microplastics on Aquatic Ecosystem

Although various attempts have been made to evaluate the toxicity of microplastics to aquatic creatures, the impacts and mechanisms involved are still unknown (Desforges et al. 2014). Microplastics' potential toxicity can be attributed to three different mechanisms: (1) ingestion stress, such as physical blockage and expenditure of energy required for egestion, (2) additive leakage from plastic, such as plasticizers, and (3) exposure to toxicants involved with microplastics (persistent organic pollutants/POPs) (Cole et al. 2011; Andrady 2011). The consequences of microplastic exposure would be expected to differ depending on particle accumulation and translocation inside tissues, the organism's ability to ingest the particles, and the possibility of trophic transmission.

13.6.1 Impact on Aquatic Organisms

Phytoplanktons Any negative impact on the primary producers can eventually jeopardize a specific ecosystem's whole food web and food chain. It has been reported that exposure of phytoplanktons to microplastics can result in a stunted growth rate (Besseling et al. 2014). The negative impact of microplastics on algal development appeared to diminish as particle size rose (Zhang et al. 2017a, b). Physical interaction was found to be the cause of the impact of nanoplastic beads on two algae species, *Scenedesmus* spp. and *Chlorella* spp. When *Chlorella vulgaris*, *Dunaliella tertiolecta*, and *Thalassiosira pseudonana* were exposed to polystyrene particles with sizes ranging from 0.05 to 6 m for 72 h, no changes in algal growth were observed, but photosynthesis was reduced by 2.5– 45%. Contrastingly, Besseling et al. (2013) found that in the *Scenedesmus obliquus*, nanosized polystyrene particle (0.22 and 103 mg/l) exposure influenced algal development and reduced chlorophyll concentration, resulting in lower photosynthesis.

The effects of MPs have been documented in the marine ecosystem, with most scientific data indicating a possible effect of MPs at the producer level. After 96 h, exposure to polyvinyl chloride (PVC) MP (1 m size) lowered the growth rate by 39.7%, but 1 mm PVC had no harmful effect on *Skeletonema costatum* (Zhang et al. 2017a, b). In contrast, when *Tetraselmis chuii* was exposed to fluorescent red polyethylene microspheres (1–5 m) in the presence and absence of copper, no significant growth rate suppression was detected, suggesting that particle size was inversely proportional to MP toxicity (Davarpanah and Guilhermino 2015).

Zooplanktons Microplastics have been detected in rotifers, copepods, and cladocerans, which come in contact with microplastics through primary surface adherence and feeding habit (Jeong et al. 2016). Ingestion of 1 μm PE microplastics resulted in the immobility of the limnic *Daphnia magna* as concentration and time of exposure increased, according to Rehse et al. (2016), whereas the 100 μm that were not swallowed by *Daphnia magna* did not produce the physical impacts. *Calanus helgolandicus* copepods swallowed 11% fewer algae when exposed to 20 μm PS microbeads and cultivated algae, resulting in lower ingested carbon biomass and decreased fecundity (Cole et al. 2011). Differing sizes of microplastics can have significant size-related impacts on zooplankton, such as lower eating capacity, decreased fecundity and growth rate, increased mortality, lengthy reproduction time, and could even affect the next generation (Zheng et al. 2020). Smaller plastic particles, such as nanoplastics, are more toxic and damaging to zooplankton (Sun et al. 2017). Furthermore, the capacity of microplastics to be excreted may be directly proportional to their particle size. In brief, consumption of microplastics by zooplanktons revealed that primary consumers could directly interact with microplastics in the ecosystem.

Invertebrates Aquatic invertebrates feed directly on primary producers and serve as a valuable food source for the carnivores, which are significant ecological players. Aquatic invertebrates are more prone to microplastic pollution due to their feeding habits and position in the food chain as primary predators. Several reports have identified arthropods (De Sá et al. 2018; Arias-Andres et al. 2019), mollusks (Abidli et al. 2019; Teng et al. 2019) and worms (Li et al. 2019; Lv et al. 2019) as potent species for accumulation and transfer of microplastics into the next trophic level. Because of the abundance and toxicity of microplastics in bivalves like clams and mussels, the species has been considered a useful bioindicator for aquatic microplastic contamination (Ward et al. 2019). Furthermore, different aquatic invertebrate species have varied life properties, which has an impact on microplastic uptake models and dispersion in invertebrates. For example, microplastic uptake into the nonfilter-feeder marine shore crab might be facilitated by respiratory exposure (Watts et al. 2014). Furthermore, Kolandhasamy et al. (2018) discovered that adherence of microplastics to soft tissues of mussels causes microplastic accumulation and subsequent ingestion. Various negative impacts of ingested microplastic particles on the growth, feeding, development, survival, and reproduction of the invertebrates have been documented (Huvet et al. 2016; Foley et al. 2018; Trestrail et al. 2020).

Fish Microplastics can be ingested by fish either directly from the aquatic ecosystem or indirectly from their prey. Fish features (e.g., species, life phases, feeding behavior, and living habitat) influence microplastic intake the most, followed by exposure conditions, plastic qualities (e.g., kind, size, shape, color) and aging of microplastic biofilms (Neves et al. 2015; Ory et al. 2018; Collard et al. 2019). Lusher et al. (2013) investigated 504 fish from ten both pelagic and demersal species caught in the English Channel and discovered plastic waste (0.13–14.3 mm) in 36.5% of the fish digestive tracts, 92.4% of which were microplastics. As per a recent global analysis, 427 different fish species are present in all freshwater, brackishwater, and marine environments, while different food chain positions (i.e., herbivore, algivore, omnivore, carnivore, and detritivore) could ingest microplastics (Lima et al. 2021). Three distinct benthic fishes, that is, *Cleisthenes herzensteini*, *Liparis tanakae*, and *Lophius litulon* collected from 14 different spots of the South Yellow Sea, were found with microplastic concentrations of 19.2, 27.5, and 5.9 particles g^{-1}, respectively (Wang et al. 2020). According to this finding, it can be concluded that microplastics had significantly contaminated the surface sediments and benthic species. Microplastics can interact with fish in various ways, including direct feeding, transfer in levels of the food chain, respiratory exposure and absorption through the skin, but their distribution in fish is difficult to predict. Hotspots for primary plastic accumulation are gastrointestinal tracts and gills (Barboza et al. 2020; Jaafar et al. 2020; Koongolla et al. 2020); especially nanoplastics are transported to various tissues and organs via complex mechanisms due to their smaller size and membrane permeability (Jacob et al. 2020; Guerrera et al. 2021; Ma et al. 2021). Microplastics in fish can affect the histological function and produce gastrointestinal obstruction, leading to reduced feeding. The very fine-sized microplastic may be absorbed through the intestinal lining as it passes through the gastrointestinal tract and eventually enters the bloodstream, translocates to other organs, and threatens survival (Barría et al. 2020). Other severe impacts are dysfunctionality of gills, disruption in neuromuscular functions, and rendering the fish vulnerable to plastic toxicity (Chen et al. 2021).

13.6.2 Toxicity from Contaminants Associated with Plastic

Microplastics' enormous surface-to-volume ratio and hydrophobicity allow them to accumulate harmful chemicals in water (e.g., heavy metals and persistent organic pollutants) at concentrations far greater than in ambient water (Mato et al. 2001; Holmes et al. 2012). Furthermore, several additives, such as bisphenol A, alkylphenols, phthalates, and polybrominated diphenyl ethers, are commonly used in the manufacture of plastics to improve the performance of the final product (Barnes et al. 2009). These plastic additives may have hazardous effects on the aquatic biota after they have leached out. Colonization of the plastic by potentially hazardous microbes could endanger the aquatic food web (Zettler et al. 2013). Despite the fact that microplastics are biochemically inert, the leaching of plastic additives and the buildup of other toxicants and pathogenic bacteria turn them into a complex cocktail of dangerous compounds (Hossain et al. 2019). The ingestion of contaminated microplastics by aquatic creatures provides a viable route for these dangerous compounds to enter the aquatic food web. Microplastics, in combination with noxious chemicals, have the potential to cause neurotoxicity (Avio et al. 2015), organ disease (Bhatt et al. 2021; Du et al. 2021), metabolic disorders (Ye et al. 2021), and mortality in aquatic biota (Phothakwanpracha et al. 2021). However, whether ingesting microplastics promotes the transmission of toxicants to aquatic organisms is still debatable, especially when compared to other exposure paths.

13.7 Bioremediation Aspects of Microplastics

Biodegradation is the breakdown of organic substances by living organisms, and this process is referred to as environmental remediation or bioremediation when it occurs in conjunction with the ecosystem and waste management (Masiá et al. 2020). The type of polymer, its characteristics, the type of organisms used, and the pretreatment form are the prime influencers of the biodegradation process. In the degradation process, the molecular weight of the polymer and the additives coated with the polymer play a major role (Artham and Doble 2008). The rate of degradation is reciprocally propositional to the molecular weight of the polymer. The degradation rate for some plastics, such as polyhydroxyalkanoates (PHA), polyhydroxybutyrate (PHB) and polylactic acid (PLA), is higher in comparison to synthetic polymers, such as polyethylene (PE), polycaprolactone (PCL), and polystyrene (PS) (Sivan 2011).

Biodeterioration, biofragmentation, assimilation, and mineralization are the four phases in the biodegradation process. The biodeterioration process begins with creating a biofilm surrounding the plastic polymer, signaling the start of the degradation process. Microbes manufacture the extracellular enzyme in the second stage, which acts on the polymer, converting it into an oligomer, dimer, or monomer, and preparing it for easy ingestion. In the third step, the oligomer/dimer/monomer assemblage on the surface of microorganisms is absorbed by microbial cells by simple diffusion or enhanced diffusion. The formation of daughter metabolites such as CO_2, H_2O, and CH_4 is the final stage (Lugauskas et al. 2003; Tokiwa et al. 2009). Each microorganism has the ability to destroy microplastics through the techniques described above. However, each microbe has the ability to release a unique enzyme for microplastic breakdown. It differs for microorganisms depending on their environment. Tanasupawat et al. (2016) reported the isolation of *Ideonella sakaiensis* bacteria from a PET-polluted environment. Palm et al. (2019) have revealed that this bacterium secretes the two enzymes responsible for PET breakdown: PETase and MHETase. Some bacteria can multiply on the flat substrate of plastic items, using them as a carbon source and forming a biofilm around them (Zettler et al. 2013). These bacteria use specific enzymes to break down the synthetic polymer into monomers. Skariyachan et al. (2021) recently reported the use of a bacterial consortium (*Pseudomonas* and *Enterococcus sp.*) to decompose microfragments of LDPE and PP.

Alcaligenes faecalis, *Pseudomonas stutzeri*, and *Streptomyces* sp.—all have polyhydroxyalkanoate (PHA) depolymerase (Jendrossek and Handrick 2002; Kadouri et al. 2005). Basidiomycetes, Deuteromycetes (*Penicillium* and *Aspergillus*), and Ascomycetes are the most common PHA-degrading fungus isolated from soil and marine habitats (Egbeocha et al. 2018). Polycaprolactone (PCL) is synthetic polyester that is quickly destroyed by the bacteria *Alcaligenes faecalis* and *Clostridium botulinum* and the fungus *Fusarium* (Grigore 2017; Egbeocha et al. 2018; Li 2018). Polylactic acid (PLA) is a polymer commonly used in biodegradable plastics; it has been shown to be degraded by a thermophilic bacteria (*Bacillus brevis*) (Duis and Coors 2016), as well as by only two *Fusarium moniliforme* fungus strains and *Penicillium roqueforti* (Egbeocha et al. 2018).

Other than microbes, the potential of various plants and animals is being researched for bioremediation of microplastics. Higher eukaryotes such as vertebrates, cephalopods, and decapods, which have been reported to be vulnerable under stress, should not be used in bioremediation. Second, microplastics should be captured, retained, and filtered/ingested at higher rates, as should their digestion/elimination; they should not be released into the environment. Species should only be used within their indigenous range, as geographical migrations must be avoided for the sake of biodiversity conservation (Molnar et al. 2008). Species with a wider distribution and easier control and management are considered potential candidates.

Microplastic retention appears to be a possibility for filter-feeding organisms. *Mytilus* mussels are pollutant-retention organisms that help bioremediation in natural habitats (Broszeit et al. 2016). Microfragments of plastics can be kept in their circulatory system for 48 days (Browne et al. 2008); nevertheless, most microplastic fibers are expelled after 24 h, compromising their elimination efficiency (Chae and An 2020). Adherence to the coral surface appears to be an effective strategy for MPs retention in other filter-feeders such as cnidarians (ingestion rates were 0.251–14.810 particles/h, whereas surface adhesion was 40 times larger; Martin et al. 2019). The sandworm *Arenicola marina* has a lifetime retention rate of 240–700 MPs (1.2 ± 2.8 particles/g), which appears to have no effect on its metabolism (Bansal et al. 2021); it could be a candidate for environmental remediation in oceanic and brackish waters, because it can tolerate salinities as low as 12 ppt. The echinoderm sea cucumber (recommended for pollution monitoring) is another intriguing organism that may be appropriate for eliminating PCB-contaminated plastic, since it preferentially ingests microplastics over other sediment particles (Alava 2019).

Higher plants have the greatest advantage over animals in that there is no sign of suffering. The bioremediation capability of algae, specifically microalgae, has been investigated in water. Roccuzzo et al. (2021) depicted that unicellular microalgae may digest endocrine-disrupting substances in wastewaters when used alone or in combination with bacteria. Seagrasses, which can grow in marine and brackish habitats, are of interest in cleaning effluents near the sea. The smooth ribbon seagrass *Cymodocea rotundata* was proposed by Huang et al. (2021a, b) for bioremediation of textile dye wastewater. Microplastics can be retained in a variety of ways in seagrasses and higher plants, with the particles collecting on the blades along with their associated bacteria. Plastic particles are found in epibiont communities on the blades in the seaweed *Thalassia testudinum* (Goss et al. 2018), but synthetic microfibers have been identified adhering to blades without epibiont communities in the seaweed *Fucus vesiculosus* (Goss et al. 2018; Gutow et al. 2016). Ali et al. (2020) recommended many freshwater Magnoliophyta (having heavy metal removing capability) for removing microplastics from WWTPs, including water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), and duckweed (*Lemna minor*). Nano- and microplastics do not appear to constitute an ecological concern to aquatic macrophytes in environmentally realistic quantities. Some macrophytes, including *Egeria densa*, and their microbiomes have been shown to collect and convert gold nanoparticles (Avellan et al. 2018); these systems could be studied for MPs bioremediation.

13.8 Development of Regulations and Policies

Plastic production has been increasing exponentially for decades, and it appears that the number of microplastic particles will continue to rise in the coming years. The original sources and classifications of plastics and microplastics entering the marine environment must be recognized to reduce the introduction of microplastics into the aquatic ecosystem. Also, raising public awareness about microplastics through education in the public, private, and government sectors will go a long way. Raw water, groundwater, bottled drinking water, and food items have all been discovered to contain microplastics (Koelmans et al. 2019; Rainieri and Barranco 2019; Jadhav et al. 2021). However, no microplastic contamination criteria have been established, and no parameters have been designed to assess the microplastic limit in drinking water. As a result, a number of organizations, government agencies, institutes, and authorities working on new pollutants must concentrate on determining microplastic limits in various resources and their potential repercussions. Microfibers and microbeads are predominantly secondary microplastics produced by washing garments; as a result, policy implications must be established to filter and catch these microplastics before they contaminate the natural ecosystem (McDevitt et al. 2017). Aside from that, corrective methods to limit and control plastic and microplastic debris with public participation must be implemented. To reduce microplastic and plastic pollution in the natural environment, comprehensive and effective measures must be implemented.

Concerns about microplastics have prompted numerous groups to propose management standards. The United Nations Environmental Programme's (UNEP) Expert Panel has called for prompt action to clear the oceans of microplastics, citing that microplastics are swallowed by a huge number of marine organisms, causing them physical and chemical harm (Wu et al. 2017). Similarly, the UNEP-MAP (United Nations Environment Program/Mediterranean Action Plan), OSPAR (the Oslo/Paris Convention—for the Protection of the Marine Environment of the North-East Atlantic), and HELCOM (Baltic Marine Environment Protection Commission—Helsinki Commission) have developed guidelines for assessing marine litter, including microplastics (Karbalaei et al. 2018). Nongovernmental organizations (NGOs) have also presented initiatives to raise awareness and assist in quantifying the extent of MPs pollution and its consequences on a national and international scale.

Many regions have established or implemented regulations prohibiting the manufacture and use of primary MPs, such as microbeads, which could minimize MPs entering the aquatic system (CEPA (Canadian Environmental Protection Act) 2016; Beat the Microbead 2016; United Kingdom Department for Environment, Food and Rural Affairs 2016) (Wu et al. 2017), as well as restrictions on the use of single-use macroplastics (i.e., bottles, carrier bags). The Netherlands was the first country to declare its intention to create microbead-free cosmetics, with a 2016 deadline (Ogunola et al. 2018). According to Kamat and Kamat, China has outlawed non-biodegradable bags and single-use straws in all cities. Korea, meanwhile, prohibited microplastic-based cosmetics in 2021. Some nongovernmental organizations (NGOs) and businesses identified microplastic-free products and promoted the certificates "Good Scrub Guid" (Flora and Fauna) and "Zero plastic within" (product label) (Jeyavani et al. 2021). Bio-based plastics have recently been employed by China and American (USA) customers in place of plastic products. According to Kamat and Kamat, the Indian government announced in 2018 that single-use plastics would be phased out by 2020. One-time used plastic bags, cutlery, and some PET bottles were all banned in India in June 2019. The airport administration certified 55 of the country's 134 airports to be plastic-free. The government of Himachal Pradesh has outlawed the disposal of plastic products (plastic cups and plates) since 2017, resulting in a significant reduction in plastic pollution (Kamat and Kamat 2021).

13.9 Conclusions and Future Perspectives

Because of their longevity and slow rate of deterioration, plastic materials survive in the environment. It has been changed into microplastics due to natural forces, which easily enter all ecosystems and, as a result, into living beings, where it accumulates, magnifies, and causes damaging consequences. Microplastics are easily swallowed by marine organisms due to their small size and have been reported to concentrate in tissues, the circulatory system, and the brain. This chapter highlights these important aspects of microplastics in an effort to promote coherent literature and future research among scientists interested in this field. Microplastics cannot be reduced without the general population's participation, the socioeconomic sectors, tourism, and waste management companies.

Microplastics are predicted to have a greater negative impact, because most countries lack sewage treatment infrastructure and have yet to minimize, repurpose, and recycle plastic products. Appropriate technological solutions must be implemented, and knowledge of waste segregation at the source and the need for plastic recycling must be raised. Strategies to limit the input of plastic into the biosphere should be included in the management protocol. Specific approaches should be used to treat primary and secondary microplastics and keep them out of the ecosystem. Furthermore, there are little research on the prevalence, fate, toxicology, and reduction of MPs in India, necessitating the scientific community's attention for further investigation.

Flaws in waste management must be addressed in order to improve current procedures and reduce the risks connected with them. Furthermore, several bacteria are being tested with properties that could degrade microplastics of aquatic origin. These microorganisms might potentially be used to clean up contaminated settings. The use of microbes for microplastic degradation is a potential and environment-friendly action plan that will allow for the management of microplastics without negative consequences and eventually support the natural clean-up of contaminated areas.

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Biodegradation of Endocrine-Disrupting Chemicals Using Marine Microorganisms

14

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Abstract

Endocrine-disrupting chemicals (EDCs) are manufactured organic (pesticides, food additives, polycyclic aromatic hydrocarbons, pharmaceuticals, etc.) or mineral (heavy metals) chemicals that are found almost everywhere in nature. Despite their presence at very low concentrations, EDCs raise paramount toxicological concerns for human health and the environment. They act by altering the functions of the endocrine system of many organisms, including humans. EDCs originate from industrial activities such as manufacturing pesticides and pharmaceuticals and daily use of household chemicals, personal care products, medicines, hygiene and cosmetic products, and consumer articles. Many EDCs are challenging to eliminate with traditional biological wastewater treatment methods and are thus emitted into the environment. Besides, isolation and characterization of new microbial strains from nature, capable of degrading EDCs more effectively, is of significant interest nowadays. Because of their tremendous diversity, marine microorganisms have an excellent bioremediation potential for virtually every contaminant compound, including EDCs. Thus, this chapter summarizes current knowledge on the ability of marine microbial species to interact and degrade or neutralize different EDCs.

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Keywords

Bioremediation · Endocrine-disrupting chemicals · Marine microorganisms · Toxicity

14.1 Introduction

With an estimated 2.01 billion tons of human waste generation worldwide in 2016 (Kaza et al. 2018), anthropogenic pollution in all its forms has reached proportions nowadays, never observed before. The occurrence of a new kind of pollutant called endocrine-disrupting chemicals (EDCs) has exacerbated human health and environmental concerns due to their various toxicologic effects at a physiological level and persistence (Marlatt et al. 2022). According to the World Health Organization (Damstra et al. 2002), an EDC is defined as “an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse effects in an intact organism, or its progeny, or (sub)populations.” EDCs are mostly anthropogenic chemicals with various chemical structures (organic and mineral) that contaminate all the environmental media. They are generated chiefly from human industrial activities, including manufacturing pharmaceuticals, pesticides, dioxins, flame retardants and other chemicals of daily use, such as cosmetics, and hygiene products. If they are not adequately treated in wastewater treatment plants because of their recalcitrance, EDCs end up in the different media of the environment, where they negatively affect living organisms (Yang et al. 2020). Numerous reports have demonstrated that EDCs and their degradation by-products are responsible for several toxicologic effects on different biological systems (Phong Vo et al. 2019; La Merrill et al. 2020; Galbiati et al. 2021).

Different strategies have been developed to degrade, neutralize, or remove EDCs from different systems. These methods can be categorized into (1) physicochemical methods, such as adsorption (Wang et al. 2021), photolysis using UV radiation applied alone (Li et al. 2022a) or combined with chemical treatment such as chlorination (Han et al. 2022), high-frequency sonolysis (Glienke et al. 2022), electrochemical oxidation using, for instance, a carbon felt electrode (Kim et al. 2021), ozonation (Doná et al. 2019), nanofiltration or reverse osmosis (Khoo et al. 2022), among others, (2) biological methods, which use the catabolic potential of specific microorganisms through the enzymes they produce (Kumari and Ghosh Sachan 2019; Kanaujiya and Pakshirajan 2022; Feng et al. 2022; Shabbir et al. 2022), and (3) hybrid systems combining physicochemical and biological methods (Bhatt et al. 2022). Because of its eco-friendly and cost-effective advantages, the biological approach is, by far, the method of choice for eliminating EDCs. Furthermore, if EDCs are present in the systems to be bioremediated at a low level, then inhibiting their biodegradation by the pollutants themselves is improbable.

An extensive number of studies have been devoted to the bioremediation of EDCs using various pure or mixed cultured microorganisms isolated from different environments, including those polluted with various xenobiotics. Examples of microorganisms include the diazinon-degrading *Candida pseudolambica* isolated from agricultural lands contaminated by pesticides (Ebadi et al. 2022), different methylotrophic recombinant yeast strains of *Pichia pastori* (Li et al. 2022b), *Rhodococcus* sp. PFS1 isolated from a paddy field soil and capable of degrading di-(2-ethylhexyl) phthalate (Kamaraj et al. 2022), tetrabromobisphenol A-degrading *Enterobacter* sp. T2 isolated from a sludge sample (Peng et al. 2022), dibutyl phthalate-degrading *Acinetobacter* sp.33F isolated from a municipal solid waste leachate (Sharma et al. 2021), activated sludge systems (Boonnorat et al. 2019; Pinpatthanapong et al. 2022), different periphytic microbial communities (Shabbir et al. 2022), a bacterial consortium of *Pandoraea* sp. PAE-2 and *Microbacterium* sp. PAE-1, which was effective in completely degrading dibutyl phthalate (Lu et al. 2020), and marine microorganisms, such as *Bacillus velezensis* MHNK1 capable of eliminating atrazine (Jakinala et al. 2019), benthic diatoms, such as *Navicula incerta* (Liu et al. 2010), *Cylindrotheca closterium* (Li et al. 2015), and other phyto (*Nannochloropsis* sp., *Chaetoceros gracilis*), zooplankton (*Brachinous* sp., *Artemia* sp. organisms (Ishihara and Nakajima 2003), and *Dunaliella salina*, *Cylindrotheca closterium*, and *Chaetoceros muelleri* (Chi et al. 2019).

The marine environment's richness in microbial metabolic and phylogenetic diversity offers excellent potential for screening new and very effective microbial strains capable of degrading EDCs with high elimination rates. Thus, this chapter summarizes the current knowledge on the efficiency of marine microbial species in degrading different EDCs.

14.2 A General Outline of EDCs and Their Hazardous Effects

Identifying substances that can act as endocrine disruptors is a priority. However, this task is not easy: based on scientific information, about a thousand compounds have been so far shown to disrupt hormone signaling pathways. Some molecules have been the subject of extensive investigations and strict regulations or have even withdrawn from the market. However, for most of these substances, only fragmentary information prevents a conclusion on their effects on the endocrine system. The molecules used can be transformed into many degradation products, indirectly impacting the hormonal system's functioning. There are three main groups of endocrine disruptors: products naturally present in the environment, synthetic products manufactured for targeted use, and synthetic substances that can disrupt the endocrine system. Among synthetic compounds, three main families stand out: persistent organic pollutants (POPs), pesticides, and plasticizers (Encarnação et al. 2019; La Merrill et al. 2020).

14.2.1 Natural Substances

Several known endocrine disruptors are naturally occurring and produced by plants or fungi. Phytoestrogens are molecules from the plant kingdom capable of binding to the estrogen receptor. The identification of reproductive disorders such as infertility in Australian sheep herds (known as "clover disease") in the 1940s highlighted the impact of these phytoestrogens. Among them, we can cite genistein, daidzein, or coumestrol. They are present in certain plants or vegetables, such as clover, bean sprouts, alfalfa, cabbage, green beans, and spinach. To date, more than 200 substances derived from plants have been recognized as having estrogenic activities. Most phytoestrogens belong to the flavonoid group, which is divided into three main categories (1) isoflavones, present in legumes; (2) lignans, found in various cereals; (3) coumestans, essentially represented by coumestrol, present in alfalfa used in animal feed (Kuhnle et al. 2009; Ionescu et al. 2021; Pool et al. 2022).

Mycoestrogens are substances produced by fungi. They are found in high concentrations after harvesting corn or barley and in cereal seeds and vegetable oil. A prominent mycotoxin is zearalenone. The latter develops in foodstuffs following a fungal infection of cereals, especially corn, oats, wheat, rice, and soybeans. Zearalenone's most concerning toxic effect is its character as an endocrine disruptor with estrogenic activity (Awuchi et al. 2021; Kinkade et al. 2021; Zhou et al. 2022).

14.2.2 Persistent Organic Pollutants

Persistent organic pollutants are compounds that resist photolytic, biological, and chemical degradation. It is, therefore, not uncommon to find this type of compound in the environment years after its use. Persistent organic pollutants are mostly halogenated molecules characterized by low water solubility and high lipid solubility, resulting in tissue bioaccumulation. Many of these compounds have been, or continue to be, used in large quantities. Due to their persistence in the environment, they can be bioaccumulated and biomagnified. Bioaccumulation is the mechanism by which chemical substances absorbed by living organisms will accumulate in specific tissues (adipose most of the time). Biomagnification is the mechanism by which the content of chemical substances is increased throughout the food chain. Because of this biomagnification, some organisms can concentrate toxic substances up to 1 million times (mussels, oysters) and therefore become toxic. This bioaccumulation is now prompting health authorities to warn pregnant women about consuming certain foods, such as shellfish or wild fish (Gui et al. 2014; Sajid et al. 2016; Guo et al. 2022).

Among the best-known persistent organic pollutants, dioxins and furans are polychlorinated compounds with similar molecular structures and modes of action. Dioxins come from waste incinerators (combustion by-products). In humans, exposure occurs almost entirely through food, particularly dairy products, fish, and meat. Dioxins are lipophilic compounds metabolized and eliminated slowly by the body, thus tending to bioaccumulation (Kirkok et al. 2020).

14.2.3 Pesticides

Pesticides have been on the market for several decades at a steady pace, generating numerous health problems. They are still dumped into the environment, causing widespread contamination of soils and groundwater. They are also responsible for many health problems in humans. While the harmful nature of pesticides is well documented, it is no less challenging to remove these substances from the market (Rani et al. 2021).

Indeed, these substances are subject to strong industrial lobbying seeking to preserve this market of more than 40 billion dollars. Part of the problem with pesticides is that they do not stay exclusively in the soil where they are applied. Due to evaporation, many of these substances are dispersed in the atmosphere; 50–90% of the used pesticides do not reach their target (Wang et al. 2020).

Among these pesticides, Chlordecone is a pesticide and a persistent organic pollutant from the organochlorine family, banned in many countries. It has a half-life of between 120 and 160 days in the body and up to 46 years in the environment (Tzanetou and Karasali 2022).

Glyphosate, the active molecule of Roundup, is the most widely used herbicide in the world. It is a phosphonic acid resulting from the oxidative coupling of the methyl group of methyl phosphonic acid with the amino group of glycine. Although degradable in the environment, the overuse of this pesticide makes it a pollutant systematically found in ecosystems. Studies have shown the presence of glyphosate in dust or aerosols from agricultural activities. This dust, carried by the wind, makes inhalation one of the potential sources of exposure to this pesticide (Martens et al. 2019).

Atrazine is a herbicide from the triazine family that interferes with photosynthesis mechanisms. This molecule has an estimated half-life of a few weeks to several months. This herbicide, forbidden in France and Poland, is widely used in the United States to treat corn cultivations at more than 400,000 tons (10 million acres) per year. Due to its extensive use, concerns about its effects on the environment and human health have been expressed. It has been demonstrated that this pesticide is capable of interfering with the functions of oxidative phosphorylation (transformation of ADP into ATP) as well as the functions of cytochromes P450, decreasing the oxygen consumption of the cell (Ackerman et al. 2014; Barchanska et al. 2017; Liu et al. 2022; Lu et al. 2022; Zhang et al. 2022).

14.2.4 Plasticizers

Every year, nearly 8 million tons of plasticizers are used to improve plastics' strength, appearance, and flexibility. The best-known bisphenol A (BPA) and phthalates are at the center of many scientific and media questions. Indeed, in few years, plastics have invaded our environment. Their ubiquity raises many environmental and public health questions, particularly regarding endocrine disruption (Martínez-Ibarra et al. 2021; Palacios-Arreola et al. 2022; Vikhareva et al. 2022).

BPA is the best-known of the bisphenols and plasticizers in general. It is widely used in industry as a monomer and precursor in manufacturing epoxy resins (internal coating of cans), polycarbonates, and thermal printing inks. One of the problems raised by this molecule is that once heated or exposed to detergents, it can migrate from food plastics to food, thus being able to be ingested. Although ingestion is the main route of exposure, exposure to BPA can also be through the skin. As a result, this molecule has been banned in France and Belgium since 2012 in baby bottles and food containers (Almeida et al. 2018; Vinković et al. 2020; Palacios-Arreola et al. 2022).

The second major group of plasticizing compounds of concern is phthalates, such as di(n-butyl)phthalate (DBP) or di(2-ethylhexyl)phthalate (DEHP). Since the 1930s, these compounds have been used as plasticizers in the composition of many products, such as cosmetics, children's toys, paints, and even food packaging. As with BPA, the main route of exposure is ingestion following the migration of residues from packaging to food. In addition, children could ingest these substances when they put their toys in their mouths. High concentrations of phthalate metabolites have been detected in the urine of women of childbearing age from everyday objects. Phthalates would also have an antiandrogenic action. It is currently assumed that instead of binding to androgen receptors, these molecules would decrease testosterone synthesis in Leydig cells, thus reducing testosterone levels during fetal life, a critical period of sexual differentiation (Hlisníková et al. 2020; Wang and Qian 2021).

14.2.5 Drugs

Many studies warn against the consumption of certain medicinal substances. If it is customary to cite contraceptive pills among these substances, it is not necessarily intuitive to also think of aspirin (acetylsalicylic acid) or paracetamol (acetaminophen).

The contraceptive pill is essentially based on estrogens or progestagens controlling the ovarian cycles in women. These molecules block ovulation and cause a thickening of the cervical mucus, preventing any fertilized egg from nesting. The molecules used in these pills (ethinylestradiol, mestranol, etc.) and their mode of action exemplify endocrine disruption (Wan et al. 2022).

Among the most commonly used drugs are paracetamol, whose worldwide consumption is constantly increasing, and anti-inflammatories (steroidal or not), such as aspirin and ibuprofen. Unfortunately, these drugs are at the origin of the development of cryptorchidism in newborns after exposure in pregnant women. This is why it is strongly discouraged for these women to take these drugs during the last trimester of pregnancy (Stukenborg et al. 2021).

14.3 Marine Microorganisms (Diversity and Distribution)

Due to the age of marine life (more than 3.5 billion years), the multiplicity of niches, and ecological situations, marine organisms are biologically much more diverse than terrestrial organisms.

14.3.1 Bacteria and Archaebacteria

The marine microbial community is dominated by numerous ultra-micro-bacteria or nano-bacteria, particularly, *Sphingomonas*, which pass easily through 0.2 μm filters. Archaebacteria and planktonic bacteria are present both in the seas and ocean depths. Bacteria concentrations are higher at the surface, but below 100 m, archaebacteria constitute a more significant portion of the total population.

The number of bacteria in the water mainly depends on the organic matter content. In clean waters, they are present in low numbers, while polluted waters contain up to several million cells per 1 ml (Overmann and Lepleux 2016; Alvarez-Yela et al. 2019; Sanz-Sáez et al. 2020).

14.3.2 Viruses

Marine environments are home to a large number of viruses. These are present in concentrations 10 times higher than that of bacteria. Most of them are bacteriophages. This virioplankton constitutes an important part of the aquatic microbial community. It can influence the functioning of the microbial loop, intervene in the horizontal transfer of genes between prokaryotes, and control the diversity of the microbial community (Middelboe and Brussaard 2017; Pang et al. 2019; Ding et al. 2021: Jian et al. 2021).

14.3.3 Fungi

Fungi are usually found in shallow waters. Zoospore fungi are adapted to aquatic life and include oomycetes (biflagellate spores), zygomycetes, and chytrids (monoflagellate spores). Some species of the latter attack algae and amphibians. The filamentous fungi, which can sporulate underwater, constitute the hyphomycetes. In addition, fungi belonging to the Ascomycetes and the Deuteromycetes are relatively frequently found (Jiang et al. 2016; Jones et al. 2022).

14.3.4 Algae

Algae are the simplest autotrophic eukaryotes, which incorporate over 20,000 species. They are important producers of organic matter and oxygen. Algae live as single cells or create multicellular bodies.

The composition of the algal community changes considerably in quality and quantity depending on the mineral salt content of a given reservoir and the characteristics of the substances that constitute the primary pollutant. The members of the phyla Dinophyta, Haptophyta, and Rhodophyta are mainly found in marine ecosystems (Heimann and Huerlimann 2015).

14.3.5 Protozoa

Protozoa are heterotrophs that feed by absorbing dissolved organic compounds or on bacteria (Sleigh 1991).

14.4 Biochemical Aspects of the Biodegradation of EDCs by Microorganisms

14.4.1 General Biochemical Aspects of Xenobiotics Degradation

Significant progress has been achieved toward comprehending the biochemical mechanisms involved in the degradation (total or partial) and neutralization of xenobiotics, including EDCs. Nowadays, bioremediation, which allies fundamental biochemical reactions and engineering techniques, is continuously gaining interest in xenobiotics removal (Tazdaït and Salah-Tazdaït 2021). There are chiefly three mechanisms involved in the microbial treatment of inorganic MPs (heavy metals) (Fig. 14.1): (1) biosorption, which occurs mainly through ions exchange, involving different cell-surface-located functional groups, including carbonyl, hydroxyl, amine, sulfhydryl, carboxyl, and phosphoryl, (2) biocomplexation (bioaccumulation), which involves metabolically produced organic chelators, such as siderophores (enterochelin of *Escherichia coli*, desferrioxamine of *Streptomyces pilosus*,

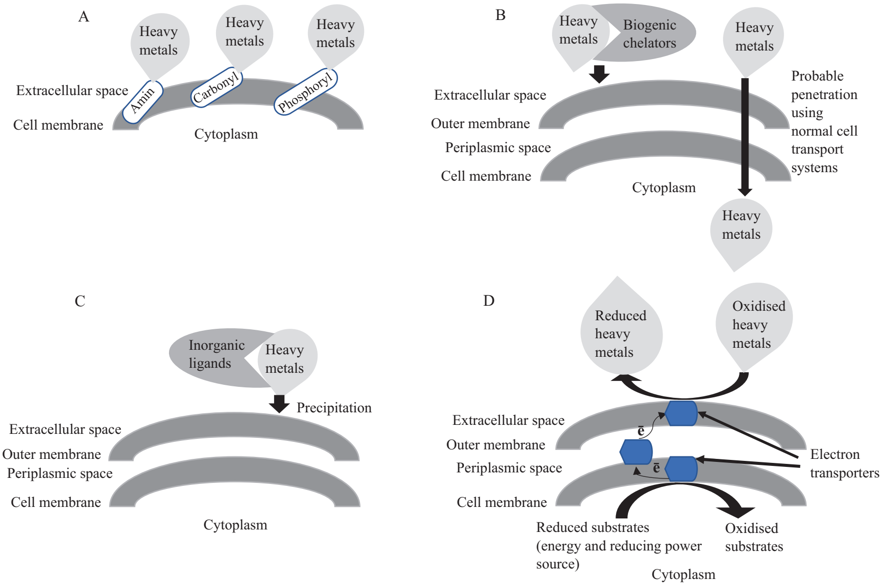

Fig. 14.1 Schematic illustration of the mechanisms of the interactions between microbes and heavy metals. (**A**) Biosorption, (**B**) biocomplexation (bioaccumulation), (**C**) biomineralization, (**D**) bioreduction

hydroxamate-type siderophores of *Synechococcus elongatus* BDU 130911), and exopolysaccharides of *Shewanella oneidensis* MR-1, or penetration into bacterial cells via normal cell transport systems followed with sequestration by chelating proteins called metalloproteins, (3) enzymatically mediated reduction (bioreduction) that acts on inorganic MPs by lowering their redox state and thereby reducing their solubility in the media they contaminate, and biomineralization (Salah-Tazdaït and Tazdaït 2022).

The microbial processes involved in the bioremediation of organic EDCs can occur aerobically or anaerobically through biotransformation or mineralization. The mineralization process refers to successive metabolic reactions that lead to the entire degradation of the pollutants into harmless mineral compounds, such as NH_3, CO_2, SO_4^{2-}, and H_2O, and during which the pollutants, called primary substrates, serve as a source of nutrients (Tazdaït et al. 2013; Kaur and Goyal 2019). During the biotransformation process, the organic pollutants, called secondary substrates, undergo slight chemical modifications cometabolically without serving as nutrients. This process is usually observed in pure microbial cultures and necessitates the presence of simple or complex growth substrates (Salah-Tazdaït et al. 2018; Tazdaït and Salah-Tazdaït 2021). It is worth noting that because the biotransformation process usually produces one or more metabolites that can be similarly or more toxic than the parent pollutant, special attention should be focused on identifying the metabolites yielded during MPs bioremediation (Fig. 14.2). On the other hand, the biodegradation of xenobiotics compounds occurs only from a certain level known as minimum substrate concentration ($S_{\min}$). If the contaminant is present at a concentration $\geq S_{\min}$, then it will serve as a substrate sustaining microbial growth (Becker and Seagren 2010).

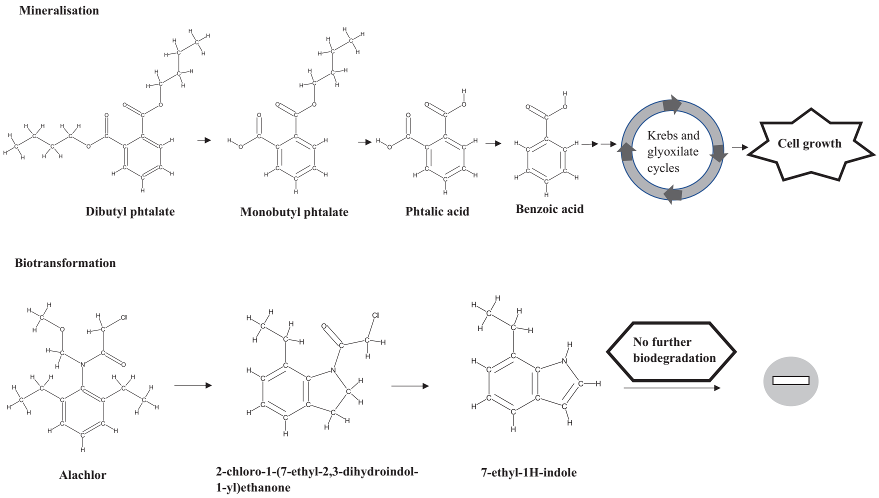

Fig. 14.2 Schematic classification of two EDCs (dibutyl phthalate and alachlor) bioremediation reactions according to the extent of biodegradation (mineralization or biotransformation). Note that successive arrows designate multiple metabolic reactions

14.4.2 Microorganisms for the Treatment of EDCs

Interest in the microbiological degradation of endocrine disruptors has increased in recent years. Some endocrine disruptors have great estrogenic activity and high toxicity. This is why the degradation of these compounds has become increasingly important. Several studies have identified microorganisms capable of degrading endocrine disruptors (Table 14.1).

14.4.2.1 Biodegradation of EDCs by Marine Bacteria and Fungi

Many studies have focused on isolating and characterizing various marine (water or sediment) microbial isolates pertaining to different microbial groups (bacteria, fungi, algae, and diatoms) capable of efficiently degrading a variety of confirmed or potential EDCs. In a study by Jakinala et al. (2019), the marine isolate *Bacillus velezensis* MHNK1 producing surfactin biosurfactant has been tested for its ability to aerobically degrade the herbicide atrazine at 200 mg/L in batch experiments. The isolated strain could mineralize the herbicide by using it as a growth substrate (carbon source) and exhibited a maximum removal rate of about 87 within 5 days of incubation in surfactin-free. However, in the presence of surfactin at 2CMC (critical micelle concentration), complete atrazine degradation was observed within four days. The authors suggested that surfactin improves atrazine degradation either by increasing the solubility of the herbicide or by enhancing the hydrophobicity of the strain cell surface. Another study investigated the aerobic degradation of dimethyl phthalate by the marine *Sphingomonas yanoikuyae* DOS01. The strain was isolated from marine sediment collected from the South Chinese Sea. The bacterial strain exhibited the ability to use dimethyl phthalate as the sole source of carbon and energy for its growth when tested at 180 mg/L and showed a biodegradation efficiency of nearly 100% after 35 h of incubation. Besides, the strain could completely mineralize 250 mg/L monomethyl phthalate within 60 h, a degradation metabolite of dimethyl phthalate (Gu et al. 2009). Another marine bacterial isolate *Pseudoalteromonas* sp. strain GCY, isolated from sediments from a coastal area of the Northern Yellow Sea (China), was assessed for tetrabromobisphenol A biodegradation. The differential involvement of different biogenic molecules, namely, hydroxyl radical, siderophore, hydrogen peroxide, and superoxide anion radical in tetrabromobisphenol A decomposition, was evidenced. The isolated strain was not capable of using tetrabromobisphenol A as the sole carbon source. The contaminant at 10 mg/L was instead degraded cometabolically through β-scission and debromination routes, reaching a biodegradation rate of 80% within ten days and producing different metabolites, namely, 4-(2-hydroxyisopropyl)-2,6-dibromophenol tribromobisphenol A, 4-isopropylene-2,6-dibromophenol, 4-hydroxybenzoic acid, 2-bromobenzoic acid, and 2,4,6-tribromophenol (Gu et al. 2018). More recently, Khandare et al. (2021) succeeded in degrading polyvinyl chloride, known to contain phthalates, using three bacterial strains, namely, *Alteromonas*, *Cobetia*, and *Vibrio*, which were isolated from a marine water sample collected in Diu Island (India). It was reported that all strains could grow on polyvinyl chloride films and mineralize them, which increased their hydrophilicity and reduced their strength. *Aeromonas*

Table 14.1 Some examples of microorganisms degrading some endocrine disruptors

Endocrine disruptors		Microorganisms	Strains	References
Plasticizers	Bisphenol A	Bacteria	*Sphingomonas bisphenolicum*	Sakai et al. (2007) Chouhan et al. (2014)
		Fungi	*Irpex lacteus*	Shin et al. (2007)
		Algae	*Stephanodiscus hantzschii* *Ulva prolifera*	Azizullah et al. (2022)
	Di-n-butyl phthalate	Bacteria	*Sphingobium yanoikuyae* TJ	Hu et al. (2021a, b)
		Fungi	*Stropharia rugosoannulata* *Trichosporon porosum* *Stachybotrys chlorohalonata*	Carstens et al. (2020)
		Algae	*Cylindrotheca closterium* *Dunaliella salina* *Chaetoceros muelleri*	Gao and Chi (2015)
Persistent organic pollutants	Azo dyes	Bacteria	*Shewanella putrefaciens* *Arthrobacter bambusae* DP-A9 *Kocuria rosea* MTCC 1532	Ngo and Tischler (2022)
		Fungi	*Aspergillus ochraceus* NCIM 1146 *Bjerkandera adusta* CX-9 *Marasmius scorodonius*	Ngo and Tischler (2022)
		Algae	*Chlorella vulgaris* *Anabaena oryzae* *Wollea sacata*	Ishchi and Sibi (2019) Ngo and Tischler (2022)
Pesticides	Lindane	Bacteria	*Paracoccus* sp. NITDBR1 *Burkholderia* sp. IPL04 *Xanthomonas* sp. ICH12	Zhang et al. (2020)
		Fungi	*Rhodotorula* sp. VITJzN03 *Bjendera audusta* *Cyathus bulleri*	Zhang et al. (2020)
		Algae	*Nannochloris oculata*	Touliabah et al. (2022)
	Atrazine	Bacteria	*Arthrobacter aurescens* TC1 *Rhodococcus* sp. NI86/21 *Klebsiella variicola* FH-1 *Citricoccus* sp. TT3 *Arthrobacter* sp., DNS10 *Bacillus velezensis* MHNK1	Bhatt et al. (2020)
		Fungi	*Metarhizum robertsii* *Pleurotus ostreatus* INCQS 40310	Szewczyk et al. (2020) de Oliveira Lopes et al. (2020)
		Algae	*Chlorella* sp.	Hu et al. (2021a, b)

isolate exhibited the best biodegradation performance with 1.73% polyvinyl chloride weight loss within 60 days, followed by *Cobetia* and *Vibrio* isolates (1.23%).

The role of fungi in degrading EDCs has been well demonstrated in many studies. In one study by Birolli et al. (2018), the authors investigated the performance of five marine sponge fungi (*Penicillium citrinum* CBMAI 1186, *Cladosporium* sp. CBMAI 1237, *Trichoderma harzianum* CBMAI 1677, *Mucor racemosus* CBMAI 847, and *Aspergillus sydowii* CBMAI 935) to bioremediate anthracene. Besides, *Cladosporium* sp. CBMAI 1237 was able to degrade six EDCs, tested at 50 mg/L in artificial seawater, with different biodegradation efficiencies: pyrene (62%), acenaphthene (78%), anthraquinone (32%), nitropyrene (64%), phenanthrene (47%), and fluoranthene (52%), within 21 days of incubation. In their study, Bhatt et al. (2014) reported the efficient application of the marine fungus strain *Cochliobolus lunatus* CHR4D, isolated from marine coast sediment contaminated by oil, to chrysene biodegradation, which occurs through the cometabolic process. In order to improve the biodegradation efficiency of the isolated fungus, the authors applied response surface methodology as a mathematical optimizing tool to optimize different cultural parameters, including pH, trace metal solution volume, and initial concentration of $MgSO_4{\cdot}7H_2O$, KH_2PO_4, $CaCl_2{\cdot}H_2O$, glucose, chrysene, and ammonium tartrate. The central composite design significantly improved the chrysene biodegradation rate by a 1.65-fold factor, passing from 56% (without optimization) to 93.1% after 4 days of culture.

Some heavy metals, although toxic at high concentrations, are required for various physiological functions; others, such as lead, cadmium, mercury, and arsenic, are known or suspected to be mutagenic, teratogenic, carcinogenic, and endocrine disrupting, even at low levels (Rahman and Singh 2019). Bioremediation of heavy metals, such as mercury, using marine bacteria has been investigated by many reports. For instance, Al-Ansari (2022) has reported using the marine gram-negative bacterium *Marinomonas* sp. RS3, isolated from the Red Sea (Kingdom of Saudi Arabia), for bioremediation of mercury. It was found that *Marinomonas* sp. RS3 exhibited a significant tolerance toward mercury as mercuric chloride at 50 mg/L, with a removal efficiency of 78% obtained within 72 h of reaction in a batch experiment. The removal rate of the heavy metal was significantly enhanced (84.5%) in the presence of glucose used as a carbon source. Additionally, the authors suggested that the biodetoxification of mercury occurs through reduction, involving a mercuric reductase enzyme encoded by the mercury-resistant *merA* gene. In their study, Dash and Das (2014) tested the marine Gram-positive strain *Bacillus thuringiensis* PW-05 isolated from the Bay of Bengal (India) for its capacity to remove mercury from seawater broth medium. Up to 94.72% of 80 ppm mercury (mercuric chloride) was removed through volatilization after 48 h of culture.

14.4.2.2 Biodegradation of EDCs by Marine Microalgae

Many studies have paid attention to the bioremediation abilities of different marine algae toward various EDCs. For instance, the marine microalga *Cyclotella caspia* was tested for its ability to degrade the xenoestrogen nonylphenol at different concentrations (0.14, 0.18, and 0.22 mg/L). The obtained results revealed that the tested strain gave low nonylphenol degradation rates of 6.5%, 31.7%, and 37.7% for nonylphenol initial concentrations of 0.22, 0.18, and 0.14, respectively, within 192 h of reaction. Besides, it was evidenced that nonylphenol had toxic effects on the strain, especially when tested at 0.22 mg/L, by inducing cell deformity, disrupting the photosynthesis process, and increasing free radicals production (Liu et al. 2013). In a set of experiments conducted by Gao and Chi (Gao and Chi 2015), three marine microalgae, *Cylindrotheca closterium* (a benthic diatom), *Dunaliella salina* (a planktonic green alga), and *Chaetoceros muelleri* (a planktonic diatom), have been screened for diethyl phthalate and di-n-butyl phthalate biodegradation in batch experiments. Both phthalate acid esters, at 2 g/L each, were degraded by the three algal species. Regarding di-n-butyl phthalate removal, the best biodegradation efficiency was exhibited by *Cylindrotheca Closterium* (93.1%), followed by *Chaetoceros muelleri* (47.1%) and *Dunaliella salina* (40.0%) after 168 h of culture. The algal removal rates of diethyl phthalate were 81.2%, 32.3%, and 26.3% for *Cylindrotheca closterium*, *Dunaliella salina*, and *Chaetoceros muelleri*, respectively. The authors also reported an inhibitory effect on the decomposition of diethyl phthalate exerted by di-n-butyl phthalate when tested together. Besides, it was shown that the enzyme-mediated biodegradation of di-n-butyl phthalate occurred intra- and extracellularly, while the biodegradation of diethyl phthalate occurred chiefly extracellularly. In another study, *Cylindrotheca closterium* (2×10^5 cells/cm^2) efficiently removed dibutyl phthalate, and diethyl phthalate spiked in marine sediment samples. After only two days of incubation, the algal species completely eliminated diethyl phthalate in both the surface and the bottom sediment, while 94.1% and 81.4% dibutyl phthalate degradation were observed in the surface and the bottom sediment, respectively (Li et al. 2015). Sun et al. (2019) investigated the potential of the marine microalga *Karenia brevis*, cultivated in seawater, to degrade a mixture of four phthalate esters (dipropyl phthalate, diethyl phthalate, dimethyl phthalate, and diallyl phthalate) with different alkyl chains length. It was shown that the growth of *Karenia brevis* was inhibited by the four toxicants in a dose-dependent manner and that the more the alkyl chain of the toxicant is long, the more toxic it is. Besides, it was found that the bioremediation efficiencies of the microalga at a high density of 6×10^5/mL were 46.7%, 57.4%, 68.2%, and 93.3% for dipropyl phthalate, diallyl phthalate, diethyl phthalate, and dimethyl phthalate, respectively, within 96 h of incubation. Furthermore, the authors reported that the biodegradation of diethyl phthalate and dipropyl phthalate occurred chiefly through transesterification, de-esterification, or demethylation.

14.5 Conclusion

There are increasing worries about the adverse effects of EDCs on human health and ecosystems. Treating these hazardous compounds before they reach the environment is of utmost importance. Considerable efforts are being made to develop eco-friendly and low-cost effective methods for EDCs treatment. Given the enormous diversity in the microorganisms that can interact and neutralize hazardous molecules, the use of biological systems, especially microorganisms, is considered nowadays a promising auspicious approach with the potential to treat virtually all existing xenobiotics (including EDCs) and probably those to come. The large availability and diverse biochemical characteristics of marine microorganisms make them an ideal tool for the bioremediation of EDCs from contaminated environmental sites. However, fundamental research aiming at further comprehending biochemical mechanisms at the base of EDCs biodegradation must be pursued and paralleled by endeavors to construct genetically engineered microbial strains with improved EDCs degradation properties. Additionally, improvement in process engineering will undoubtedly help apply marine microorganisms in EDCs bioremediation in real field conditions.

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Bioreactors for Bioremediation of Polluted Water

15

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Abstract

Nowadays, the bioremediation of water bodies is necessary. Several microorganisms have been reported as pollutant degraders, this capacity is due to their ability to adapt to hostile environmental conditions. Bioreactors are a promising biodegradation technique for hydrocarbons, plastics, heavy metals, and pesticides bioremediation. The design of bioreactors depends on biological systems, characteristics of water polluted, target pollutants, and its scalability. An optimum bioreactor design is expected to have improved productivity, operational viability, better capacity, and removal efficiencies of pollutants in a cost-effective manner.

The important challenge for researchers now is an adequate large-scale implementation, the exploitation of metabolic pathways, and the better use of enzymes.

Remediation of the sea represents the material and cultural foundation of the way of life of people affected by the environment and promotes environmental justice. In this sense, the practice of biotechnologists represents an ethical contri-

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bution to restoring the health of the sea and thereby protecting the livelihoods of people who depend on the marine ecosystem.

Keywords

Bioreactor · Polluted water · Bioremediation

15.1 Introduction

Three-quarters of our world is covered with water; this chemical compound is in the air, sea, land, rivers, lakes, ice, and groundwater. The total global water reserves are 1.4 billion km^3, of which around 97.5% is saltwater and 2.5% is freshwater (Arshad Ullah et al. 2018).

The marine environment acts as a highly productive zone, is a complex ecosystem with rich biodiversity, and covers 71% of the earth's coverage. Marine ecosystems provide important ecological, nutritional, economic, cultural, and coastal protection benefits; they are interconnected with terrestrial and freshwater ecosystems; therefore, changes in one have impact on another, including pollution (Thushari and Senevirathna 2020; Selig et al. 2018).

Unfortunately, marine ecosystems are highly exploited and affected by environmental pollution (Lau et al. 2019).

Pollution is the presence of some substance in a concentration different from the balance in an ecosystem, which can negatively affect it. Global pollution is largely caused by human activities and affects human health to the same extent (Fereidoun et al. 2007). The presence of chemical, physical, or biological components producing a deterioration in a water body, usually due to human activity, is called water pollution (Schweitzer and Noblet 2018). Water pollution occurs from directly identifiable sources such as oil spills, effluents coming out from industries, wastewater effluent, storm sewer discharge, or from different nonidentifiable sources such as runoff from agricultural fields, cars, or urban waste (Sarker et al. 2021; Schweitzer and Noblet 2018). Contaminated marine water affects all aquatic ecosystems' biodiversity and produces climate change impacts (Kılıç 2020). Marine ecosystems are vital for all living beings, and their preservation can be prioritized. For that, nowadays, effective and economical remediation water technologies are required.

This chapter describes the importance and relevance of using bioreactors to remediate polluted water.

The following sections first show the primary water pollutants (hydrocarbons, plastics, heavy metals, and pesticides). Subsequently, bioremediation is defined. After that, the application of bioreactors for bioremediation is presented for each primary water pollutant. Later, it discusses why biotechnology contributes to the realization of human rights. Finally, some conclusions and perspectives are shown.

15.2 Main Water Pollutants

Marine waters are polluted firstly by effluents from industry (Ighalo et al. 2020) and urbanizations (Kumar et al. 2020): fertilizers and pesticides from agriculture (Chaudhry and Malik 2017; Mateo-Sagasta et al. 2017), antibiotics and hormones from the pig industry (Cheng et al. 2020), hydrocarbons, detergents, sewage (Ossai et al. 2020), plastics (Alda-Vidal et al. 2020), and heavy metals (He and Li 2020; Kumar et al. 2020). Human activities mostly cause pollution of marine water. The list of pollutants in the world is highly extended. Among the most abundant emergent pollutants entering aquatic systems are hydrocarbons, plastics, heavy metals, and pesticides due to their environmental and human health impacts (Table 15.1) (Kour et al. 2021; Kılıç 2020; Jayaswal et al. 2018). We will refer to these four pollutants in the subsequent paragraphs.

Hydrocarbons are organic compounds naturally found in the earth as crude oil, gas and coal consist mainly of hydrogen and carbon. Several products are obtained

Table 15.1 Main water pollutants

Pollutant	Example	Source	Reference
Hydrocarbons	Diesel, gasoline, petroleum, kerosene	Aquatic transport, petrochemical industries, petroleum drilling/refining process, accidental spilling, sabotage of petroleum facilities, and domestic and industrial discharges	Sheikh-Abdullah et al. (2020), Al-Hawash et al. (2018) and Ite et al. (2018)
Plastics	Polypropylene (PP), polyethylene (PE), polystyrene (PS), polyvinyl chloride (PVC), and polyethylene (PET)	Carried by rivers, streams, washed by stormwater runoff, sewage overspills, wind, or where human directly litter, such as roadsides, beaches, or rivers	MacLeod et al. (2021), Thushari and Senevirathna (2020) and Schweitzer and Noblet (2018)
Heavy metals	Cd, As, Pb, Ni, Cu, Hg, Zn, or Cr	Chemical weathering of rocks, decomposing vegetation and animal matter, atmospheric deposition, metal refineries, fertilizers, electronics production wastes, rubber-product-processing industry, painting, cadmium-nickel battery manufacture, and vehicle exhausts	Madhav et al. (2020), Singh et al. (2020), Hiew et al. (2018) and Schweitzer and Noblet (2018)
Pesticides	Carbamate, organophosphate, organochlorine, and pyrethroid	Control weeds, private gardens, agricultural, livestock activities, and for public health for prevention of vector-borne diseases	Hassaan and El Nemr (2020), Jayaswal et al. (2018) and Rodríguez et al. (2018)

from crude oil as diesel, gasoline, or kerosene (Sheikh-Abdullah et al. 2020). For that, hydrocarbons are considered the main energy source and materials for different industries (Meng et al. 2019). Hydrocarbons contamination is generated by aquatic transport, petrochemical industries, petroleum drilling/refining process, accidental spilling, sabotage of petroleum facilities, and domestic and industrial discharges (Al-Hawash et al. 2018; Ite et al. 2018). The improper disposal of hydrocarbons causes severe damage in various ecosystems, particularly aquatic ecosystems. An important adverse effect is bioaccumulation on flora, fauna, and humans; when hydrocarbons enter in the food chain could have mutagenic, neurotoxic, and carcinogenic effects (Tursi et al. 2019; Ite et al. 2018). This pollutant affects organisms through indoor exposure when the contaminated water is drunk or by outdoor exposure, inhalation, or dermal absorption (Sheikh-Abdullah et al. 2020).

The growing amount of plastic is one of the most alarming concerns for the environment due to its resistance to decomposition (Schmaltz et al. 2020). Rivers can carry plastics, streams, washed by stormwater runoff, sewage overspills, wind, or where humans directly litter, such as roadsides, beaches, or rivers (MacLeod et al. 2021; Schweitzer and Noblet 2018). On December 2019, the world was affected by a pandemic originated by a coronavirus (SARS-Cov-2), and the use of personal protective equipment (PPE) such as masks and gloves by health workers and later by ordinary citizens became essential. PPE waste now also contributes to plastic pollution in aquatic ecosystems (Silva et al. 2021).

The accumulated plastics in aquatic ecosystems can be classified based on their sizes: megaplastics, macroplastics, mesoplastics, and microplastics (Thushari and Senevirathna 2020). Plastic debris can accumulate on water bodies' surfaces, columns, or floors (MacLeod et al. 2021). Polypropylene (PP) and polyethylene (PE) float in water due to low density, while polystyrene (PS), polyvinyl chloride (PVC), and polyethylene (PET) with higher density are through the water column (Thushari and Senevirathna 2020). Plastic debris has a negative impact on the environment, including the transport of toxic pollutants such plastic additives or chemicals that are absorbed from the surrounding. Aquatic fauna is affected by entanglement or ingestion of plastic waste, even affecting human health (Schmaltz et al. 2020; Thushari and Senevirathna 2020).

Heavy metals such as Cd, As, Pb, Ni, Cu, Hg, Zn, or Cr are regarded as a metals having high atomic weight (63.5–200.6 Dalton) and high specific density (>5 g/mL) and are toxic even at low concentrations (Hiew et al. 2018). Heavy metal sources may be geogenic or anthropogenic. Chemical weathering of rocks, decomposing vegetation and animal matter, and atmospheric deposition are natural sources (Madhav et al. 2020). Main anthropogenic sources of heavy metals in marine water are discharge from mining industries, petroleum refineries, metal refineries, fertilizers used in agricultural activities, electronics production wastes, rubber-product-processing industry, painting, cadmium-nickel battery manufacture, and vehicle exhausts (Singh et al. 2020; Schweitzer and Noblet 2018). The accumulation of high heavy metals in the food chain causes negative effects on aquatic organisms, plants, and humans in general. Heavy metals damage the cell membrane, affect enzymes involved in chlorophyll production, can cause some types of cancer and human nervous disorder (Pooja et al. 2020; Inyinbor-Adejumoke et al. 2018; Bolisetty et al. 2019; Hiew et al. 2018).

Pesticides (herbicides, insecticides, fungicides, rodenticides) are classified depending on their sources: carbamate, organophosphate, organochlorine, and pyrethroid (Hassaan and El Nemr 2020). Pesticides are applied to control weeds, private gardens, agricultural and livestock activities, and for public health to prevent vector-borne diseases, such as dengue (Jayaswal et al. 2018; Rodríguez et al. 2018). Their indiscriminate use to satisfy demands plays a major role in polluting water (Khanna and Gupta 2018). Pesticides are considered chemicals of high impact, because they cause serious health hazards to organisms as they are soluble in fat and can bioaccumulate (Khanna and Gupta 2018). In aquatic ecosystems, pesticides can affect fish, algae, and plankton, deteriorating aquatic habitat (Hassaan and El Nemr 2020). Cancer, allergies, neurologic, reproductive, and endocrine and mutagenic disorders have been related to pesticides exposure (Rodríguez et al. 2018).

An example is the case of the cenotes of Quintana Roo, Mexico. The cenotes of Quintana Roo present evidence of environmental deterioration that affects the very basis of trophic relationships from the level of microbial biota (Alvarado-Flores 2019), which are the sustenance of other species, including even large marine mammals, that live in the ecological niche of the Caribbean Sea (Niño-Torres et al. 2015). It turns out that the cenotes, part of the aquifer, are intimately linked to the mangrove and ocean system (Ramírez et al. 2018). The nutrients that come from the mangrove provide food for the reef. Pollution in the cenotes can be derived from the mangrove or the sea and vice versa.

The contamination of water bodies of Quintana Roo has been reported since 2002:

Presence of neurotoxic heavy metals in marine mussels in the Bay of Chetumal: mercury, lead, cadmium (Díaz López et al. 2006), nickel, and vanadium (García-Ríos and Gold-Bouchot 2002). Also, in Chetumal presence of organic matter, sewage discharge decreases biological diversity. Polychaetes, a type (phylum) of the annelids, were used as bioindicators (Kuk 2007). Fertilizers, herbicides, and pesticides in coastal areas of Othón P. Blanco, in the south of the State, from agricultural activity (Euán-Ávila et al. 2002).

Microbiological contamination in the coastal area of Akumal showed fecal coliforms, with water of poor sanitary quality in December in the high season. Untreated wastewater effluent in coastal regions can affect the communities of the reef biota and modify the biocenosis (set of organisms in a place or biotope) with definitive effects (Barrera-Escorcia and Namihira-Santillán 2004).

Pharmaceutical and personal care products have been found in the aquifer near the coastal area of the Riviera Maya (Tulum and Puerto Aventuras), originating from domestic wastewater. Stimulants and illicit drugs, cocaine, have been found in cenotes, possibly from sewage in tourist areas. The hydrocarbons indicated contamination due to runoff from highways and other impervious surfaces, specifically in Puerto Aventuras. In Tulum and Puerto Aventuras, herbicides and organochlorines pollutants, are from golf course grass.

The current situation of marine water pollution is delicate, so it is necessary to develop economically viable and ecologically safe remediation methods such as bioremediation.

15.3 Applied Bioreactors for Bioremediation of Polluted Water

Remediation water strategies include biotic or abiotic degradation processes (Schweitzer and Noblet 2018). Biotic treatment is an alternative pollutant removal method, because it does not elicit deleterious environmental effects and may also be less expensive than other techniques (Al-Hawash et al. 2018).

Bioremediation can be defined as a biological process to remove, degrade, or transform pollutants into harmless or less harmful substances. Bioremediation can employ microorganisms (bacteria, fungi), plants, algae, or enzymes with complete or partial mineralization. Many studies have shown that bioremediation has received worldwide attention for cleaning most pollutants types, such as hydrocarbons, plastics, heavy metals, and pesticides (Shukla et al. 2019). In particular, water remediation strategies include biotic or abiotic degradation processes (Schweitzer and Noblet 2018). Biotic treatments are effective pollutant removal methods that do not elicit deleterious environmental effects and may also be less expensive than other physic-chemical techniques (Al-Hawash et al. 2018).

Moreover, bioremediation approaches are generally classified as in situ or ex situ. In situ involves the treatment at the site and ex situ elsewhere (Yuniati 2018). In situ technologies include bioventing, biofilters, biostimulation and bioaugmentation; they usually require longer periods of treatment, extended monitoring and depend on environmental factors. Ex situ strategies comprise of biopiling, composting and bioreactors; they allow a greater process and monitored control (Luka et al. 2018). Many critical factors affect the rate of bioremediation as biological activity, nutrients, moisture, temperature, pollutants proprieties and mass transfer limitations (Sheikh-Abdullah et al. 2020; Al-Hawash et al. 2018).

A bioreactor is defined as an engineered device designed to efficiently operate biological processes by controlling culture parameters and managing its optimal conditions (Krychowska et al. 2020). Bioreactors are a promising technique for removing hydrocarbons, plastics, heavy metals or pesticides from the environment, mainly due to their remarkable properties, such as a higher degree of control over process upsets and maintaining the desired biological activity during the culture.

Bioreactors have been used for bioremediation purposes, finding advantages such as a higher degree of control over process upsets and maintaining the desired biological activity. The bioreactor is designed to promote an environment that ensures favorable conditions for mass transport and adaptation of the biological entities. Temperature, pH, agitation, moisture, aeration, and pollutant and nutrient concentration are among the main factors to be monitored or controlled in the reactor. After laboratory-scale testing, these systems can be optimized, standardized, and scaled up. Bioreactors can be classified based on their operating mode (batch, fed-batch, and continuous bioreactors); the reactor shape, which can be pneumatically (bubble column, draft-tube airlift bioreactors, etc.) or mechanically agitated reactors (stirred tank, wave bioreactors, etc.); and their mechanisms (packed bed, fluidized, hybrid, membrane, or photobioreactors, etc.). Figure 15.1 shows different types of bioreactor shapes which have been used for bioremediation purposes. These systems are useful devices that can provide a suitable environment for microbial species with naturally pollutant degradation capacity. The use of bioreactors ensures better degradation rates than degradation under natural environmental conditions. Therefore, a wide variety of bioremediation applications have been developed and evaluated in bioreactors to degrade several contaminants (Tekere et al.

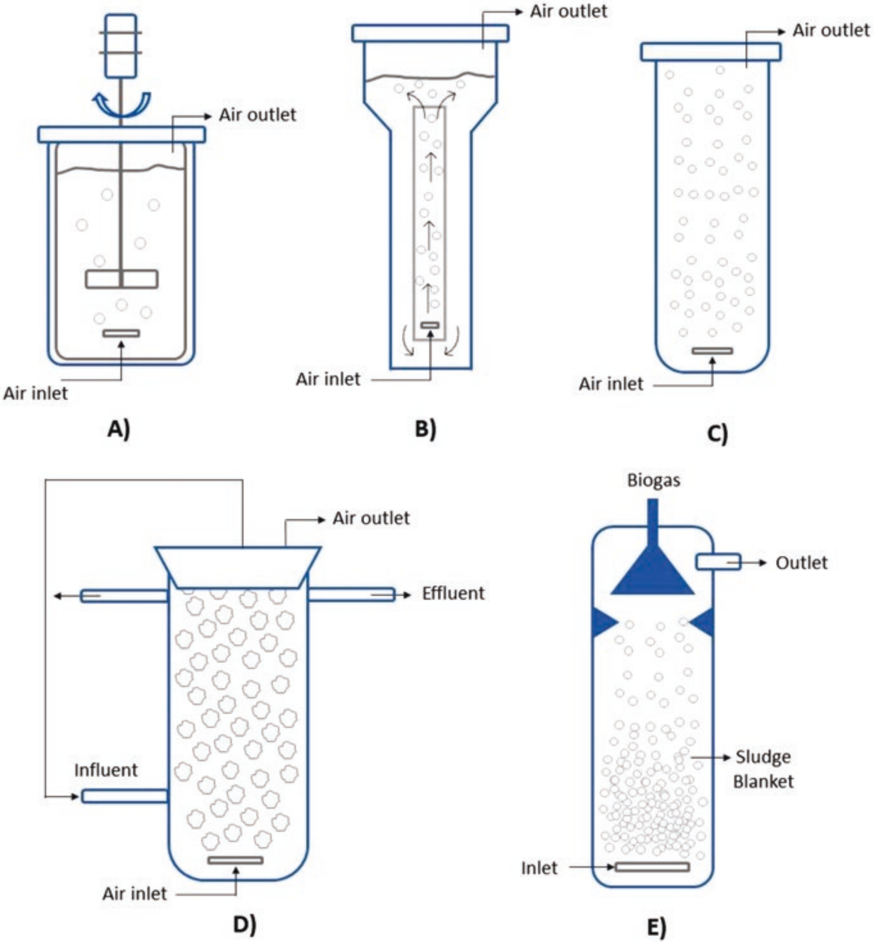

Fig. 15.1 Bioreactor configurations employed for bioremediation purposes. (**a**) Stirred tank reactor, (**b**) airlift reactor, (**c**) bubble column, (**d**) packed-bed bioreactor, and (**e**) upflow anaerobic sludge blanket bioreactor (UASB)

2019; Luka et al. 2018). Different applications for bioremediation purposes are presented below. Various applications of bioreactors for bioremediation polluted water purposes are presented in Fig. 15.1.

15.3.1 Hydrocarbon-Polluted Water

Hydrocarbons have heavily contaminated marine environments due to intensive offshore exploitation, processing, and transport of these chemicals. This has led to large oil spills, such as the Deepwater Horizon oil spill, where nearly four million barrels of oil were spilled (Environmental Protection Agency 2022). The presence of contaminants modifies the balance of ecosystems, favoring the proliferation of microorganisms capable of growing under such environmental conditions. Native microorganisms native to these environments have been isolated to assess their ability to degrade a wide variety of hydrocarbons in order to propose a solution to this global concern. Several laboratory-scale bioremediation studies have been conducted in different bioreactor configurations, the application of which has been suggested to restore oil-contaminated sites (Narciso-Ortiz et al. 2020). Pneumatically agitated bioreactors, such as airlift bioreactors and bubble columns, have attracted attention due to their low energy consumption, efficient mixing, homogeneous shear stress and simple construction (Tec-Caamal et al. 2018).

Our research group has tested studied airlift bioreactors of 0.9 L for hydrocarbons polluted marine water as diesel, gasoline, phenanthrene, hexadecane and pyrene (Sandoval-Herazo et al. 2020; Narciso-Ortiz et al. 2020) using a bacterial consortium, with more than 90% of hydrocarbons degrade. The bubble column is another bioreactor studied by our work team with 3.9 L of volume, where Mayan crude oil was degraded entirely in 12 days (Castañeda-Chávez et al. 2020).

In model medium airlift bioreactors have been studied by hydrocarbons degradation in different volumes: 3 L (Dutta et al. 2018; Tec-Caamal et al. 2018), 7 L (Denis et al. 2017) and 10 L (Medina-Moreno et al. 2013; Lizardi-Jiménez et al. 2012) using the same bacterial consortium, the results were similar, with more than 90% of hydrocarbons degrade.

Interesting results have also been observed in other bioreactor configurations, such as slurry bioreactors (Robles-González et al. 2008), immobilized cells systems (Partovinia and Rasekh 2018), and multistage-designed bioreactors (Antwi-Akomea et al. 2018).

Rodríguez-Calvo et al. (2020) developed a 1 L bioreactor sorbent with 33% filling material (granulated hydrophobic cork and meltblown polypropylene) where hydrocarbon-polluted industrial wastewater was recirculated for 7 days with indigenous microbiota; they showed a retention rate of 97.5% of total petroleum hydrocarbons with granulated hydrophobic cork. Studies carried out in model medium are excellent candidates for the bioremediation of marine water.

Other interesting research on the topic is the investigation of Antwi-Akomea et al. (2018). They studied a process that involved the treatment of hydrocarbon-contaminated salt water in a locally designed multistage bioreactor (eight with a volume of 9.1 L) incorporated with three media filters using a mixed consortium, the coupling effect recorded over 99% total petroleum hydrocarbon removal.

In 2017, Germano-de Almeida et al. studied the biodegradation of marine fuel by a microbial consortium isolated from seawater in 7.5 L stirred bioreactor operated for 24 days; their result was a total hydrocarbon biodegradation of 93.5%.

Blaise-Chikere et al. (2016) used a 2.5 L stirred tank bioreactor for 64 days. The bioreactor medium composition was 1 kg of crude oil-polluted sediment with indigenous bacterial population, 1 L of seawater, 20 mL of crude oil, 0.2 g of anthracene and 20 g of fertilizer as biostimulated, the decrease in petroleum hydrocarbon concentration was 97.2%.

The above experiments have been useful in understanding microbiological and engineering design aspects of the biodegradation process of bacteria cultivated in bioreactors, but also to identify critical factors taking place in real polluted environments (Kapellos 2017; Medina-Moreno et al. 2020). Nature of the bacterial cells (hydrophobicity or bacterial shape), the strategies by which bacteria access hydrocarbons (emulsification, cell attachment to oil droplets or biofilm formation), hydrocarbon droplet size, bubble diameter, dispersed-phase fraction, reactor geometric relations and turbulence regimes in the bioreactor, are among the most important factors found in lab-scale experiments. The challenge for the implementation of bioreactors for bioremediation purposes remains their scale-up. Therefore, future work should address the implementation of mathematical modeling, optimization, bioreactor design and control strategies to make the process viable.

15.3.2 Plastic-Polluted Water

Plastic degradation rates depend on the properties of polymer material, environmental conditions, biological activity, and salinity (MacLeod et al. 2021; Thushari and Senevirathna 2020). The plastic biodegradation process generally includes four steps: biodeterioration, the formation of biofilm over the plastic; biofragmentation, the extracellular enzymes production; assimilation, the absorption of oligomer/dimer/monomer by microbial cells; and mineralization, the production of metabolites such as CO_2, H_2O, and CH_4 (Jeyavani et al. 2021).

Biotechnology bets on the use of enzymes for plastic biodegradation, which is essential to understand these enzymes' mechanisms for engineering aimed to improve biocatalytic applications (Kumari and Chaudhary 2020). Yoshida et al. (2016) reported *Ideonella sakaiensis* as polyethylene terephthalate- degrading (PET) and PET-mineralizing bacteria by PETase and MHETase enzymes. Until now, *I. sakaiensis* and its enzymes are the plastic-degrading biologic factors more studied.

Carniel et al. (2020) studied postconsumed PET hydrolysis catalyzed by *Humicola insolens* cutinase in stirred reactors. They propose changes to reduce the costs process: using 0.5 M NaOH by pH control in place of Tris-HCl buffer and reducing enzyme loading, resulting in a 1.16-fold increase of hydrolysis products. Membrane bioreactor technology has also proven the ability to remove microplastics (>90%) from water, but the filtration module is relatively expensive and can be easily blocked, resulting in higher operating costs (Patrício-Silva 2021).

Lopardo and Urakawa (2019) studied the biodegradation of two polymers, polycaprolactone (PCL), a petroleum-based polymer, and polyhydroxyalkanoate (PHA) a bacterial-based polymer, in marine recirculating aquaculture system wastewater treatment with microbial community naturally present in the system, for 3 months in 1 L bioreactors. The effect of increasing the C/N ratio and nutrient removal efficiency was explored. Results showed a greater degradation efficiency of 75.9% for PHA and 64.5% for PCL during nitrate-nitrite removal.

In 2020, Briassoulis et al. evaluated the biodegradation of non-floating plastic materials (low-density polyethylene, polyhydroxybutyrate, polybutylene sebacate and polybutylene sebacate-co-terephtalate) in a seawater-sediment interface of the coastal marine. The bioreactor volume was 4 L containing 170 g of sediment with indigenous bacterial population and 400 mL of seawater in stirring, the results showed that all materials were degraded by over 80% except low-density polyethylene.

15.3.3 Metal-Polluted Water

Heavy metals are released into the environment by natural sources, such as weathering processes, atmospheric deposition of particles, forest fires, and volcanic activity, or by anthropogenic inputs, such as sewage, agricultural practices, industrial discharges, industrialization of coastal areas, among others. Metals become persistent and toxic to the environment when they are present above threshold concentrations, and the metals and medium physicochemical characteristics and the affected organisms determine such limits. In particular, the introduction of metals into marine water bodies is a cause for concern, since the imbalance of the environment has led to the contamination of marine flora, with the accumulation of metals in higher animals and invertebrates, which can affect human health, since some organisms serve as food. Arsenic, chromium, cadmium, lead, and mercury are some of the most important metals affecting human health. Therefore, great efforts have been made to develop and implement effective and low-cost procedures to remove these contaminants from water, ensure a safe water supply for human consumption, and preserve natural environments. Among these techniques, the deployment of bioreactors is an attractive green alternative. Several microorganisms are capable of removing heavy metals by biosorption, which is the formation of a surface layer of solutes on the adsorbent material and results in the accumulation of metal ions in the cell components.

Due to their adaptation capacity, the microorganisms have qualified as biosorption agents or biosorbents. Batch, stirred-tank, continuous-flow stirred tank, fixed packed-bed, fluidized-bed, multiple-bed, airlifts, and bubble column bioreactors have been applied in Cr(VI) biosorption (Vendruscolo et al. 2017).

An example is the study of Corral-Bobadilla et al. (2019); they used the Spent Mushroom Substrate of *Agaricus bisporus* in 20 L working volume of the down-flow bioreactor to remove heavy metals from industrial waters (contained chromium, lead, iron, cobalt, nickel, manganese, zinc, copper, and aluminum). The heavy metals were removed more than 50%, pH was a limiting factor in the process.

In 2019, Ibrahim et al. tested different bacterial isolates obtained from a polluted lake to remove heavy metal ions (Cd^{2+}, Fe^{3+}, Cu^{2+}, Mn^{2+}, Co^{2+}, Zn^{2+}, Ni^{2+}, Pb^{2+}) from polluted wastewater samples and designed a fixed bed column bioreactor packed with a solid supporter. The better-identified bacteria was *Pseudomona aeruginosa* and it obtained better results in fixed bed column bioreactor than batch bioreactor, increasing removal efficiency by about 20% more and decreasing consuming time from 48 to 24 h.

Fulekar et al. (2012) studied the bioremediation of Fe, Cu, and Cd by biostimulated microbial culture isolated from heavy metals waste disposal–contaminated site. The biodegradation process was in a salt medium in a laboratory stirred bioreactor for 21 days. The microbial consortium showed effectiveness in bioremediation in heavy metals: 98.5% Cd, 99.6% Cu, and 100% Fe.

The marine environment has many microorganisms with different capacities, including pollutant degradation. De et al. (2006) reported that mercury-resistant marine bacteria, *Pseudomonas aeruginosa* and *Alcaligenes faecalis*, removed approximately 70% of Cd in a seawater nutrient broth in 72 h.

Airlift bioreactors have also been employed for metal removal using extremophile microbes (Tec-Caamal et al. 2021). In this study, the archaea *Acidianus brierleyi* was shown to be able to oxidize iron (FeII)) and arsenic (As(III)), which react and precipitate in the form of biogenic scorodite. An airlift bioreactor was appropriate for arsenic removal, since the gentle mixing allowed biofilm formation, enhanced metal oxidation, and promoted biomineral nucleation. It is worth mentioning that this process is available on an industrial scale (PAQUES Technology 2020).

15.3.4 Pesticide-Polluted Water

Membrane bioreactors combine biological treatment with membrane filtration to remove contaminants; pesticide-polluted water is mainly treated with this type of bioreactor.

Dash and Osborne (2020) demonstrated that *Kosakinia oryzae* is a bacterial strain with pesticide tolerance, efficient biofilm formation, and capable of secreting organophosphate-degrading enzymes in vertical-flow packed bed biofilm bioreactor using *Agaricus bisporus* as a biofilm carrier in salt media. They showed over 90% of organophosphorus biodegradation capacity in 120 min.

Another interesting investigation is the study of Asif et al. (2018); they probed laccase degradation of trace organic contaminants (including pesticides) by a laboratory-scale (1.5 L) membrane distillation–enzymatic bioreactor system. Biodegradation of pesticides was over 50%, better than previous reports (<10%).

Ravi et al. (2021) presented a study for the biodegradation of chlorpyrifos in a two-phase partitioning bioreactor using bacterial consortia isolated from cow dung slurry as an aqueous phase and hexadecane as an organic phase. They reported that

93.44% of pesticide was degraded in 15 days. This two-phase system is the baseline to salt-out or salt-in the specific pollutant across the phases to maintain the higher surface area.

15.4 Biotechnology to Remediate Marine Ecosystems: A Contribution to the Realization of Human Rights

In Latin America, a critical perspective of human rights in the Marxist tradition is currently gaining relevance (Kala and Vargas 2020). This perspective is critical of the idealist philosophical tradition of law, which proposes the concept of the realization of human rights based on the good design of the law and its good practice (Curcó 2018; Redondo 2018). From the critical perspective of human rights, their realization is not based on the law but rather on the existence of concrete material and historical conditions that contribute to the just life of humanity (Médici 2020; Rosillo 2013).

From the critical perspective of law, the concept of the realization of human rights refers to the search for an experiential practice of justice. While justice may be stipulated in local, national, and international laws and norms, it is only formal. From the critical perspective of human rights, justice is defined as a practice of permanent "cultural production" (Herrera 2005) in the hands of individuals, collectives, or peoples who, living in a context plagued by injustice, seek to experience just social relations.

From a critical human rights perspective, marine ecosystems constitute the material basis for producing and reproducing human and nonhuman life. In the same sense the Global Ocean Observing System Steering Committee (GOOS) (COI 2018a) recognizes that the ocean "sustains planetary life, produces most of the oxygen we breathe, and regulates the climate." Hence, the ocean is "essential for our sustainable development and well-being and our security and prosperity."

Considering the sea as the support of life on the planet became relevant in international forums in the middle of the last century. In 1960, UNESCO's Intergovernmental Oceanographic Commission (COI) was created. Since then, the COI has been dedicated to implementing a strategy of scientific observation of the oceans to "address climate change, generate predictions and protect the health of marine ecosystems" (COI 2018b). Currently, the United Nations 2030 Agenda includes an explicit ocean goal, the Sustainable Development Goals (SDG) 14, to: "conserve and sustainably use the oceans, seas and marine resources for sustainable development" (UN 2022).

The sustainable use of the marine ecosystem is a global challenge, considering that the economic potential of the ocean has doubled in the last 20 years. According to the UN (2021: 1), "the contribution of the ocean to the global economy is projected to double from $1.5 trillion in 2010 to $3 trillion by 2030." Productive development is due to "Offshore wind, offshore aquaculture, seabed mining and marine genetic biotechnology were practically non-existent 20 years ago and are now growing into prominence as the relevant technology becomes available" (UN 2021:

1). In addition to the above, the diagnosis of marine pollution by hydrocarbons, plastics, heavy metals, and pesticides makes the challenge of combining development and sustainability of the sea a major one.

In this chapter, we consider that applying bioreactors for bioremediation to revert the water pollutant presented in the previous section contributes to the sustainability of the sea and the realization of human rights. If biotechnology serves to remediate the marine ecosystem, the practice of biotechnology aims ethically to heal the sea, that is, to return it to a state that represents a safe and sustainable material source of life. From a critical human rights perspective, seeking and applying biotechnological (safe) solutions to remediate marine ecosystems is fundamental to experiencing environmental and social justice.

In general terms, the application of biotechnology to a legitimate purpose, such as decontaminating the sea, attributes an ethical property to the work of biotechnologists, but this may not be the only one. In the Latin American context, cases of environmental pollution leave economic, social, and environmental impacts on entire populations (Tetreault et al. 2019). In many cases, these populations are made up of people who consider the sea not only a material source of life but also a symbolic element that gives them a sense of identity. Environmental damage to the maritime space generates people affected by the environment, that is, people who have an ecological need to remediate marine ecosystems, because the continuity of their livelihood and identity depends on it. The intervention of biotechnologists as allies of people affected by the environment can add another ethical attribute to their practice.

In the case of Mexico, the people affected by the environment due to the pollution of the marine ecosystem number in the thousands, and these people affected by the pollution have different identities (Table 15.2). The people affected by the environment are identified as fishermen, farmers, and indigenous people, who form social collectives. Among the objectives of these social collectives is to form alliances with scientists committed to the affected society to remediate marine ecosystems that have already been damaged. Taking the Atlas of Environmental Justice (2022) as a reference, it is possible to document at least three socio-environmental conflicts in Mexican territory that seek the remediation of the marine ecosystem after the environmental impacts suffered in terms of contamination by hydrocarbons, heavy metals, and pesticides in the sea.

The remediation of the marine ecosystem represents, for these collectives in struggle, the recovery of a broad set of rights and values that have been violated. Table 15.2 shows the names of the socio-environmental conflicts in Mexico that see the practice of remediation and the contribution of scientists to repair marine ecosystems and thus contribute to the realization of a broad set of rights.

Table 15.2 Socio-environmental movements in Mexico that see marine ecosystem remediation as the realization of human rights

Conflict name	Environmental impacts	Mobilized groups	Rights infringed
Dispute over maritime space between oil industry and fisheries, Sonda de Campeche, México	Oil spills	– Women's organizations – Fishermen	Losing local knowledge, know-how, practices and culture, deterioration of the landscape and loss of a sense of local identity
The Riverine Pact against oil exploitation in Tabasco, México	Oil spills	– Farmers – Indigenous communities – Citizens (neighbors) – Social movements – Local scientists/professionals – Fishermen	Losing local knowledge, know-how, practices and culture, deterioration of the landscape and loss of a sense of local identity
Socio-environmental conflict on the southern coast of Jalisco	– Heavy metals – Pesticides	– Fishermen – Farmers	Violation of the right to health, loss of food sovereignty, dispossession of public goods. Deterioration of the landscape and loss of a sense of local identity

Source: Based on data from the Atlas of Environmental Justice

15.5 Conclusions

Nowadays, the bioremediation of water bodies is necessary. Several microorganisms have been reported as pollutant degraders, this capacity is due to their ability to adapt to hostile environmental conditions. Bioreactors are a promising biodegradation technique for hydrocarbons, plastics, heavy metals, and pesticides bioremediation. The design of bioreactors depends on biological systems, characteristics of water polluted, target pollutants, and its scalability. A good bioreactor design is expected to have improved productivity, operational viability, better capacity, and removal efficiencies of pollutants in a cost-effective manner.

The important challenge for researchers now is an adequate large-scale implementation, the exploitation of metabolic pathways, and the better use of enzymes.

Remediation of the sea represents the material and cultural foundation of the way of life of people affected by the environment and promotes environmental justice. In this sense, the practice of biotechnologists represents an ethical contribution to restoring the health of the sea and thereby protecting the livelihoods of people who depend on the marine ecosystem.

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Part IV

Others Applications and Perspectives

Industrial Importance of Marine Algae

16

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Abstract

The biosphere is now a busy, thriving place due to the microbial occupants of the globe ocean. Additionally, marine microflora may have been the first lifeforms on earth and, therefore, the progenitors of all lifeforms, a distinction that places marine microorganisms in a central spot in the saga of evolution. This is due to the fact that existence on this biosphere most likely apparently started in water. The biosphere's workhorses, marine bacteria, complete many of the processes in these biogeochemical cycles. Marine bacteria, which possess a wide range of potential activities, are responsible for the majority of the planet's metabolic

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processes that maintain the elemental cycle. Additionally, marine microbial populations have high metabolic rates. Even while terrestrial species make up the great bulk of the planet's biomass (3200 gigatons or more, or 1015 kg), marine plankton, which weigh around 0.4 gigatons, are responsible for 45% of the planet's overall oxygen respiration. Microalgae inhabit unique environments that compete for space and nutrients, and have excellent adaptation strategies to live under different physicochemical conditions. Therefore, they evolve with fantastic defense strategies to generate new secondary metabolites (natural bioproducts). In general, the production of these bio-products varies between species and even within the same algae. The production of organic products depends on factors such as environmental conditions, season, geographic location, and life cycle stage. Biologically active compounds (bioproducts) are physiologically active substances that have functionality in the human body.

Keywords

Marine plankton · Biogeochemical cycles · Fisherelin · Cyanobacteria

16.1 Introduction

Microbial life in the waters altered the chemical equilibrium of the oceans and atmosphere over millions of years following the development of the earliest life forms and introduced gradients of oxidizing (electron-scavenging) and reducing agents (electron sources) (Adrian et al. 2009). Molecular oxygen was first introduced to the atmosphere by early bacteria, which paved the way for the emergence of plants, creatures, and eventually humans. A new era of chemistry, based mostly on redox chemistry—the transfer of electrons from one molecule to another—was ushered in by these microbe-induced alterations. The stable biogeochemical and climatological oscillations that support life on this planet are now based on redox chemistry (Bahr et al. 1996).

Salinity, currents, terrestrial inputs, and climate are just a few of the many forces and factors that have an impact on the microbial ecosystems in the seas (World Health Organization (WHO) 2008). In the open ocean, salinity is generally constant, although it is less consistent along the coast. Our knowledge of transport systems in the deep sea has been significantly affected by the discovery that ocean and seafloor currents operate differently than previously believed. Gradients of nutrients, contaminants, and other material are produced by terrestrial inputs, which have an impact on habitats. The highest scale of influence on microbial ecosystems comes from climate effects. Marine communities can be impacted by temperature, precipitation, and wind (especially windborne particulate matter) (Todar 2009; Scheutz and Strockbine 2005).

Importantly, marine microorganisms themselves have an impact on their environments through the consumption, production, and sequestration of various chemicals. As a result, microbe-controlled mechanisms frequently regulate gradients of materials crucial to both micro- and macroorganisms in the ocean (Wilson 2005).

Microbial environments change throughout many time scales—diel (day to day), occasional, decadal, and longer. A significant number of the progressions prompted by human exercises can affect marine microbial networks and, thus, can influence the ways by which those networks tweak the climate and environment (Biavati and Mattarelli 2003). Transient changes in marine microbial natural surroundings can be outlined by portraying three unique living spaces: the focal Pacific gyre, the Chesapeake Bay, and aqueous vents. The focal Pacific gyre is an untamed sea environment that changes on a diel premise, yet it has likewise displayed changes over a long time as movements happen between local area mastery by diatoms and by picoplankton (Ishii et al. 2006; Carpenter et al. 2005). The Chesapeake Bay displays diel changes, checked occasional changes, and significant decadal changes over the recent hundreds of years as human exercises have caused significant damage. Aqueous vents display both short and significant stretches of variance, adjusting over the direction of minutes, hours, and many years. This fluctuation makes a fleeting and erratic living space for microorganisms (Chaffron et al. 2010; Comté et al. 2006; Eiler and Bertilsson 2004).

The issue of either sea microbial variety are either wordlywise, or are limited and restricted to sure terrestrial regions, was produced decades in the past. Current evidence displays that most coastal bacteria are not wordlywise, but, alternatively, are limited to distinguishing resistance types or terrestrial parts (Lindeman 1942; Lindström et al. 2005; Liu et al. 2009). However, skilled are any instances of really worldly-wise structures, containing the open ocean-ocean sea group I archaea. Many sea surface waters claim constant sales of nearly 106 microorganisms and 107 viruses per milliliter, a condition that concede possibility be contingent on mineral disadvantages and predatoriness controls required by protists and viruses. In few cases, microbial society numbers are retained at about an order of size beneath the giving competency of the habitat—presumably by predatoriness (Logares et al. 2009; Newton et al. 2006). Viruses grant permission synchronize accompanying their hosts, serving to makeup societies and variety, but few studies have proves that there placement of viruses from a method has no effect on bacterial affluence and society form. Protists, play a more opposing, less critical part in stable state support of bacterial container abundances by absorbing many various types of bacterial dupe (Nold and Zwart 1998; Rheinheimer 1980).

Marine microorganisms seize any of metabolic wherewithal that cannot reside earthly microorganisms. As in added environments, the geochemical residence drives the progress of various metabolic proficiencies in the sea atmosphere. For example, cold surroundings familiar the cool drive various reworking than extreme heat, press residences forthcoming hydrothermal vents. Methane seeps are another model of a particularly sea surroundings that has instigated novel metabolic wherewithal. In poison gas seeps, extreme sulfate concentrations integrate accompanying extreme poison gas concentration to favor the anaerobic corrosion of poison gas, an absorption raise only in the oceans. The light compelled protons send proteorhodopsin, that is established only in sea microorganisms, giving an understanding to play a main act in the strength balance of the residence by way of allure strength to capably produce strength from light. The use of sodium weak transporters is more restricted to sea microorganisms. Marine symbioses, containing the symbioses betwixt macroorganisms and bioluminescent microorganisms and middle from two points shipworms and nitrogen repairing cellulolytic microorganisms, have likely encourage in activity many singular metabolic actions.

16.2 Natural Algal Products and Biological Significance

Microalgae are microscopic organisms commonly found in freshwater, estuarine, and marine environments (Mobin and Alam 2017, 2018). These are usually single-celled microorganisms that exist individually, in chains, or in groups. Sizes vary from a few microns to hundreds of microns depending on the species (Mobin and Alam 2018; Alam et al. 2014; Suganya et al. 2016). They are photoautotrophic organisms that can make complex compounds using simple substances normally available in the environment. It is estimated that there are between 200,000 and 800,000 species of microalgae, of which about 50,000 have been described (Mobin and Alam 2018; Richmond 2004). Because they live under a variety of environmental conditions, they have evolved many defense mechanisms to survive under adverse climatic conditions (varying salinity, temperature, nutrients, ultraviolet radiation, etc.) and eventually new structures. They create a wide range of attractive compounds with a wide range of bioactivity (Herrero et al. 2013). Microalgae therefore provide a commercial source for a variety of high-value bioproducts.

16.3 High-Quality Commercial Bioactive Natural Products

Microalgae have been described as true natural reactors for the production of bioactive natural products and are an excellent alternative to chemical synthesis of certain bioactive natural products of commercial interest (Plaza et al. 2009). We have a great interest in developing and manufacturing various bioactive compounds that may be used as functional ingredients such as carotenoids, phycocyanins, polyphenols, fatty acids, and polyunsaturated compounds. Microalgae-derived bioactive compounds such as proteins, fatty acids, vitamins, and pigments are obtained directly from primary metabolism or synthesized from secondary metabolism (de Morais et al. 2015). In most microalgae, bioactive compounds are rich in biomass. However, in some cases, these metabolites are seccreted into the medium (de Morais et al. 2015).

16.3.1 Antioxidants

Microalgal biomass is a rich natural source of antioxidants with potential applications in human food and aquatic feeds, cosmetics, and pharmaceuticals (de Morais et al. 2015). The use of antioxidants for health has been stimulated by the observation that free radicals and oxidation are involved in many physiological functions that lead to disease states. In the process of evolution, they have adapted uniquely to extreme habitats. A photosynthetic lifestyle leads to high oxygen levels and severe stress. This leads to the development of numerous effective defense systems in the body to prevent the accumulation of free radicals and reactive oxygen species, and this mechanism protects microalgae from cell-damaging effects.

16.3.2 Cosmetics

Cosmetics refers to substances applied to the human body for cleansing, healing, antisun, antibacterial, antiaging, anticellulite, moisturizing, beautifying, perfuming, altering appearance, and other purposes. No more harm to human health than soap (Saraf 2012). Numerous formulas have been developed to smooth, revitalize, and beautify skin, hair, and more (Novak et al. 2014). Microalgae extracts are already used as raw materials in cosmetics containing biologically active ingredients claimed to have medicinal or drug-like benefits (Borowitzka 2013). These extracts are commonly found in cosmetic packaging, especially as ingredients for face, hand and body creams or lotions (Anbuchezhian et al. 2015). The microalgae and cyanobacteria like *Arthrospira, Donaliella, Haematococcus,* and *Chlorella* are mainly used in cosmetics (Borowitzka 2013). Extracts from these microalgae are added to regenerating creams and lotions. It is also used in sunscreens, shampoos, and hair masks. *Chlorella vulgaris* extract has been reported to stimulate collagen synthesis in the skin. It also contributes to fiber regeneration and reduces wrinkles on the skin surface (Codif, France). Protein-rich *Arthrospira* extract slows skin aging (Exsymol, Monaco).

16.3.3 Antifungal Properties

Cyanobacteria (cyanobacteria) are a highly diverse group of Gram-negative prokaryotes and the oldest photosynthetic organisms: 2000 different strains of freshwater and marine cyanobacteria have been found worldwide, demonstrating remarkable ecological diversity. Its ability to grow under adverse conditions and its autotrophic properties make it suitable for cultivation in nutrient-poor lakes, ponds, and seas that pose a serious threat to water and cause eutrophication (Chorus and Bartram 1999; Duy et al. 2000). This can lead to unpleasant tastes and odors in water due to the release of volatile compounds (Jones and Korth 1995). According to Hunter, several genera of cyanobacteria have been reported to produce toxic aquatic flowers and various toxins (Hunter 1995). Known for their diverse antibacterial activity, microalgae are used worldwide to manufacture a variety of value-added products. Pharmaceutical companies, especially drug discovery departments, have continued this 40-year research to extract new compounds and drugs from cyanobacteria. Development without the addition of organic substrates has practical advantages over microorganisms. Lesser availability and high cost of next-generation antibiotics led to the search for new agents with antibacterial activity. The medicinal and nutritional properties of cyanobacteria were first recognized in 1500 BC when *Nostoc* species were used to treat gout, fistula, and some cancers. Cyanobacterias are also antibiotic resistant. Cyanobacteria are a source of antifungal compounds: Flavanoid-type compounds, fisherelin A, phytoalexin, tritoxin, laxaphycin, ambigin, calophycin, scutophytin, and laxaphycin are found in *Oscillatoria, Cytonemapseudohoffmani*, and *Anabaena laxa*. It is isolated from another species of cyanobacteria that is thought to have antifungal properties. It is isolated from another species of cyanobacteria that is thought to have antifungal activity (Chauhan et al. 2020).

16.3.4 Anti-Inflammatory and Analgesic Agents

A significant portion of pharmaceutical waste in 4444 wastewater consists of anti-inflammatory (AI) and analgesic (AN) agents, which are used as analgesics and anti-inflammatory agents, respectively (Fent et al. 2006). Both groups of chemicals are in widespread nonprescription use, with estimated annual consumption in developed countries of several hundred tons (Daughton and Ternes 1999). Because they contribute significantly to the total amount of pharmaceutical contamination in water, this study explores the most common and commercially available AN and AI chemicals, their occurrence and fate in the aquatic environment, and their therapeutic potential. We aim to provide a thorough and critical review of sewage and drinking water treatment plants. Occurrence and Fate of AN and AI in the Aquatic Environment Individual concentrations in surface waters are shown to be within the GLA 1 range, indicating a high proportion of municipal wastewater (Heberer 2002; Mompelat et al. 2009; Boyd et al. 2003; Quintana et al. 2005; Buser et al. 1999). Therefore, most of the AI/AN agents and metabolites used are ultimately discharged into water bodies regardless of the wastewater treatment efficiency of wastewater FAH. The much lower volume of surface water than sewage treatment plant effluent is due to dilution effects and the potential for removal by natural pathways such as hydrolysis, sorption, biodegradation and photolysis. However, some PhAC residues (such as ANs/AIs) can enter aquifers under recharge conditions and have been detected in groundwater samples from water supplies behind urban sewage treatment plants (Heberer 2002). Studies on the fate of anti-inflammatory and analgesic PhACs in received water indicate that many of them and their metabolites are metabolized by a combination of abiotic and biotic processes and direct or indirect phototransformation (Tixier et al. 2003; Miège et al. 2009; Sedlak and Pinkston 2001). Mechanism-determined: In surface waters throughout the food chain, some of the residues may undergo biotransformation, but the extent of transformation by abiotic reactions is much greater (Rahman and Lowe 2006). In the literature we reviewed, we found that hydrolysis is a minor elimination pathway for environmentally relevant human drugs, most of which are transformed by photolytic and biodegradative processes. We also found that human drugs that cannot absorb solar radiation (nonphotolabile) are relatively biodegradable, and poorly biodegradable or partially biodegradable drugs are photoreactive.. IBP was the only human drug (among those examined in this study) characterized by a high sorption coefficient. Therefore, they migrate into sediments as an additional removal pathway (Tixier et al. 2003; Ziylan and Ince 2011).

16.4 Biotransformation of Arsenic in Algal Cells

Polysaccharides, proteins, chitin, lipids, and vitamins. In the food web, the main producers are edible macroalgae, commonly known as algae. As a medicine and food source, algae are mainly used to produce nutritious products. Algae are also called sea vegetables (Zhao et al. 2018). In the Hawaiian Islands, seaweed is used by humans as a seasoning instead of pepper, oregano, or mustard (McDermid and Stuercke 2003). A myriad of bioactive molecules, including proteins, amino acids, lipids, fatty acids, carbohydrates, sterols, vitamins, and bioactive pigments, is derived from microalgae and is useful in the production of a wide range of nutritious foods and industrial-scale pharmacological applications. (Zhao et al. 2018). Iodine is abundant in freshwater algae. Although food is obtained from terrestrial plants, the iodine content is low and seaweed is a cheaper food option because of the human iodine need (Rajapakse and Kim 2011). The use of freshwater algae is valuable because of its potential applications in industries such as the pharmaceutical, nutraceutical, and cosmetic industries (Gopeechund et al. 2020; Sansone et al. 2020). Many algae antioxidants, such as peptides, polyphenols, polysaccharides, and carotenoids, have been found to be antibacterial, anticancer, antidiabetic, anti-Alzheimer's, Antifibrotic, and neuroprotective (Hosseini et al. 2020). Freshwater organisms and their metabolites produce successive extracts in the form of antioxidants, pure substances, and biomass (El-Shafei et al. 2021). A rich source of natural antioxidants such as sponges, sea squirts, bryophytes, lichens, bacteria, fungi, algae and microalgae. Freshwater algae are the best source of natural antioxidants. The main purpose of antioxidant production in freshwater microalgae and the cleaning ability of such antioxidants is to produce microalgal compounds such as vitamins, sterols and phenolic compounds (Widowati et al. 2017). The potential value and market demand of freshwater microalgae related to carotenoid accumulation is high due to their rapid growth, active metabolism, balanced biochemical precursors and bioproducts (Batista et al. 2013).

16.5 Classification of Algae

Phaeophyte Brown algae (Phaeophytes): There are approximately 1780 species of brown algae and are currently classified in the family Fucoidaceae (or Pheophyta) of the phylum Zooxanthora (De Reviers et al. 2007); this class includes 17 orders Brown algae are notaclose relative of red and green algae, although they are macroscopically similar and intermingled on rocky shores. They belong to another kingdom (Chromista) and their closest relatives are microscopic algae (diatoms, chrysophytes, zooxanthellae) that live in marine and lake plankton. Brown algae are found in all these as of the world, but are more diverse and abundant in coldwaters; especially their largest and most spectacular representatives (Laminariares and Desmareschares species) wholly confined to cold polar and temperate waters. The morphological diversity of brown algae is not inferior to that of green algae and red algae. Filamentous species is composed of finely branched rows, such as *Ectocarpus* and *Pylaiella*, grow on intertidal rocks or larger algae in many parts of the world. However, most brown algae are larger and look like branching ribbons, shrubs, or small trees. Members of Fucales are algae of particular ecological importance, as they form dense areas in the intertidal zone of many rocky coasts of temperate seas. For this reason, they generate habitat that supports enormous biodiversity and are considered keystone species; the Fucus Belt in the North Atlantic and Cystoseira in the Mediterranean are well-known examples. The order also includes the only example of macro algae that float permanently and never clings to the bottom of a rock: the Sargasso Sea in the middle of the North Atlantic Ocean is an area bounded by ocean currents where large amounts of Sargassum are constantly floating around.

TBrown seaweed is largest known seaweed. It belongs to the order of seaweeds represented by the word kombu Ecklonia, Eisenia, Laminaria and Lessonia species produce similar underwater forests in other parts of the world. Anatomically and morphologically, seaweeds are also the most complex algae. Their tissues contain a large number of cells and structures, and their complexity is comparable to that of vascular plants. Based on information about their DNA sequences, their classification has recently changed. Green algae are currently defined as not forming homogeneous, cohesive entities. Instead, they belong to a larger group called Viridiplantae, which also includes terrestrial plants (Lewis and McCourt 2004). Alga is the name of a class that includes all marine green algae. About 920 species inhabit the algae family, which is widely distributed in the world's oceans. The algal bodies of green algae have a wide variety of morphologies, but most of them are very simple. Cladophora and Chaetomorpha are two common taxa, both containing branched or unbranched thin filaments. The sea lettuce species is sometimes called sea lettuce because of its appearance, which is a leaf composed of two layers of cells. These algae grow quickly and are well known for their ability to absorb nutrients from seawater. The growth of sea lettuce is a typical phenomenon in eutrophic waters. If this development is left unchecked, huge masses of sea lettuce accumulate, causing the so-called green tide, which requires mechanical removal of the algal biomass. A specific type of body tissue that exists. Two orders of siphonic green algae—Bryopsidales and Dasycladales—are among the most ecologically effective algae.

These algae consist of a single giant cell with multiple nuclei that serve as its body. *Caulerpa* is the best known genus of siphonal algae. Tropical and warm temperate waters are home to a wide variety of *Caulerpa* species. They are widely used in tropical aquariums and are very popular with aquarium enthusiasts due to their beautiful habits (Stam et al. 2006). Unfortunately, this type of algae tends to grow uncontrollably and rapidly. The spread of *Caulerpa taxfolia* in the Mediterranean Sea after its accidental release from the Maritime Museum of Monaco was one of the most notable instances of land invasion by marine organisms. In the years that followed, *Caulerpa racemosa* var. Cylindracea, introduced from Australia by an unidentified route, has also actively overgrown the Mediterranean Sea (Verlaque et al. 2003; Piazzi et al. 2005).

Different types of eatables like pasta, vegetable soups breads and snacks (Nunes et al. 2020) have contributed to huge growth in industries. The algal industrial revolution has marked an impressive step toward achieving the Sustainable Development Goals (SDGs) and increasing energy demand. Algae production uses closed, lighted culture vessels to process biomass. It can be defined as a photobioreactor (PBR) that seals a nonenvironmental structure without actively transporting gases or contaminants. PBR allows control of pH, temperature, light intensity, and carbon dioxide rate. Low carbon dioxide content and minimal water evaporation enable high concentration production of complex biopharmaceuticals. Many types of this PBR have been developed to culture algae for bioenergy and bioproducts production (Ugwu et al. 2008). PBR plays a valuable role in algae production, producing large amounts of microalgae for universal cultivation of many species. The PBR architecture ensures uniform illumination of the culture surface and rapid mass distribution of carbon dioxide and oxygen. Microalgae cells are highly adherent and can exchange light on the reactor surface. The reactor architecture aids in algal production, reaching rapid levels of mass transfer by not damaging or inhibiting the cultured cells. Products made from seaweed are available in large quantities in the global market. Microalgae are photosynthetic eukaryotes that grow in both freshwater and seawater. The bioactive components of microalgae consist of lipids (12–48%), proteins (18–46%), carbohydrates (18–46%), and carotenoids (10–14%) (Khoo et al. 2020; Koyande et al. 2019; Tang and Ying 2020; Tibbetts and Injaian 2014). In the biopolymer industry, algae are a potential starting material due to their rapid biomass productivity, five to ten times faster than conventional food crops (Makareviciene et al. 2013). Algae are useful not only as an excellent food source, but also because the biodegradability of algae-based bioplastics is beneficial for a variety of purposes in industries such as packaging of polymers in the food industry, agriculture, and horticultural polymers. (Zeller et al. 2013). In the circular economy, algae-based bioplastics are being investigated as a sustainable solution. This is because it can be converted into a natural material by composting using plastic bags, making it a useful alternative to peat to end the life cycle of bioplastics (Khoo and Tan 2010). From indoor photobioreactors, microalgae cultures can be easily grown and do not require cultivated land (Karan et al. 2019). For microalgae, the culture medium is saved with the help of wastewater resources compared to chemical-based culture medium (Karan et al. 2019; Leong et al. 2019; Gómez-López et al. 2019). Algae-based biopolymers offer key advantages for green production and help improve waste management (Rendon et al. 2016). In algae production, the biorefinery has revealed that early development stages do not cause damage or pollution to the atmosphere. In nature, algae absorb CO_2 and release oxygen into the environment (Khan et al. 2018). To produce algal biopolymers, this process can operate with less maintenance and energy consumption compared to other polymer manufacturing industries (Ortelli et al. 2019). The production of microalgae culture consists of two types: indoor photobioreactor and outdoor open pond cultivation. Davis et al. (2011). Scale-up systems and indoor PBR manufacturing require proper design to support construction and cleaning-in-place (CIP) systems (Zhu et al. 2017). In biopharmaceuticals, algae have valuable properties such as low production costs, absence of toxic compounds in many species, high biosynthetic capacity, and potential use as oral delivery vehicles. They are important hosts. Microalgae are single-celled microorganisms that can be easily grown on very inexpensive media. Photosynthetic algae require minimal nutrients in the system for sunlight to drive biomass production. Photosynthetic algae are very useful in sewage treatment. The primary energy source for this algae is light, and it absorbs carbon dioxide and nutrients for metabolism.

16.6 Conclusion

Thus, the production of algal species is a major source of food ingredients that do not require rigorous purification, and these species do not produce toxic compounds. Algae lack harmful endogenous compounds for this unique property of using algae as hosts to facilitate biopharmaceutical production. In the global market, genetically engineered algae for the production of biopharmaceuticals for the production of algae-based biopharmaceuticals are firstly genetic coding and to generate working vectors that aid in the development of transformed algae clones. Develop cloning. Algae production rates are increasing worldwide as algae are used as biostimulants, increasing crop yields and reducing the need for fossil-based fertilizers (Mona et al. 2021). Therefore, marine algae are a source of great natural products for the human need.

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17

Sargassum-Derived Agents for Potential Cosmetic Applications

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Abstract

Significant advances in the cosmeceutical sector have been achieved by brown macroalgae named *Sargassum*, it is a species inhabited in the subtropical, tropical, and temperate regions. It is widespread throughout the three (Pacific, Atlantic, and Indian) ocean basins. Different species based on their geographical location have been widely explored for their antioxidant, anti-inflammatory, skin repair, and photoprotective applications in cosmeceutical industry. *Sargassum* species are an excellent source of essential minerals (potassium, calcium, sodium, and phosphorus) carbohydrates, dietary fibers, essential amino acids (tryptophan, arginine, and phenylalanine), beta-carotene, and vitamins. Hence, it is also considered as a probable caloric source for future global food requirements. We consider the primary constituents in *Sargassum,* which includes sulfated polysaccharides (fucoidan), polyphenols (fucoxanthin and phlorotannins), and mycosporine-like amino acids. Furthermore, their mechanisms are being explained for achieving the antioxidant and anti-inflammatory activities. *Sargassum* has been a widely recognized macroalgae, exhibiting its promising effects in the treatment of UV-induced inflammation and adverse effects. Hence, in this chapter, we elucidate the effect of treatment of *Sargassum*-based formulations on the human skin. We also update the recent advancements in its formulations such as development of *Sargassum*-based nanoparticles, owing to its extensive prominence.

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Keywords

Fucoidan · Fucoxanthin · Phlorotannins · Shinorine

17.1 Introduction

Sargassum is a diverse genus of brown macroalgae, belonging to the class of Phaeophyceae and order Fucales. It spans across the Indian, Pacific, and Atlantic—three ocean basins with numerous species thriving across the tropical, subtropical, and temperate habitats. They are generally found to populate shallow water and coral reefs (Pendleton et al. 2014; Yip et al. 2020). They are often known for their planktonic and pelagic existence; hence, the Sargasso Sea's has been named after the macroalgae (Pendleton et al. 2014; Helm 2021). *Sargassum* genus species are often known to grow up to a length of quite a few meters and are known to have a rough and sticky texture to withstand the strong water currents (NOAA 2021). The list of common *Sargassum* species is compiled in Table 17.1 along with their geographical location, constituents, and therapeutic properties.

17.2 Major Constituents

It is inferred from Table 17.1 that the major constituents in the genus are fucoidan, fucoxanthin, and phlorotannins. Apart from the ones mentioned, *Sargassum* species also have mycosporine-like amino acids (MAAs) that are equally responsible for the antioxidant and anti-inflammatory properties in cosmeceutical applications (Suh et al. 2017a, b). Hence, we highlight these primary constituents and their role in skin antiageing below.

17.2.1 Fucoidan

Fucoidan is the principal component responsible for the antioxidant, antiphotoaging, and cosmetic application of *Sargassum* species (Pangestuti et al. 2021). It is a sulfated polysaccharide exhibiting a potent antioxidant activity. Fucoidan's photoprotective activity has been established by utilizing UVB-irradiated human fibroblast (Pangestuti et al. 2018). It exhibits the primary antioxidant activity by reacting with the generated free radicals and converting them into a stable and less reactive nonradical product (Wang et al. 2009a, b). However, the secondary antioxidant activity is associated with its degree of sulfation and molecular weight (Pangestuti et al. 2021). Fucoidan molecules with a low molecular weight have a greater antioxidant potential than those with a higher molecular weight (Pangestuti et al. 2021). This conclusion implies that sulfate groups present in low-molecular-weight fucoidan are more accessible, hence better absorbed before irradiation and exhibit better secondary antioxidant activity (Koh et al. 2019; Pangestuti et al. 2021). The

Table 17.1 Geographical location, main constituents, and therapeutic properties of common *Sargassum* species

Species	Geographical location	Main constituents	Therapeutic properties	References
S. horneri	North-Western Pacific coasts including China, Japan, and Korea	– Phlorotannin polysaccharide (alginates) – Plastoquinone (sargachromenol) – Monogalactosyl-diacylglycerols – Proteoglycans	Antioxidant, anti-inflammatory, antiphotoaging, skin barrier repair	Fernando et al. (2018), Han et al. (2020) and Murakami et al. (2021)
S. fusiforme	Zhejiang, South China	– Plastoquinone polysaccharide (alginates) – Carotenoid (fucoxanthin) – Lectin – Glycyrrhizin – Fucosterol – Saringosterol	Antioxidant, anti-inflammatory, antiphotoaging, skin barrier repair	Ye et al. (2018), Wang et al. (2018), Zhang et al. (2020) and Li et al. (2021)
S. muticum	Western Pacific, spanning from China and South Korea to Japan and Southern Russia	– Phlorotannins – Meroterpenoids – Polysaccharides (fucoidan, alginate, laminarin)	Antioxidant, anti-inflammatory, antiphotoaging, skin barrier repair	Han et al. (2016), Song et al. (2016), Lee et al. (2017) and Susano et al. (2022)
S. pallidum	East Asia and South-east Asia	– Polysaccharides (alginates, fucoidan) – Oligosaccharides	Antiphotoaging, skin barrier repair	Yang et al. (2019) and Fernando et al. (2020a)
S. siliquastrum	Jaeju Island, Korea	– Chromanols (sargachromanol D, E, K) – Sulfated polysaccharide (fucoidan) – Carotenoid (fucoxanthin)	Antioxidant, antiphotoaging, antimelanogenesis	Cha et al. (2011) and Fernando et al. (2020b)
S. thunbergii	Northwest Pacific	– Phlorotannin – Polysaccharides – Sulfated galactofucan – Isopentadiene	Antioxidant, antimelanogenesis	Ren et al. (2017), Jin et al. (2019), Kang et al. (2020) and Gam et al. (2021)

(continued)

Table 17.1 (continued)

Species	Geographical location	Main constituents	Therapeutic properties	References
S. fulvellum	Coasts of Eastern and southern sea of Korea	– Phlorotannin (fucols, phlorethols, fucophlorethols) – Polysaccharides (fucoidan, alginates, laminaran) – Carotenoid (fucoxanthin)	Antioxidant, antiinflammatory	Lee et al. (2013a) and Wang et al. (2019a)
S. polycystum	Warm seas of Pacific, Indian and Atlantic Oceans	Polysaccharides (fucoidan, alginates)	Antioxidant, antimelanogenesis	Chan et al. (2011), Motshakeri et al. (2014), Palanisamy et al. (2017), Palanisamy et al. (2019) and Fernando et al. (2020b)
S. wightii	Southern Indian coastline	– Fucoidan and fucoxanthin – Phloroglucinol – Phlorotannin, phenolic acids – Flavonoids	Antioxidant, anti-inflammatory	Kumar et al. (2021)

Sulphated polysaccharide of Fucoidan

Supression of NF-κB

Supression of Matrix metallo-proteinase

Supression of collagen damage and photoaging

Fig. 17.1 Fucoidan's mechanism of action for photoprotective, antiaging, and antioxidant activities

low-molecular-weight fucoidan is studied to exhibit a greater photoprotective effect rather than an UV-filtering effect (Kim et al. 2018). Fucoidan's photoprotective and antioxidant activity is mediated by the sulfated polysaccharides, which are responsible for suppressing the active expression of NF-κB. This results in the inhibition of matrix metalloproteinase (MMP-1), an enzyme accountable for degradation of collagen, and subsequent photoaging of human skin (Pangestuti et al. 2018). Other factors such as monosaccharide composition, purity, glycosidic linkage, branching site, and environmental factors (months/seasons) also affect the overall therapeutic activity of different *Sargassum* species (Kong et al. 2007; Kim et al. 2018; Salehi et al. 2019). Fucoidan also stimulates the production of different factors responsible for cell growth, reinforces the differentiation and proliferation of fibroblasts, ultimately leading to restoration of the dermal tissue and exhibiting potent antiaging activity (Kim et al. 2018). Fucoidan's mechanism pathway as a potent antiphotoaging agent is demonstrated in Fig. 17.1.

17.2.2 Fucoxanthin

Fucoxanthin is a natural lipid-soluble pigment found in large amounts in *Sargassum* species. It has been studied to inhibit H_2O_2 and ultraviolet-induced oxidative stress in human fibroblasts (Heo and Jeon 2009; Urikura et al. 2011; Wang et al. 2019b). It reduces the oxidative stress by two broad mechanisms. Firstly, by inhibiting MTOR, AKT, or PI3K pathway, which initiates cell apoptosis mechanism. Secondly, by inhibiting p38-MMP-2/9 pathways, which suppress the invasion and migration of glioblastoma cells (Liu et al. 2016b). Fucoxanthin also exhibits photoprotective effects and reduces UVB-induced photoaging via Reactive Oxygen Species (ROS) scavenging activity. It disperses the excess absorbed energy as heat and subsequently returns to the primary ground state, thereby diminishing the adverse effects caused by UV irradiation. Hence, it is also known as a singlet oxygen quencher (Sachindra et al. 2007) In the recent trends, fucoxanthin has shown to promote the activity of a UV-sensitive gene filaggrin in UV-induced sunburn cases. This stimulation of filaggrin by fucoxanthin suggests that *Sargassum* significantly exerts promotion of skin repair and barrier formation, which is useful in postacne treatment. In humans, efficient absorption of fucoxanthin primarily depends on various factors, such as the degree and form of dietary lipids that is consumed, the stability and integrity of the matrix to which fucoxanthin is attached, and supplementary dietary factors including the amount and intake of dietary fiber (Urikura et al. 2011; Liu et al. 2016a; Chen et al. 2016; Matsui et al. 2016; Pangestuti et al. 2018). Furthermore, in a study conducted on hairless mice, a fucoxanthin-based topical formulation was applied on an erythema model that was previously irradiated by UV-B. It revealed that fucoxanthin exhibited the photoprotective properties by suppression of iNOS and COX-2 while upregulating the expression of HO-1 protein through Nrf-2 pathway (Saha et al. 2020). Moreover, when fucoxanthin was topically administered prior to UV-B radiation in the same model, it showed significant antiangiogenic effects as well (Urikura et al. 2011). A study conducted on reconstructed human skin concluded that fucoxanthin improved the expression of proinflammatory mediators, such as TNF- α, COX-2, iNOS, IL-6, and IL-8. It was also observed that fucoxanthin was efficient in reducing expression of MMP-13 and occurrence of epidermal hypertrophy in the epidermis of the skin (Han et al. 2021) Other studies also revealed that fucoxanthin treatment ameliorated corneal damage that was induced by UV-B irradiation. It achieves this by downregulating the expression of Vascular endothelial growth factor (VEGF) (Urikura et al. 2011). In a study, the antiphotoaging effect of Fucoxanthin (isolated from *Sargassum siliquastrum*) was studied in human fibroblasts in which a significant reduction in oxidative stress through ROS-scavenging activity was achieved. In this, fucoxanthin was applied 24 h before the quantification of results. Fucoxanthin was observed to stabilize the UV-B-induced cell damage in a dose-dependent manner (Heo and Jeon 2009).

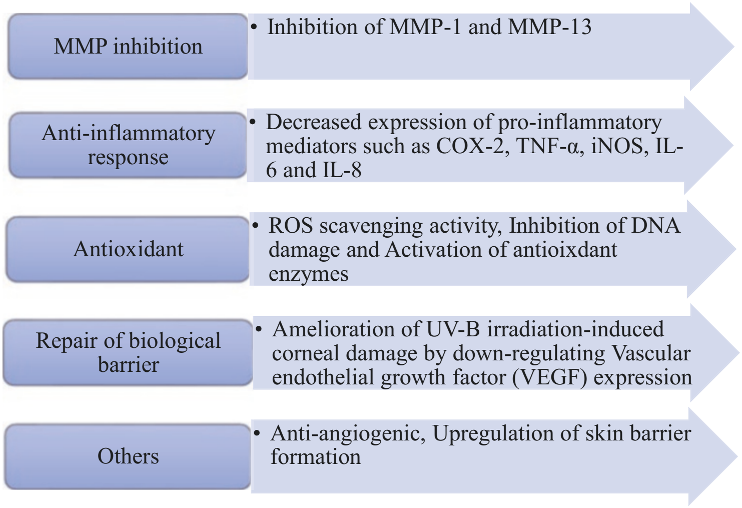

Fig. 17.2 Various activities exhibited by Fucoxanthin

Furthermore, fucoxanthin also exhibited an equally notable ROS-scavenging activity in HaCaT and HDF cells of UV-B-irradiated mice (Rodríguez-Luna et al. 2019). From the structural viewpoint, fucoxanthin possesses an uncommon allenic bond and 5,6-monoepoxide, which has a vital role in its ROS-scavenging activity. Moreover, in fucoxanthin's structure, the functional groups present in the terminal ring also influence its activity as an antioxidant. Fucoxanthin, being electron rich, is an efficient radical scavenger in the carotenoids class. Hence, it can be collectively understood that fucoxanthin could be a potential natural adjuvant molecule for inhibiting photoaging (Peng et al. 2011). Figure 17.2 summarizes the different pathways through which fucoxanthin exerts excellent photoaging and anti-inflammatory properties.

17.2.3 Mycosporine-Like Amino Acids (MAAs)

MAAs have been shown to be among the most potent naturally occurring UVA-absorbing molecules (Pangestuti et al. 2018). Some examples of seaweed-derived MAAs found in *Sargassum* species are Porphyra-334, Shinorine, and Palythine. Their antioxidant activity has been tested using a variety of assays, some of which include the Oxygen Radical Absorbance Capacity (ORAC-fluorescein) Assay, 2,20-Azinobis-(3-Ethylbenzothiazoline-6-Sulfonic Acid Assay (ABTS+) radical scavenging, ROS scavenging (De La Coba et al. 2009; Kim et al. 2018). MAAs exhibit substantial antioxidant properties although the underlying mechanisms are not well explained or understood. This discrepancy necessitates further research and testing to determine the precise antioxidant pathways of MAAs. In HaCaT cells, seaweed-derived MAAs have additionally demonstrated photoprotective activity by fending off DNA damage brought on by UV-B radiation (Suh et al. 2017a, b). Recent studies have revealed that the therapy with Porphyra-334 and Shinorine activates the Nrf2 or Kelch-like ECH-associated protein 1 (Keap1) pathway. Through this, Nrf2 and Keap1 dissociate, hence inhibiting the oxidation process, therefore exhibiting their potent antioxidant activity (Gacesa et al. 2018). Before and after exposure to UV light, the levels of mRNA expression of oxidative stress defense proteins, such as endogenous antioxidant and malondialdehyde, were measured. Shinorine and Porphyra-334 therapy was observed to boost the expression of endogenous antioxidants (SOD, GSH-Px, and CAT) and reduce the expression of malondialdehyde in UV-irradiated mice (Ying et al. 2019). This regulation emphasizes the antioxidant benefits of MAAs obtained from seaweed. Several mechanisms exhibit this activity, including significant UV absorption, protection against macromolecule damage, and antioxidant efficiency. Seaweed-derived MAAs have also been evaluated for their anti-inflammatory activities in HaCaT cells irradiated by UV (Cho et al. 2014). Porphyra-334 demonstrates the anti-inflammatory effect by inhibiting the production of COX-2 and a primary cytotoxic mediator involved in the innate response in mammals (Pangestuti et al. 2011). In addition, treatment of LPS-stimulated macrophages with Shinorine and Porphyra-334 has shown potential anti-inflammatory characteristics (Lee et al. 2021). In a study conducted on UV-irradiated mice, Poprhyra-334 drastically decreased the production of pro-inflammatory mediators by restricting the activation of the NF-κB and MAPK signalling pathways (Becker et al. 2016; Terazawa et al. 2020). It also demonstrated potential antiphotoaging capabilities by inhibiting MMP1 and MMP-3 levels. When human dermal fibroblast cells were treated with Porphyra-334, an increased concentration of extracellular matrix components such as type I collagen, procollagen, and elastin was observed while inhibiting advanced glycation end products (AGEs) (Orfanoudaki et al. 2019). According to the findings of this study, by absorbing UV light, Porphyra-334 treatment preserves the structural integrity of collagen fibers. It has also been observed that Porphyra-334 inhibits the expression of the caspase-3 protein in UV-irradiated HaCaT cells, indicating that it possesses anti-photoaging capabilities. Therefore, Porphyra-334 has shown promising anti-photoaging properties. It exhibits the photoprotective effect by the downregulating the pro-apoptotic proteins (Suh et al. 2017a, b). Suh et al. (2017a, b) investigated the expression profiling of Porphyra-334 genes and microRNAs (miRNAs) that were modified in response to UV exposure, as well as the functional networks of these genes. Both the Wnt (Wingless/integrase-1; linked to UV-repressed genes) and the Notch signalling pathways were shown to be mediated by Porphyra-334. In addition, it is hypothesised that Porphyra-334 shields cells from UV-induced photoaging by modulating the expression patterns of genes involved in UV-mediated biological processes. This would have the effect of preventing the cells from ageing prematurely (Suh et al. 2017a, b). In general, the capacity of MAAs to convert potentially damaging UV rays into heat that is then released into the environment without producing any reactive photoproducts allows them to protect the skin cells. The treatment with MAAs was successful in reducing the depth of wrinkles and roughness of the skin while maintaining suppleness. This lends credence to the idea that MAAs are efficient and possibly useful antiphotoaging agents. In a recent article, it was discovered that sunscreens made with MAAs showed the same Sun Protecting Factor (SPF) and UV-B Biological Effective Protection Factors (BEPFs) as reference sunscreens, but a slightly lower UVA-BEPFs (De La Coba et al. 2019). Collectively, these studies have shown that MAAs have the ability to act as excellent antioxidant and anti-inflammatory agents when triggered by UV light.

17.2.4 Phlorotannins

Phlorotannins, a class of polyphenol complexes, are found solely in brown seaweeds. They are synthesized via acetate–malonate pathway, which is also known as the polyketide pathway (Koivikko et al. 2005). Phlorotannins are often heralded as *Sargassum*'s only chemical defense: their presence in seaweeds protects them against grazers. They are an essential component of seaweed, present in the cell walls, and are responsible for the absorption of UV radiation. This bioactive agent has been isolated from distinct brown seaweeds including *Sargassum thunbergii and Sargassum piluliferum*. Compared to other phlorotannins isolated from brown seaweed species, phloroglucinol showed a stronger cytoprotective effect when tested in HaCaT cells that were irradiated by UV-B (Pangestuti et al. 2021). Phloroglucinol exhibits a stronger activity as an antioxidant by downregulating the expression of superoxide radical, hydroxyl radical, and ROS, while inducing the expression of enzymes responsible for its antioxidant activity. Phloroglucinol can achieve this by stimulating the expression of nuclear factor erythroid 2 (NFE2)-related factor (Nrf2) and signaling of heme oxygenase-1 (HO-1). Milanović et al. (2020) compared and studied the overall antioxidant activity of 2,4,6-Trihydroxypyridine and phloroglucinol towards HO· radicals. Their study concluded that phloroglucinol possesses a more powerful antioxidant activity compared to 2,4,6- Trihydroxypyridine (Milanović et al. 2020). From this study it was also observed that the chemical and structural modifications in the molecule directly affected the ROS-scavenging ability of phloroglucinol. In addition, various studies have revealed that the photoprotective activity exhibited by phlorotannin is strongly associated with their radical-scavenging activity (Pangestuti et al. 2021). In Phloroglucinol structure, the (–OH) hydroxyl group attached to the aromatic ring donates an electron and gives it to a reactive species or a free radical present. This can be one of the underlying mechanisms followed by phlorotannin for limiting the ROS and ROS-induced damage on macromolecules. This effect is responsible for limiting the signaling pathway such as mitogen-activated protein kinase (MAPK) and signaling transduction pathway NF-κB (Pangestuti et al. 2021). Overall phlorotannin demonstrates a great capacity as an active antiphotoaging substance by exhibiting a variety of actions including anti-inflammatory, antioxidant, downregulation of proapoptotic factors, and MMP-inhibition. Phlorotannin does not exert any toxic effect till a specific level of concentration, which proves its probable and safe use as a photoprotective component in cosmetic products. Its additional biological activity as an antibacterial agent demonstrates its effectiveness as a natural preservative in cosmetics and skincare goods. As a result, it has tremendous promise for usage as a skincare and cosmetic agent with additional potential skin benefits (Pangestuti et al. 2021).

17.3 Effect of Sargassum Treatment on Skin

As we know that when human skin is exposed to sunlight for an extensive period, ultraviolet (UV) rays damage our skin cells. Before understanding the effect of *Sargassum*-derived agents on the skin, let us first have a basic understanding of the skin layers. Skin is organized into two primary layers: epidermis and dermis. The epidermis of ectodermal origin is the outermost layer and plays an important role in resisting against the environmental stressors such as chemical agents, pathogens, and UV (Elwood and Jopson 1997; Lowe 2006; Fuchs 2007; Slominski et al. 2012). The mesoderm gives rise to the dermis, which is below the epidermis and contains hair follicles, nerves, sebaceous glands, sweat glands, nerves, and hair follicles. The dermis also has a lot of immune cells and fibroblasts, which are responsible for the skin's normal functions (D'Orazio et al. 2013). The basement membrane separates the epidermis from the dermis. The layers of the epidermis are mostly based on the shape, size, nucleation, and keratin expression of the keratinocytes. (Fuchs and Raghavan 2002). Keratinocyte is an effective physical barrier and is also involved in accumulation of melanin. This melanin, accumulated in keratinocytes, is often referred to as "natural sunscreen" as it is a forerunner in providing protection against incoming UV photons (D'orazio et al. 2013). UV exposure contributes to a variety of skin troubles including cancer and photoaging. Exposure can be either in the form of occupational, the most prominent, or recreational UV exposure (Fartasch et al. 2012). UV is a part of the electromagnetic spectrum, and the wavelengths fall between that of visible light and gamma radiation. UV energy is categorized into UV-A (315–400 nm), UV-B (320–280 nm), and UV-C. (100–280 nm) (D'orazio et al. 2013). The longer wavelength UV-A penetrates deeply into the dermis and is efficient at generating Reactive Oxygen Species that potentially damages the DNA via photosensitizing reactions. UV-B is almost completely absorbed by the epidermis and very little reaches the dermis. It is directly absorbed by DNA and causes molecular rearrangements, leading to formation of undesirable photoproducts. Studies conducted on human dermal fibroblasts have shown to cause DNA mutations, inflammation, and photoaging (Heo and Jeon 2009; Martinez et al. 2015; Chung et al. 2015; Daré et al. 2020). It also induces a release of cascade of cytokines, as well as vasoactive and neuroactive mediators in the skin that result in an epithelial inflammatory response, a sunburn (D'orazio et al. 2013). However, UV-C is fully absorbed by the atmospheric ozone layer; hence, it is not known to cause much damage to human skin. If the dose of UV exceeds the normal threshold damage response and the damage is significantly greater, the keratinocytes activate apoptotic pathways, which signal the cells to die. This pathway is carried out by activating the damage signals (P53), which alter keratinocyte physiology and mediate cell cycle arrest, ultimately causing the cells to die (Coelho et al. 2009). Another pathway the keratinocytes follow when exposed to UV is the activation and expression of Matrix Metalloproteinase-1 (MMP-1) enzyme. When UV-B penetrates the epidermis layer, it facilitates the upregulation of MMP-1, a collagenase enzyme, this results in an increase in activity and protein expression of MMP-1, which leads to degradation of cutaneous proteins, collagen and gelatin, ultimately leading to loss of elasticity and increased wrinkle formation in the exposed area (Ryoo et al. 2010). However, treatment with fucoidan showed significantly increased suppression of MMP-1, leading to the suppression in collagen degradation, hence restoring the skin elasticity and overall increase in suppleness of the skin. In a study, UV-B light was used to stimulate the expression of keratinocytes in two keratinocytes samples (Song et al. 2016). Sample A was treated with *Sargassum muticum* extract (SME), while Sample B was used as control. It was observed that in Sample A, treatment of cells with SME reversed the deleterious effects of UV-B exposure by almost 90%, while Sample B exhibited the peculiar characteristics of wrinkle formation and loss of elasticity. Furthermore, SME was also observed to inhibit UV-B-induced increase in epidermal thickness, wrinkle length, and collagen fiber loss. SME also inhibited UV-B-induced upregulation of MMP-1 in keratinocytes. Considering the relation between antiphotoaging and antioxidant activity of SME, it can be hypothesized that fucoidan, fucoxanthin, flavonoids, MAA, and phlorotannin present in SME may be the primary constituents responsible for photoprotective and antiphotoaging properties of SME (Song et al. 2016). The UV protection activity of macroalgae is also demonstrated by the polyphenol constituent of fucodiphlorethol G that has been studied to increase the viability of human keratinocytes that were exposed to UV-B. Polyphenol fucodiphlorethol-G exhibits this effect by absorbing the incoming UV-B irradiation and removing intracellular Reactive Oxygen Species, thereby also reducing the harmful effects on DNA fragmentation and its subsequent mutation. Phlorotannin fucofuroeckol-A, present in *Sargassum* species, also demonstrates excellent protective effects against UV-B-induced allergic inflammation in mast cells by decreasing the histamine release, increasing intracellular Ca2+ uptake, and scavenging ROS (D'orazio et al. 2013). According to a study, restoration of collagen fibers was also observed in the skin tissue of mice post treatment with *Sargassum* extract. Based on the results obtained and effects observed, the study concluded that extracts obtained from *Sargassum* species are a suitable candidate as a photoprotective agent (Song et al. 2016). Apart from its widespread use in overcoming photoaging and improving elasticity of the skin, *Sargassum* species can also be used to overcome the problems of hyperpigmentation and dark spots via inhibition of melanogenesis. Melanogenesis is a physiological process that results in production of melanin pigment, which largely prevents skin damage due to UV radiation. (Jung et al. 2009; Heo et al. 2010; Huang et al. 2012). However, its abnormally excessive production and accumulation often causes pathological and cosmetic problems such as hyperpigmentation. In humans, melanin biosynthesis is upregulated by proteins such as tyrosinase (TRP-1, tyrosinase-related protein-1) and TRP-2 (Pillaiyar et al. 2018). The expression of tyrosinase, TRP-1, and TRP-2 is activated by MITF (microphthalmia-associated transcription factor), which is regulated by MAPK (mitogen-activated protein kinase) signaling pathways, including ERK (extracellular signal-regulated kinase) and JNK (c-Jun N-terminal kinase) (Jung et al. 2009; Lee et al. 2013b; Azam et al. 2017). Fucoidan present in *Sargassum* species inhibits this melanogenesis process by effectively reversing the melanin stimulatory effects via reducing the expression of these proteins (Azam et al. 2017; Wang et al. 2020). Fucoidan inhibits the alpha-MSH-stimulated melanin biosynthesis by regulating the ERK-MAPK pathway, which inhibits MITF, thereby downregulating the levels of tyrosinase (TRP-1 and TRP-2) (Pillaiyar et al. 2018). Overall, these results suggest that fucoidan has strong melanogenesis inhibitory activity and would therefore be a potential candidate for skin-whitening products. Other than fucoidan, polyphenols and polysaccharides present in *Sargassum* species were also found to have an excellent antioxidant activity. Among polyphenols and polysaccharides, polyphenols were found to possess a good scavenging ability against hydrogen peroxide and hydroxyl radicals when compared to polysaccharides, while polysaccharides exhibit a better tyrosinase inhibitor activity and increase in moisture absorption and retention ability when compared to polyphenols. *Sargassum*-derived polyphenols were observed to absorb the UV-A and UV-B better than polysaccharides. The results obtained suggested that a combination of *Sargassum*-derived polyphenols and polysaccharides would prove to be a promising duo against photoaging and wrinkle formation. However, the precise mechanism by which these constituents confer these effects and safeguard the skin against photoaging remains to be fully elucidated.

Apart from the previously mentioned mechanisms, species of *Sargassum* have also been observed to exhibit its antioxidant activity via inhibition of mitochondrial apoptosis. According to a study, the pretreatment of human keratinocytes with *Sargassum* species extract was observed to inhibit the mitochondrial apoptosis mechanism by increasing the expression of Bcl-2 protein post UV-B exposure. In contrast, it downregulated the expression of proapoptotic proteins (Bax, P53 and PARP), which were found to increase post a UV-B exposure. This result suggested that fucoidan in *Sargassum* inhibits apoptosis by regulating the apoptotic pathway that is activated by UV-B exposure in human keratinocytes. The list of macroalgae-derived cosmetic products is compiled in Table 17.2 along with their company, country of origin, and therapeutic uses. A complete overview of some of the mechanisms exhibited by various bioactives in *Sargassum* species has been illustrated in Fig. 17.3, while a detailed role of fucoidan in prevention of cell apoptosis has been explained in Fig. 17.4.

17.4 Future Scope

The current state of nanotechnology calls for the creation of a dependable and environmentally acceptable approach to synthesize silver-containing nanoparticles for therapeutic purposes. Through effective green nano chemistry, silver nanostructures of marine seaweed *Sargassum* species can be developed by eliminating the presence and usage of toxic and hazardous solvents and waste. Silver particles with a coating of macroalgae showed strong antibacterial and antimycobacterial activity

Table 17.2 Brown macroalgae-derived commercial cosmetic products

Product	Company name	Place of origin	Therapeutic uses	Algae used	References
Bottom Bay Coconut *Sargassum* Bath Bar	Oasis Laboratory	Barbados	Restores the moisture and suppleness of the skin	*Sargassum* sp.	Desrochers et al. (2020)
Thalgo Micronized Marine Algae Sachet (Bath care)	Thalgo	France	For soothing of skin during psoriasis and eczema	*Laminaria*, *Fucus*, *Lithotamnium*	https://www.thalgo.in/body/thalassotherapy/vt16021-micronised-marine-algae/
Haeckels Photo Algae Mattify	The modern man	United Kingdom	Controls the production and building up of sebum	*Laminaria digitata*	https://www.themodernman.co.uk/us/haeckels-photo-algae-mattify-30ml.html
Kerstin Florian Aloe Gel with Algae (serum)	Kiwla health beauty	United States of America	Protects from inflammation, damaging free radicals, and decreases photoaging	*Laminaria digitata*	https://kiwla.com/products/Kerstin-Florian-Aloe-Gel-with-Algae-for-Face-and-Body?srsltid=AR5OiO3afTJF__hmOyH0zfdfqqid1r8WvT4FMPQ90RIDcSaY3wLy7wEWOL8
Hyaluronic Acid + Algae (hair serum)	Blushlin	India	Protects hair from environmental pollutants by keeping it hydrated and frizz free	*Himanthalia elongata*	https://blushlin.com/product/hyaluronic-acid-algae

Product	Company name	Place of origin	Therapeutic uses	Algae used	References
Undaria Algae Oil	Osea	Malibu, USA	Restores the natural elasticity of skin, provides antioxidant effects, hydrates, softens, and firms the skin	*Undaria pinnatifida*	https://oseamalibu.com/products/undaria-algae-oil

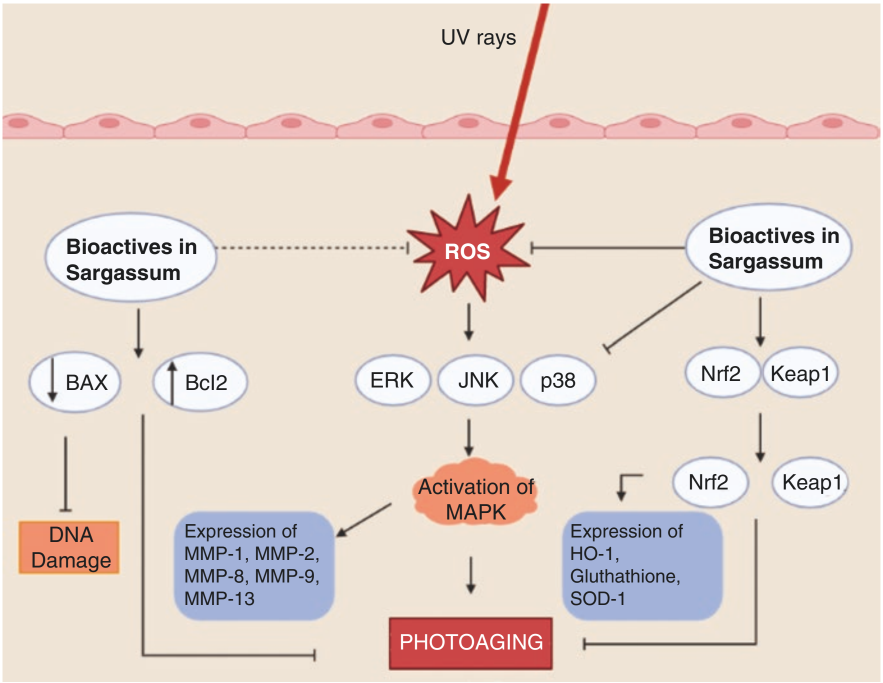

Fig. 17.3 Mechanism of action of various bio-actives in *Sargassum* species against photo aging and DNA damage

(Thiurunavukkarau et al. 2022). Excellent antibacterial activity was revealed by the biosynthesized silver nanoparticles utilizing *S. cinereum* extract, demonstrated by the sizeable zone of inhibition against the multidrug-resistant *Proteus vulgaris*, *Enterobacter aerogenes*, *Salmonella typhi*, and *Staphylococcus aureus*. Comparing *S. aureus* to other pathogens revealed that it had somewhat less resistance. The zone of inhibitions for *S. cinereum* extract generally ranged from 10 to 29 mm. The best activity against these pathogens could be obtained even at the lowest concentration of 2.5 μL of the sample tested. This excellent antimicrobial activity exhibited by *Sargassum* species is of utmost importance in treatment of acne, a condition usually caused by *S. aureus* (Mohandass et al. 2013). *S. polycystum*-coated silver nanoparticles showed notable effectiveness against infections among three distinct types of seaweed. According to a study, the potential bioactivity of various crude extracts (n-hexane, ethyl acetate, and ethanol), alkaloid, and flavonoid extracts from S. fusiforme as antimicrobial agents against diseased Porites lutea led to the conclusion that n-hexane extract and total alkaloids exhibited effective antimicrobial properties in the in vitro assay. (Thiurunavukkarau et al. 2022; Ahmed et al. 2022). This study also makes a strong case for the possibility that *S. fusiforme* extracts have phyto pharmaceutical properties that could be used as natural alternatives to enhance the health level of economically valuable coral species and serve as a good starting point for more in-depth research to address the issue of coral diseases. The

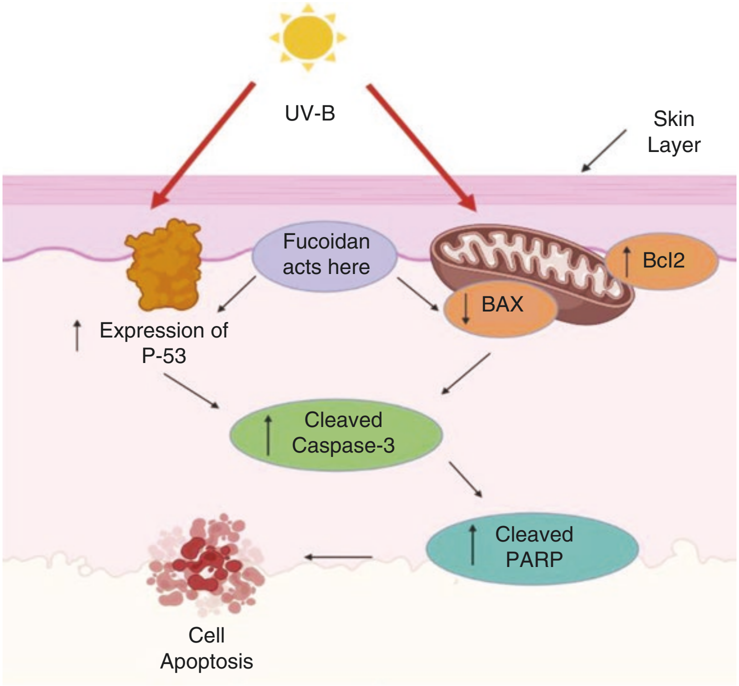

Fig. 17.4 Fucoidan's mechanism of action for inhibition of cell apoptosis mechanism

synthesized vesicles were called phyto-nanovesicles, because they were nanoscale (PNVs). PNVs' antibacterial efficacy against *B. subtilis*, *E. coli*, *S. aureus*, *P. aeruginosa, S. pyogenes,* and *K. pneumoniae* proved that it is a promising formulation. The seaweed *Sargassum binderi*, which was found in the Red Sea in Jazan, Saudi Arabia, is a relatively rare species, and its biological activity has not yet been thoroughly investigated. However, the spectrum of antimicrobial activity was found to be significantly increased when formulated as a PNV formulation. Hence, development of nanoparticles of *Sargassum* species can be considered as a highly promising novel technique for delivering bioactive substances inside the cell (Moni et al. 2019). These nanoparticles showing promising bactericidal and other medical effects will be vital in large-scale manufacturing in the future. We believe that the compiled list of brown macroalgae-derived cosmeceuticals in this book chapter would provide the necessary impetus for researchers to enhance the manufacturing of *Sargassum*-derived cosmetic products.

17.5 Conclusion

Through this chapter, we highlighted the significance of *Sargassum* genus in cosmetic industry owing to its varied properties of antioxidant, anti-inflammatory, anti-melanogenesis, and skin barrier repair. It opens up avenues for further research on the primary constituents responsible for these activities and subsequent development of newer technologies.

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18

Utilization of Seaweed as Partial Replacement to the Fish Meal in Aquaculture Diets

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Abstract

Marine macroalgae are the seaweed, which is found living in shallow coastal waters. They are thallus like in structure and are capable of adhering to many rocky and solid substrata. The eukaryotic algae are rich in polysaccharides, minerals, and other important bioactive components, which makes them commercially important organism globally for food production and nutraceuticals. They are broadly classified into different types based on the pigment composition. Seaweed is considered as super-food as its nutritive profile is rich in protein, lipids, carbohydrates, vitamins, and minerals. Aquaculture industry demands high use of dry fish meal, which makes the aqua feed expensive. Utilization of seaweed in the place of dry fish meal in fish farming is very scare and less experimented. This study is an attempt to prepare a seaweed pellet feed by replacing

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dry fish meal with the seaweed meal for the sustainable development of aquaculture.

Keywords

Super-food · Seaweed · Aqua feed · Aquaculture · Fish meal

18.1 Introduction

Algae form the basis of marine food web and they are broadly classified as micro- and macroalgae. While the former (microalgae) can be seen with aid of microscope, the latter (macroalgae) is naked to the eye. Macroalgae is the commonly known term for the seaweed, which is predominantly found in marine environment. They are neither single celled nor micro in size, but their structure resembles like a thallus. They are eukaryotic group of organisms, which exhibits an autotrophic mode of nutrition. These phototrophic organisms convert the stored chemical energy into macromolecules for its growth and reproduction. Microalgae form the kingdom of Protista, which is plant-like in nature with a profound characteristic feature that lacks water vascular system (internal transport system). They have a rich source of myriad high-value bio-nutrients, chemical components, multivitamins, and other food additives, making them an attractive option for the food industry (Øverland et al. 2019; Lafarga et al. 2020). This chapter addresses the worth of seaweed as an alternative dietary ingredient in formulated aqua feeds.

18.2 Habitat

Seaweed beds form the habitat for many marine invertebrate species and also help in nutrient recycling in the ocean (Duggins et al. 1989). They are primitive type of plants, which forms an important component in marine living resources. They can thrive even in rocky, sandy, or coral environment and are mostly found adhered to rocks, stones, or other substrata in coastal areas.

18.3 Distribution

Seaweeds are submerged aquatic plants, a renewable natural resource, found growing in large quantities along the coasts of India. Gulf of Mannar, Gulf of Kutch, Palk Bay, Lakshadweep, and Andaman and Nicobar islands are reported to be the flourishing prime areas of seaweed growth. Other places include Tamil Nadu (Krusadai Island, Mandapam, Portonovo, Kanyakumari) Goa, Gujarat (Vumani reef near Port Okha; Saurashtra, Veraval), Maharashtra, Andhra Pradesh (Visakhapatnam, Gangavaram), Kerala (Thiruvananthapuram, Kovalam), and Puducherry.

18.4 Seaweed Classification

Seaweeds are categorized into three taxonomic groups based on their pigmentation: Green seaweed (Chlorophyta), Brown seaweed (Phaeophyta), and Red seaweed (Rhodophyta). Seaweed is appreciated for its encumbered health benefits besides nutrition and stands among the world's important marine source commodities, with a remarkably high species diversification (Sugumaran et al. 2022). Among the seaweeds, brown seaweeds have low nutritional value, containing 3–15% crude protein, whereas the green and red seaweeds are reported to contain 26% and 47% crude protein (Fleurence et al. 2018).

18.4.1 Green Seaweed

Green seaweed is mostly found in sublittoral zone. Commercially important species is Sea lettuce (*Ulva* sp.). This genus is found globally and causes "green tides," affecting the water quality.

18.4.2 Brown Seaweed

This group of species reveals a brown color pigmentation, which is due to the rich alginate composition. One of the important and globally known species includes the Kelp, which is often considered as the larger species, which grow dense under the sea. They are found to be thick leather like in texture, which is about 20 m long and are called the giant seaweeds.

18.4.3 Red Seaweed

Red seaweed species are known for its biocomponent carrageen and agar as they are used as thickening agents in the food processing. Most frequently used species for the production of carrageen are Irish moss (*Chondrus crispus*). In the Indo-pacific region, *Kappaphycus* and *Porphyra* species are the largest source of food among the red seaweeds.

18.5 Seaweed: The "Super-Food"

According to the FAO (2020) report in the year 2018, there are 50 countries that are found to be actively engaged in seaweed culture and the farmed seaweeds comprises of about 97.1% by volume of the total of 32.4 million tons of wild-collected species (FAO 2020; Chopin and Tacon 2021). They are packed with biochemical compounds and form the natural food for the fishes to consume. Seaweeds are considered as "super-food" because of its potent bioactive components and are largely used as a diet in many parts of the Asian countries as raw, dried, and cooked form. Presently, it is accepted that seaweed cultivation does not utilize cultivable land, fertilizers, and pesticides compared with terrestrial crop cultivation. Its high-growth capability with less manpower encourage us with a sustainable source of biomaterial and biomass for the future (Venkatesan et al. 2015; Valero et al. 2017).

18.6 Biochemical Property of Seaweed

The seaweed contains many nutritional properties and potent bioactive compounds, which are proven to have antimicrobial, antiviral, antioxidative, anti-inflammatory, Neuroprotective, stress tolerance, and free radical scavengers (Prabu et al. 2016). Seaweeds are rich in polysaccharides and contain water-soluble compounds such as mannitol, laminarin, fucoidan, or alginate in brown seaweed; mannans, starch, and sulfated galactans in red seaweed; and Ulva in the green seaweed, respectively (Perez et al. 2016).

Seaweeds help in feeding process by its efficient gut metabolism in the fishes (Kumar et al. 2012). Carbohydrate is one of the important components for metabolism as it supplies the energy required for respiration and other most important process. Carbohydrates are considered to be the first among the organic nutrients that can be utilized to generate required energy. The type and quality of extracellular polysaccharides, which is secreted in certain algae, can interfere with nutrient absorption or, conversely, be useful binding agents in forming the pellet feed.

Protein content of the seaweed varies with its species diversity and seasonal fluctuation. Protein is the major growth-facilitating factor in any feed. Lipid-enriched diets are considered to play a nutritive role in animal health as they focus on energy reserve, functioning of biological membranes, structural integrity, and also function as precursors for certain steroids (Corraze 2001). Further, seaweeds secrete a lot of colored pigments and secrete storage molecules called the specialized metabolites that could have positive effect on the farmed fishes (Wan et al. 2018).

Seaweeds are rich in micro- and macronutrients and are consumed as preferable food. They also possess a great potential as sustainable resources in terms of its production (Bizzaro et al. 2022). In most cases, seaweeds are used as human or animal food for their mineral contents. Minerals are measured as ash in the recipe. They include phosphorous, calcium, sodium chloride, magnesium, potassium, etc.

18.7 Importance of Seaweed in Fish Culture

Fish farming is one of the fastest booming sectors of global food production. Fish meal and fish oil are key components of farmed fish diets. Seaweeds have been used as food, animal feed, fertilizer, and as sources of traditional medicine by many civilizations. Algal components are publicized for their sustainability and their added functional attributes in aquatic farm animals (Larsen et al. 2022).

18.7.1 Fish Meal Substitution

Fish culture and nutrition mainly depends on the dry fish meal and its protein content. Fish meal is an essential but expensive component in a fish diets as it is abundant in protein and crucial for all biological processes. It is extensively used in fish feed and other animal feeds. Dry fish meal is easily accessible source of animal by-product, which is very rich in protein, yet it is expensive. In recent years, seaweeds have been used as alternative source to protein for fish nutrition (Patel et al. 2018). Seaweed formulation helps to achieve a standard feed composition in pellet feeds as it is proven to enhance the fish feed with its biochemical ingredients (Ismail 2019).

Seaweeds such as *Ulva* and *Enteromorpha* are shown to exhibit a positive effect on the growth performance of rabbit fish fry and have also reduced the cost of feed; further, the replacement of pellet feed with fresh seaweeds did not have any negative impact on the growth performance of rabbit fish fry (Abdel Aziz and Ragab 2017). The efficacy of aquatic macroalgae (seaweeds) in aquatic nutrition is now promising and helps to cut off the fish meal cost. Fish growth is mainly based on the capacity of the feed ingredient that is being digested. The palatability of a feed ingredient is an important criterion in the selection of that ingredient for fish feed. The balanced nutrition of feed reflects in the feed utilization and fish growth factors. Experiment on replacement of fish meal with the brown seaweed (*Lobophora variegata*) showed higher growth profile and enhanced the feed utilization parameters in seabass *Lates calcarifer* fingerlings (Udayasoundari et al. 2016).

18.7.2 Importance of Seaweed in Aquaculture

In aquaculture practices, the use of seaweed as a source of protein remains scarce and underutilized as an ingredient in aqua feeds. The aquaculture production cost required to make an aquafeed is closer to 50–80% of the total finance; hence, the feed usage and management has to be carefully considered and put to use (Saleh 2020). The recent trend in aquaculture feed practices is the search for alternative sources of feed ingredients, especially the cheapest and one which is easily accessible.

Brown seaweed (AquaArom) supplementations have been reported to increase the growth performance, antioxidant potential, and stress and temperature tolerance in Atlantic salmon (Kamunde et al. 2019). Big challenge in aquaculture feed industry is the selection of feed mix and buoyancy of those ingredients that do not dissolve in the water; hence, they rely upon extrusion cooking process to prepare feeds that are nutritionally available and also move down the water system slowly so that the feed is available to the organisms for ingestion.

Seaweeds, such as *Gracilaria* species and *Ulva rigida*, was evidenced to have high potential alternative ingredient in European seabass juveniles diet, which showed significant improvement in growth performance (Valente et al. 2006). Fishes feed well on pellet diets and juveniles are easy to wean to pellets. Seaweed

Ulva sp. has high levels of natural colorants and short chained polysaccharides, which are useful for flesh coloring of the fish and improving gut health, respectively. The process of formulating a feed by using dry seaweed powder as a feed inclusion for the replacement of fish meal is discussed below. Thus blending seaweed mix with aquatic pellet feeds could offer a low-cost way of utilizing the valuable effects of seaweeds in fish culture.

18.8 Methodology for Seaweed Meal

18.8.1 Collection of Feed Components

The basal feed components are purchased from local market; vitamin capsules, mineral powder, and cod liver oil are purchased from the medical shops (Table 18.1). These ingredients are dried to powder form and should be stored in desiccator.

18.8.2 Sampling of Seaweed

The seaweeds were collected from Mandapam coast (South East Coast of India), Ramanathapuram district, Tamil Nadu, during the low tide. Seaweed samples were handpicked and rinsed with fresh water to remove any grit and debris. Then it was transferred to ice box and brought to the laboratory within a day for feed preparation. In the laboratory, once again, the seaweeds were rinsed with running tap water to remove the salt. Excess water was drained by spreading on to a blotting paper and air-dried under room temperature.

Table 18.1 List of feed ingredients for control and experiment formulated diet

	g/100 g	
Feed ingredients	Experiment diet feed	Control diet feed
Dry fish ground powder	12	14
Ground nut oil cake	25	25
Coconut oil cake	13	15
Soya bean meal	25	25
Green gram	10	10
Tapioca powder	3	3
Egg albumin	2	3
Cod liver oil	3	3
Dried seaweed mixture	5	0
*Vitamin tablet mix (Becosules capsules)	1	1
Salt	1	1
Total composition	100	100

18.8.3 Processing of Seaweed Powder

The seaweed and other ingredients were ground and appropriate amounts were mixed to achieve the desired level of seaweed supplementation.

18.8.4 Pellet Feed Preparation

18.8.4.1 Grinding of the Ingredients

The feed ingredients are firstly weighed to the known measurement and ground well using micro-pulverizer and sieved to obtain a fine form using commercial sieve.

18.8.4.2 Mixing Of Feed Powder

All the dry grounded feed ingredients are mixed together and blended along with the dry fish and seaweed meal.

18.8.4.3 Steam Cooking with Feed Mixer

The ground feed mixer along with seaweed meal was steam-cooked at 95–100 °C using steam cooker and further made to cool down at room temperature for 5 min.

18.8.4.4 Inclusion of Vitamin, Cod Liver Oil, and Egg

To the steam-cooked feed mix, inclusion of vitamin and cod liver oil is added and finally made to homogenized dough. Egg albumin and distilled water are added to the dough to bring them to thickened form.

18.8.4.5 Preparation of Pellet and Drying

The thick consistency of feed mix was loaded in the manual pelletizer with 3 mm diameter disc size and pelleted out using aluminum tray. Moist feed was then transferred to electrical trays at 75–80 °C and allowed to dry until the moisture is dehydrated.

18.8.5 Feed Quality Check

Physical properties of the feed such as color, texture, appearance, and smell were observed. Further proximate analysis of the pellet feed was carried out. The water stability test was also tested to the pellets.

18.8.6 Preparation of Experimental Feed

As shown in Table 18.1. Different compositions of the feed ingredients are taken for the experiment feed along with the incorporation of dried seaweed powder in known ratio. Same methodology was followed for the preparation of control diet except that no seaweed powder was added. Thickening agents such as tapioca flour and egg

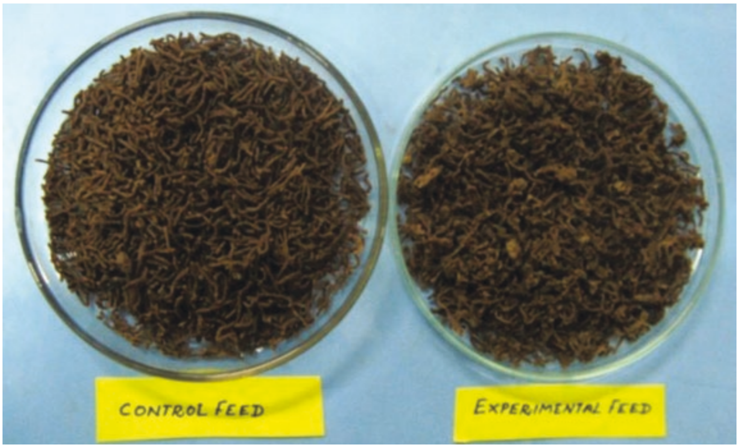

Fig. 18.1 Prepared formulated feeds

albumin were used to make the feed mix into a colloidal form. Using a pelletizer, the pellets were made and dried in hot air oven at 27 °C for about 48 h (Fig. 18.1).

18.9 Conclusion

The present study documents the efficacy of seaweed formulated feed. Effective dietary profile of the formulated feeds could be the greater replacement for fish meal in pellet feeds. Hence, fishmeal inclusion levels need to be reduced and replaced with cost-effective and sustainable feedstuffs like seaweed for the futuristic growth of the aquaculture sector.

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19

Nanobiotechnology of Marine Organisms: Mechanisms and Applications

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Abstract

One of the most emerging fields of research in recent decades is nanobiotechnology, which has wide applications in therapeutics, agriculture, and environment. The nanomaterials can be synthesized using physical, chemical, or biological approaches. Unlike physical and chemical methods, biological synthesis of nanoparticles does not need any hazardous conditions or chemicals for reduction of metal ions to their corresponding nanoparticles and their further stabilization. Various bacteria, fungi, algae, plants, and their metabolites are reported for synthesis of metal and metal oxide nanoparticles. Although there is a great diversity in the marine microflora, relatively less research has been carried out with respect to the exploration of their nanobiotechnological potential. This chapter gives an elaborate account on the marine-microbe-mediated synthesis of nanoparticles and their applications. Marine bacteria such as *Enterococcus* sp., *Marinobacter pelagius, Paracoccus haeundaensis, Streptomyces rochei*, and *Vibrio alginolyticus* can synthesize cadmium sulfide (CdSNPs), gold (AuNPs) and silver (AgNPs) nanoparticles. Similarly, halophilic cyanobacteria such as *Phormidium formosum* and *Phormidium tenue* isolated from sea water can synthesize AgNPs and CdSNPs, respectively. Various marine fungi like *Aspergillus sydowii, Cladosporium cladosporioides, Cladosporium halotolerans,* and yeasts such as *Candida* sp. and *Rhodosporidium diobovatum* can synthesize AuNPs and AgNPs

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with antimicrobial, antioxidant, and anticancer activities. Likewise, numerous marine algae such as *Amphiroa rigida*, *Caulerpa racemosa, Cystoseria baccata*, *Padina* sp., *Portieria hornemannii*, *Sargassum muticum, Sargassum tenerrimum*, *Spyridia filamentosa*, and *Turbinaria conoides* can synthesize a wide range of stable nanoparticles with promising therapeutic applications. The marine-microbe-synthesized nanoparticles are not only stable but also very small with more surface area and high medicinal properties. Hence, it would be interesting to explore these nanoparticles for development of potent nanomedicine by drug functionalization in order to aim targeted delivery and sustained release.

Keywords

Marine microbes · Nanoparticles · Biogenic synthesis · Bacteria · Fungi · Algae

19.1 Introduction

Marine microorganisms living in the hypersaline environment include several halotolerant bacteria, cyanobacteria, fungi, yeast, and algae. These microbes are widely used for production of various bioactive metabolites that are used in cosmetics, pharmaceutics, food, and agriculture (Zhang et al. 2005; Zhang and Kim 2010). Several anticancer compounds like fucoidans, fucoxanthin, dehydrothyrsiferol, didemnin B, ecteinascidin 743, aplidine, and others are reported from the marine microbes (Shah and Ghosh 2020). Likewise, numerous antimicrobial compounds such as loloatins A–D, myticin, and psammaplin A are reported from marine ascidians, algae, bacteria, bryozoans, sponges, and soft corals. It is important to note that the marine microbes have surface-active functional groups that make them efficient biosorbents. *Marinomonas communis, Marinobacter santoriniensis, Pseudomonas aeruginosa, Enterobacter cloacae, Alteromonas haloplanktis,* and *Vibrio harveyi* of marine origin are well known for removal of toxic metals such as lead, copper, cobalt, cadmium, chromium, and arsenic (Ghosh et al. 2022).

The extracellular polymeric substances (EPSs) produced by marine bacteria is a complex mixture of polysaccharides, glycoproteins, humic-like substances, lipids, uronic acid, and nucleic acids. Likewise, complex capsular polysaccharides (CPSs) or slime polysaccharides are composed of neutral carbohydrates such as arabinose, fructose, galactose, glucose, rhamnose, ribose, and xylose apart from galacturonic and glucuronic acids. Both of these EPS and CPSs play a major role in the adsorption of metal ions (Ruangsomboon et al. 2007). Many marine bacteria can produce siderophores such as aerobactin, anguibactin, alterobactin, aquachelin, biscaberin, vulnibactin, and marinobactin for chelating metals followed by their uptake (Gaonkar and Borkar 2017). Certain marine manganese-oxidizing bacteria (MOB) such as *Bacillus* sp., *Marinobacter* sp., and *Roseobacter* sp., and members from *Algoriphagus, Brevundimonas, Idiomarina,* and *Nitratireductor* genus exhibit high metal bioremoval efficiencies (Zhou et al. 2016). Mercury-resistant bacteria (MRB) with functional *mer* operon can reduce mercury to a water-soluble form, facilitating its biosorption, uptake, bioaccumulation, precipitation, and even efflux (Dash and

Das 2012). Marine *Pseudomonas aeruginosa* and *Pseudomonas putida* express *bmtA* gene-encoded metallothionein protein that can bind to multiple Zn^{2+} ions with enhanced affinity, promoting its bioaccumulation (Naik et al. 2012).

In view of the background, it is interesting to explore the nanobiotechnological potential of the marine microflora. More recently, marine microorganisms are considered as promising cell factories for fabrication of novel metal and metal oxide nanoparticles with exotic shape, size, physico-chemical and opto-electronic properties (Manivasagan et al. 2016). Marine microbe-mediated nanoparticle synthesis is an environmentally benign, rapid, and efficient method (Golinska et al. 2014). This chapter gives a detailed account on recent reports of nanobiotechnological prospects of marine bacteria, cyanobacteria, fungi, yeast, and algae as listed in Table 19.1. Further, the mechanisms of intracellular and/or extracellular syntheses are discussed along with the biomolecules serving as reducing, capping, and stabilizing agents during the nanoparticle synthesis.

19.2 Bacteria

Marine bacteria have developed mechanisms to synthesize nanostructures either intracellularly or extracellularly. Rajeshkumar et al. (2014) isolated *Enterococcus* sp. RMAA from marine water. Initially, the bacteria was cultured in the sterile nutrient broth for 24 h at 37 °C followed by recovery of the cell-free supernatant by centrifugation at 7000 rpm. The cadmium sulfide nanoparticle (CdSNPs) were synthesized by reaction of the supernatant with 1 mM $CdSO_4$ for 24 h at 37 °C for 24 h. UV-visible spectroscopy indicated an intense peak at 410 nm confirming the synthesis of the CdSNPs that were mostly spherical with size ranging from 50 nm to 180 nm as revealed by the scanning electron microscope (SEM) analysis. The strong peaks at 3272 cm^{-1} and 1630 cm^{-1} in Fourier transform infrared (FTIR) spectrum were specific to primary and secondary amines or amide linkages in the protein that might play a significant role in the synthesis and stabilization of the CdSNPs. Promising antimicrobial activity against both bacteria and fungus was exhibited by the CdSNPs. The zones of inhibition (ZOIs) against *Serratia nematodiphila, Escherichia coli, Klebsiella planticola*, *Vibrio* sp., and *Planomicrobium* sp. were 14.16 ± 0.088 mm, 16.16 ± 0.334 mm, 23.27–0.120 mm, 14.30 ± 0.153 mm, and 18.27 ± 0.146 mm, respectively, when treated with 200 μL of the CdSNPs. Similarly, the CdSNPs also showed potent fungicidal activity against *Aspergillus niger* and *Aspergillus flavus*.

Nandhini et al. (2021) derived gold nanoparticles (AuNPs) from marine *Enterococcus* sp. RMAA and analyzed the cytotoxicity of NPs on hepatocellular carcinoma cells (HCCs). The organism was inoculated in nutrient broth and incubated for 24 h in a shaking condition. About 1 mM of gold chloride was added to the broth after 18 h. The UV-Vis spectrum identified the absorbance peak in between 540 and 560 nm for the AuNPs. The TEM analysis revealed the spherical shape of particles. The average size of NPs was 7 nm. The IC_{50} (concentration at which 50% cells are killed) of AuNPs in HepG2 cells was found to be around 10 μg/mL. The

Table 19.1 Nanoparticles synthesized by marine microbes

Source	Nanoparticle	Size (nm)	Shape	Activity/ application	Reference
Bacteria					
Enterococcus sp. RMAA	CdSNPs	50–180	Spherical	Antibacterial and antifungal	Rajeshkumar et al. (2014)
Enterococcus sp. RMAA	AuNPs	7	Spherical	Anticancer	Nandhini et al. (2021)
Marinobacter pelagius RS 11	AuNPs	<20	Spherical, triangular	–	Sharma et al. (2012)
Paracoccus haeundaensis BC74171	AuNPs	20.93 ± 3.46	Spherical	Antioxidant and anticancer	Patil et al. (2019)
Stenotrophomonas	AgNPs,	40–60	Circular, triangular, pentagonal, hexagonal, irregular	–	Malhotra et al. (2013)
Stenotrophomonas	AuNPs	10–50	Spherical, irregular	–	Malhotra et al. (2013)
Streptomyces rochei MHM13	AgNPs	22 to 85	Spherical	Antibacterial and anticancer	Abd-Elnaby et al. (2016)
Vibrio alginolyticus	AuNPs	50–100	Irregular	Anticancer and antioxidant	Shunmugam et al. (2021)
Cyanobacteria					
Phormidium formosum	AgNPs	1–26	Spherical	Antibacterial	Elkomy (2020)
Phormidium tenue NTDM05	CdSNPs	5	Spherical	–	Mubarak Ali et al. (2012)
Fungi and yeast					
Aspergillus sydowii	AuNPs	8.7–15.6	Spherical	–	Vala (2015)
Cladosporium cladosporioides	AgNPs	30–60	Spherical	Antioxidant and antimicrobial	Hulikere and Joshi (2019)
Cladosporium halotolerans	AgNPs	20	Spherical	Antioxidant, antifungal, and anticancer	Ameen et al. (2021)
Candida sp. VITDKGB	AgNPs	87	Spherical and rod shaped	Antibacterial	Dinesh et al. (2011)
Rhodosporidium diobovatum	PbSNPs	2–6	Spherical	–	Seshadri et al. (2011)

(continued)

Table 19.1 (continued)

Source	Nanoparticle	Size (nm)	Shape	Activity/ application	Reference
Algae					
Amphiroa rigida	AgNPs	20–30	Spherical	Antibacterial, anticancer, larvicidal	Gopu et al. (2021)
Caulerpa racemosa	AgNPs	10	Spherical	Antibacterial	Kathiraven et al. (2015)
Caulerpa serrulata	AgNPs	10 ± 2	Spherical	Antibacterial	Aboelfetoh et al. (2017)
Cystoseria baccata	AuNPs	8.4 ± 2.2	Spherical	Anticancer	González-Ballesteros et al. (2017)
Padina sp.	AgNPs	40–45	Spherical, oval, and irregular	Antibacterial	Bhuyar et al. (2020)
Portieria hornemannii	AgNPs	60–70	Spherical	Antibacterial	Fatima et al. (2020)
Sargassum muticum	AuNPs	5.42 ± 1.8	Spherical	–	Namvar et al. (2015)
Sargassum tenerrimum and *Turbinaria conoides*	AuNPs	27.5 and 35	–	Catalytic activity	Ramakrishna et al. (2016)
Spyridia filamentosa	AgNPs	20–30	Spherical	Antibacterial and anticancer	Valarmathi et al. (2020)

AuNP-treated cells showed internal accumulation of reactive oxygen species (ROS) that inhibited the proliferation of HepG2 cells through intracellular ROS-mediated apoptosis and by reducing the concentration of proliferating cell nuclear antigen (PCNA).

Sharma et al. (2012) isolated a novel strain (RS 11) of *Marinobacter pelagius* from solar-salterns that were used for synthesis of gold nanoparticles (AuNPs). The bacteria were grown in Zobell marine broth at 37 °C till they reach the stationary phase. The bacterial biomass was recovered by centrifuging at 12,500 rpm for 15 min at 4 °C followed by washing. The washed cells were resuspended in 10 mL $HAuCl_4$ solution (250 mg/L), and the pH was adjusted to 5–6. Appearance of pink color and an intense peak at 540 nm in UV-visible spectra specific to the surface plasmon resonance confirmed the synthesis of the AuNPs. Mostly spherical and triangular AuNPs were observed, which were less than 20 nm in size as seen in Fig. 19.1. Primary and secondary amines as indicated by the prominent bands at 3027 cm^{-1} and 2977 cm^{-1} were revealed by the FTIR. Further, the bands at 1650 cm^{-1} and 1541 cm^{-1} were also observed that were attributed to the carbonyl stretch and the N-H bonds vibration, respectively, that are associated with the amide I and amide II linkages.

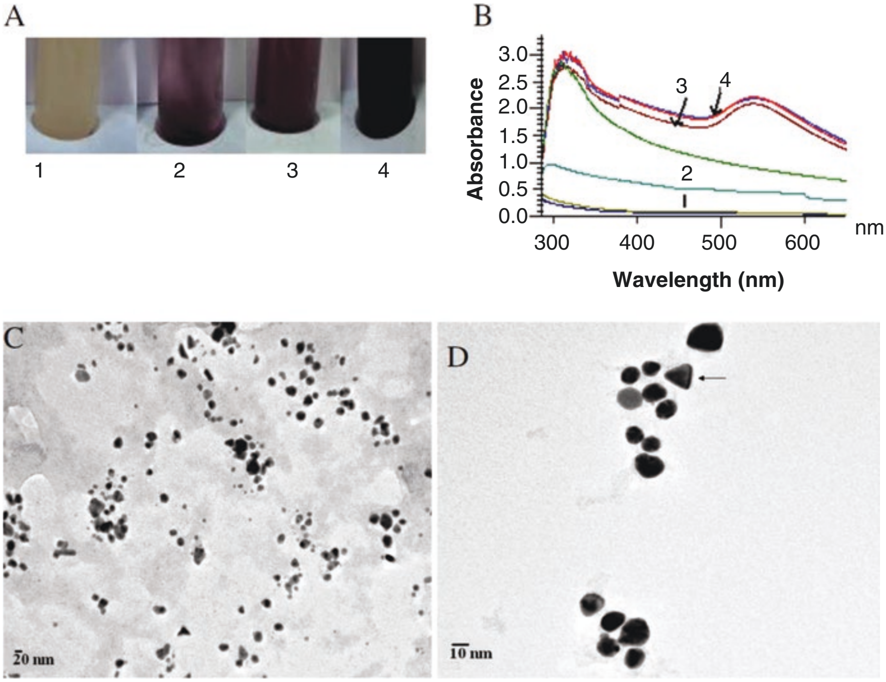

Fig. 19.1 (**a**) Pictures of test tubes containing the bacteria *M. pelagius* cells before (test tube 1) and during incubation in an aqueous of $HAuCl_4$ solution at pH 7.8; (**b**) UV–Vis absorption spectra of gold nanoparticles after the incubation of *M. pelagius* in 1×10^{-3} M aqueous $HAuCl_4$ solution at 7.8 pH. The numbers (Zhang et al. 2005; Zhang and Kim 2010; Shah and Ghosh 2020; Ghosh et al. 2022) indicate the absorption spectra taken at different time intervals 24,48,72, and 96 h, respectively; (**c**, **d**) TEM images of gold nanoparticles produced by the reaction of 1×10^{-3} M aqueous $HAuCl_4$ solution with bacteria *M. pelagius* biomass at 7.8 pH. Particles are mostly spherical with nanotriangles also present (arrow). Bar = 20 nm (**a**) and 10 nm (**b**). (Reprinted from Sharma N, Pinnaka AK, Raje M, Fnu A, Bhattacharyya MS, Choudhury AR (2012) Exploitation of marine bacteria for production of gold nanoparticles. Microb Cell Factories 11:86. https://doi.org/10.1186/1475-2859-11-86 (Open access))

Patil et al. (2019) reported the synthesis of AuNPs by using marine *Paracoccus haeundaensis* BC74171 strain of marine bacteria and also investigated their antioxidant and anticancer properties. A reaction mixture containing 10 mL chloroauric acid (2 mM) and 10 mL of the cell-free supernatant obtained after centrifuging bacteria at 3500 rpm for 30 min at 4 °C was shaken at 70 °C for 15 min in the water bath for synthesis of AuNPs. The pure AuNPs powder was obtained after separation by centrifuging at 13,000 rpm for 25 min followed by washing and lyophilization. The formation of AuNPs was indicated by the appearance of ruby-red color. The UV-Visible spectroscopy indicated the highest absorbance with a sharp peak at 537 nm. The spherical shape, polydispersed nature, and average size of 20.93 ± 3.46 nm were confirmed by TEM analysis. The zeta potential of the NPs was - 4.26 mV. The presence of elemental gold was confirmed by the signal peak at

2.15 keV using EDX (energy dispersion X-ray) analysis. The FTIR (Fourier transformed infrared spectroscopy) analysis of the NPs showed different vibrational bands at 3394, 1639, 1547, 1338, 1408, and 1157 cm^{-1} corresponding to N-H stretching, N-H bending, nitro compounds stretching, C-C aromatic stretching, and C-O stretching, respectively. The proteins and enzymes present in the surrounding reaction mixture were reported to act as reducing and capping agents during AuNPs synthesis. The antioxidant activity of AuNPs tested using 2,2-diphenylpicrylhydrazyl (DPPH) assay revealed the highest radical-scavenging activity of 73.04 ± 3.01% at 320 μg/mL concentration of AuNPs. The anticancer activity of the particles was analyzed against human cell lines such as HEK293 and HaCaT, which were normal cells and AGS and A549 that were cancer cells. The cell viability of AGS cells was 76.46 ± 2.53%, while no effect was observed on the proliferation of normal cells when treated with 200 μg/mL of AuNPs. The mechanism of inhibiting cancer cell proliferation by AuNPs was mainly the alteration of cell growth proteins, DNA fragmentation, and apoptosis.

Malhotra et al. (2013) reported synthesis of AuNPs and AgNPs from *Stenotrophomonas* isolated coral sample, *Pocillopora damicornis* collected from the Mandapam coast, Bay of Bengal, India. Synthesis of AgNPs by the cell-free supernatant was indicated by appearance of intense brick red color, while AuNPs synthesis was confirmed by development of violet color. After 4 h of synthesis, UV-visible observation showed a strong peak at 440 nm that was specific for AgNPs, while the peak for AuNPs was centered at 580 nm. The bacteriogenic AgNPs were circular, triangular, pentagonal, hexagonal, and even irregular in shape, which were in a range 40 to 60 nm. However, the AuNPs were more spherical and irregular shaped with an average particle size ranging between 10 and 50 nm.

Abd-Elnaby et al. (2016) evaluated the antibacterial and anticancer activities of AgNPs synthesized using marine *Streptomyces rochei* MHM13. About 50 mL of 1 mM $AgNO_3$ was mixed with bacterial extract (50 mL) at pH 8.5. The reaction mixture was incubated in darkness at 37 °C under shaking condition (200 rpm) for 5 days. The change in color to yellow-brown gave the visible confirmation of the successful synthesis of the AgNPs. The UV-Vis spectroscopy identified the maximum surface plasmon resonance (SPR) peak at 410 nm for the synthesized AgNPs. The intense absorbance peak of N-H stretching of the amine group was obtained at 3420.14 cm^{-1}, which was revealed from the FTIR spectrum. The amide groups of protein served as a stabilizing or capping agent for the NPs. The spherical shape and size range of 22 to 85 nm of the particles was showed by SEM analysis. The antibacterial activity of AgNPs varied in terms of zone of inhibition against different pathogenic bacteria such as *B. subtilis* (18 mm), *S. aureus* (18 mm), *P. aeruginosa* (18 mm), *Bacillus cereus* (16 mm), *Salmonella typhimurium* (18 mm), *E. coli* (16 mm), *Vibrio fluvialis* (19 mm), and *Vibrio damsela* (16 mm). The antifouling activity (inhibition of bacterial cell density) of AgNPs revealed 99.9% inhibition of *E. coli*. The significant anticancer activity of AgNPs was observed against different cancer cell lines like Hep-G2, HCT-116, A-549, and MCF-7. The highest reduction in viable cells of Hep-G2 (21.63%), MCF-7 (36.42%), HCT-116 (17.97%), PC-3

(48.13%), A-549 (38.59%), CACO (54.31%), HEP-2 (63.82%), and HELA (56.79%) was observed at 50 μg of AgNPs.

Shunmugam et al. (2021) investigated the use of the marine bacteria *Vibrio alginolyticus* for the synthesis of AuNPs and further studied its anticancer and antioxidant activities. The bacterial strain was inoculated, incubated, and centrifuged at 8000 rpm for 15 min to collect the supernatant. The precursor chloroauric acid (HAuCl) was added to the cell free supernatant and the mixture was incubated under shaking conditions for 24 h at 40 °C with 120 rpm. After that the sample was lyophilized and the powder was stored. The initial indication of successful synthesis of AuNPs was the change in color of the final solution from light to dark yellow to reddish brown. The confirmation of AuNPs synthesis by the organism was indicated by the absorbance band obtained at 530 nm when analyzed by UV-Vis spectroscopy. The morphological analysis of AuNPs carried out by SEM revealed the irregular shape of the particles. An average size of 50–100 nm of the AuNPs was identified using TEM. The FTIR results of the particles identified the peaks specific to different functional groups at 1644, 1399, 3542, 2926, and 557 cm^{-1} depicting vibrational stretches of alkene, CH_3, C-H, C-F, and C-Br, respectively. The antioxidant activity of AuNPs analyzed using the DPPH assay showed the maximum radical-scavenging activity at 10 μg/mL concentration of NPs. The AuNPs used for metal-ion-chelating assay showed enhancement by 22.06% in ferrous-ion-chelating activity compared to the untreated samples. The IC_{50} value of 15 μg/mL was obtained for AuNPs while testing the anticancer activity of the particles against colon cancer cell lines (HCA-7). The AuNPs also showed prominent fluorescence in the nucleus.

19.3 Cyanobacteria

Various cyanobacteria are rich in proteins, amino acids, carbohydrates, and sugars, which can be exploited for biosynthesis of diverse nanoparticles (Nitnavare et al. 2022). However, only few reports exist on the nanobiotechnological potential of marine cyanobacteria. In one such report, Elkomy (2020) synthesized AgNPs from a marine cyanobacterium *Phormidium formosum* isolated from the Mediterranean coast of Egypt. Initially, 5 g *P. formosum* biomass was collected from an exponential phase culture growing in F/2 medium, which was then thoroughly washed and reacted with 100 mL of 1 mM aqueous $AgNO_3$ solution for 24 h at pH 7. After completion of the AgNPs synthesis at 28 °C, the reaction mixture was centrifuged at 1200 rpm for 30 min and the pellet was collected followed by drying at 45 °C. Appearance of brown color and absorbance at 437 in UV-visible spectra confirmed the synthesis of AgNPs that were spherical in shape and 1–26 nm in size. FTIR analysis revealed a strong broad band at 3223.11 cm^{-1} that is specific to O-H stretch carboxylic group. The biogenic AgNPs exhibited antimicrobial activity against both *Vibrio* spp. and *Staphylococcus aureus*, with a ZOI equivalent to 27 mm and 22 mm, respectively.

In another interesting study, CdSNPs were synthesized using C-phycoerythrin (C-PE) which is a pigment extracted from the marine cyanobacterium, *Phormidium* *tenue* NTDM05 (Mubarak Ali et al. 2012). C-phycoerythrin extract was reacted with 0.25 mM $CdCl_2$ and 1 mM Na_2S aqueous solutions for 5 days. Appearance of orange color and UV-visible absorption peak centred at 470 nm confirmed the synthesis of CdSNPs which were spherical in shape, 5 nm in size and stable up to 8 months. FTIR spectra strongly rationalized the involvement of the thiol group in capping the biogenic CdSNPs.

19.4 Fungi and Yeast

Mycogenic synthesis of nanoparticles is considered most promising due to the rapid, efficient, and stable nature. Vala (2015) reported AuNPs from marine fungus, *Aspergillus sydowii*, isolated from Gulf of Khambhat, West Coast of India. Fungal biomass was generated by inoculating 1 mL of spore suspension (~ 10^6/mL) in potato dextrose medium prepared in 75% "aged" seawater. After 4 days of incubation, the biomass was recovered by filtration, which was then washed in distilled water. Various concentrations of gold chloride (0.25 to 3 mM) were reacted with 5 g of fungal biomass under static condition for 72 h at 27 °C. Development of purple-lavender color within 18 h or reaction with 0.25 and 0.5 mM gold chloride indicated formation of larger AuNPs. On the other hand, pink color was obtained with 2 and 3 mM gold salt concentration, suggesting formation of AuNPs of small size. It was speculated that the fungal enzymes might be responsible for the reduction of Au^{3+} to Au^0, while the proteins might have capped the mycogenic AuNPs, making them more stable. Monodispersed spherical AuNPs were in a size range of 8.7–15.6 nm with a mean diameter of 10 nm.

Hulikere and Joshi (2019) employed marine endophytic fungus, *Cladosporium cladosporioides*, for the AgNPs synthesis and also tested its antioxidant and antimicrobial activities. The mycelial biomass was cultivated, filtered, and washed with distilled water. The biomass was further incubated for 48 h at room temperature and the final synthesis of AgNPs was carried out after mixing 10 mL of filtrate and 90 mL of 1 mM $AgNO_3$. Appearance of dark brown confirmed the formation of AgNPs. The strong peak for the AgNPs at 440 nm was noted by UV-Vis spectroscopy. The field emission SEM (FESEM) revealed the uniformly spherical shape of the particle. The size of the particles were in a range from 30 to 60 nm. The crystalline nature of the particle was identified by XRD analysis. The FTIR analysis identified O-H stretching vibrations of alcohol and phenolic groups (3338 cm^{-1}), O-H stretching of carboxylic acids (2331 cm^{-1}), C-O amide stretching (1640 cm^{-1}), and C-N amine stretching (1323 cm^{-1}). Capping and reduction were attributed to the fungal proteins, enzymes, and polyphenols present in the extract. The biosynthesis of AgNPs was achieved by the combination of electron shuttles and NADPH-dependent reductases. The mycogenic AgNPs showed promising antimicrobial activity with a ZOI of 7.5 ± 2.0 mm, 9.2 ± 0.4 mm, 10.5 ± 2.5 mm, 8.4 ± 0.1 mm, 7.2 ± 3.5 mm against *S. aureus, S. epidermis, B. substilis, E. coli,* and *C. albicans*, respectively. ROS associated cell membrane disruption and damage to DNA and proteins were speculated as prominent mechanisms for the antimicrobial activity.

Ameen et al. (2021) reported the synthesis of AgNPs from the marine fungus *Cladosporium halotolerans* and investigated its antioxidant, antifungal and anticancer properties. About 90 mL of biomass filtrate and 10 mL of $AgNO_3$ (1 mM) were mixed and incubated in a shaker for 30 min. The color change from pure white to black represented the visual confirmation of AgNPs formation. The peak obtained at 500 nm using UV-Vis spectroscopy confirmed the formation of AgNPs. The XRD analysis identified the crystalline structure of the particle. From the classical Scherrer formula the size of particle was evaluated as 20 nm. The FTIR analysis revealed the peaks defining different functional groups like -OH group (1078 and 3315 cm^{-1}) and alkenes (1644 cm^{-1}) that might have taken part in the synthesis and stabilization of the AgNPs. SEM showed uniformly spherical AgNPs with 64.2% silver as analyzed using EDX. The zeta potential of the particles was −51.8 mV. TEM analysis showed that the spherical AgNPs with smooth surface were 20 nm in size as evident from Fig. 19.2. DPPH radical-scavenging activity of the mycogenic AgNPs was dose dependent where 78% antioxidant activity was noted within 30 min of incubation. The IC_{50} value for anticancer activity of AgNPs was 34.27 μL/mL against MCF-7 cells. The antifungal activity of AgNPs against *A. niger* showed dose-dependent response that was equivalent to 45% and 70% inhibition at 500 ppm and 1000 ppm of AgNPs, respectively.

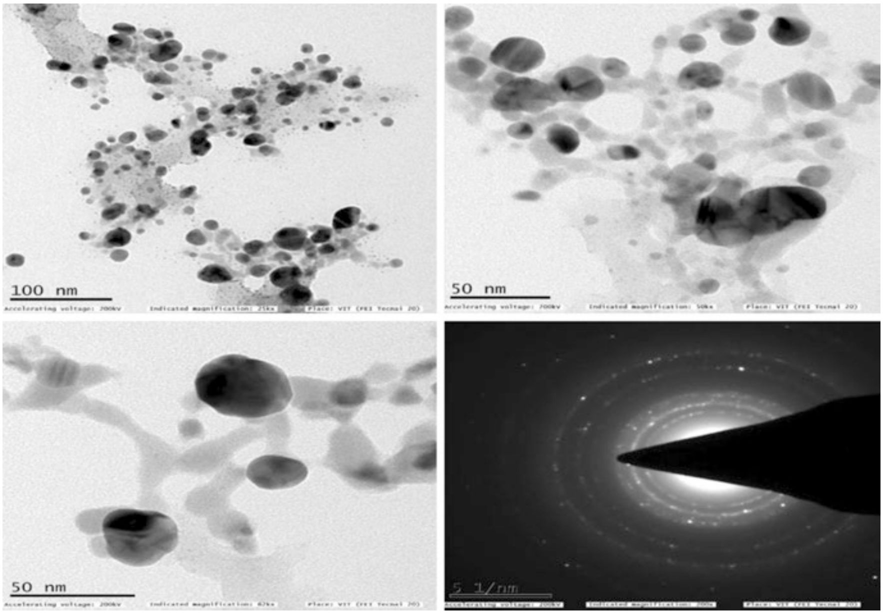

Fig. 19.2 TEM images of AgNPs synthesized using *C. halotolerans*. (Reprinted with permission from Ameen F, Al-Homaidan AA, Al-Sabri A, Almansob A, AlNAdhari S (2021) Anti-oxidant, anti-fungal and cytotoxic effects of silver nanoparticles synthesized using marine fungus *Cladosporium halotolerans*. Appl Nanosci 1–9. https://doi.org/10.1007/s13204-021-01874-9. Copyright © 2021, King Abdulaziz City for Science and Technology)

Novel marine yeast, *Candida* sp. VITDKGB, isolated from the coastal areas of Nicobar Islands, India, was used for synthesis of AgNPs with promising antimicrobial activity against multidrug-resistant (MDR) pathogens (Dinesh et al. 2011). The silver-tolerant strain of the yeast was inoculated in 100 mL sterile Sabauroud's dextrose broth prepared in 50% marine water and incubated 48 h at 35 °C under shaking condition (120 rpm). The biomass was then recovered by centrifuging the broth 10,000 rpm for 10 min at 4 °C. The supernatant was collected and reacted with 1 mM $AgNO_3$ for 48 h at 35 °C in darkness under shaking condition (120 rpm). UV-visible spectra showed an intense peak at 430 nm attributed to the SPR of AgNPs, which was mostly spherical and rod shaped. The particles were 87 nm in size as evaluated from atomic force microscope (AFM) measurements. FTIR analysis revealed the bands at 3442.97 cm^{-1}, 1383.16 cm^{-1}, 2927.34 cm^{-1}, 1631.31 cm^{-1}, 1224.76 cm^{-1}, 1062.49 cm^{-1}, and 643.72 cm^{-1} which are associated with stretching vibration of primary amines, C-N stretching of amines, aliphatic—CH_3 and CH_2 stretching, -NHCO of amide, ester carbonyl group/phenol, C-O stretching of polysaccharides/Si-O asymmetric stretch and CH out of plane bending of carbohydrate, respectively. The AgNPs exhibited antibacterial activity with ZOI equivalent to 7.33 ± 0.57 and 5.66 ± 0.57 mm against MDR *S. aureus* and *K. pneumoniae*, respectively.

Seshadri et al. (2011) reported synthesis of lead sulfide nanoparticles (PbSNPs) using *Rhodosporidium diobovatum*, a marine yeast isolated from Indian Ocean. The cells in the log phase accumulated 55% lead intracellularly when grown in lead-nitrate-supplemented culture medium. Also 26% and 90.52% lead accumulation was noted in the beginning and the end of the stationary phase (96 h), respectively. The resulting PbSNPs were spherical and well dispersed showing a large blue shift in the absorption edge to ~320 nm. The size of the nanoparticles was in a range from 2 to 6 nm. Phytochelatins were reported to cap the PbSNPs, resulting in their stability. Such thiol compounds not only detoxify the nanoparticle but also prevent agglomeration of the same.

19.5 Algae

Algae are aquatic photosynthetic microbes that are widely used for synthesis of various nanoparticles. Gopu et al. (2021) utilized marine red algae *Amphiroa rigida* (AR) collected from coastal area of Kanyakumari District, Tamil Nadu, India, for the synthesis of AgNPs. Initially, the algal biomass was shade-dried and pulverized into fine powder in order to increase the surface area. Then 10 g of the algal powder was suspended in 500 mL of double-distilled water and extracted for 20 min at 80 °C for 20 min under stirring condition. The extract was filtered and centrifuged at 10,000 rpm for 10 min. A 10 mL of AR supernatant was mixed with 90 mL $AgNO_3$ (1 mM) solution. The mixture was incubated at 37 °C till reddish brown color appeared. After the completion of the reaction, the AgNPs were centrifuged at 9000 rpm for 15 min (4 °C). The particles were washed and repeatedly centrifuged using double-distilled water for complete removal of the impurities. The successful formation of AgNPs was confirmed by the absorption band obtained at 420 nm as indicated in the UV-Vis spectroscopy. The average size of the particle from Debye-Scherer's equation was around 25 nm. The TEM analysis showed spherical particles with an average size of 20–30 nm. The FTIR spectroscopy showed the prominent peaks for OH stretching and CH stretching vibrations of hydroxyl groups (2382.87 cm^{-1}), C=O stretching (1701.22 and 1512.19 cm^{-1}), and C-O stretching in ether groups (1147.65 cm^{-1}). The bioactive compounds such as phenolic, ether, and polysaccharides were involved in the bio-reduction of Ag^+ to Ag^0. The resulting AgNPs exhibited antibacterial effect against *S. aureus* and *P. aeruginosa* with ZOI equivalent to 21 ± 0.2 mm and 15 ± 0.2 mm, respectively as seen in Fig. 19.3. The minimum inhibitory concentration (MIC) values were evaluated as 3.125 and 6.25 μg/mL, respectively. The AgNPs inhibited breast cancer cell lines (MCF-7) with an IC_{50} value of 20 μg/mL. The highest mortality (100%) against third instar larva of *Aedes aegypti* was achieved on treatment with 20 μg of AgNPs, while for the fourth instar larva, the highest mortality was caused by 40 μg of AgNPs.

Kathiraven et al. (2015) demonstrated the synthesis of AgNPs using marine algae *Caulerpa racemosa* collected from the Gulf of Mannar, Southeast coast of India. The synthesis of AgNPs was carried out by reacting 10 mL of algal filtrate with 90 mL of $AgNO_3$ (10^{-3} M) at room temperature. The synthesis of AgNPs was accompanied by initial appearance of light-yellow color that gradually intensified to

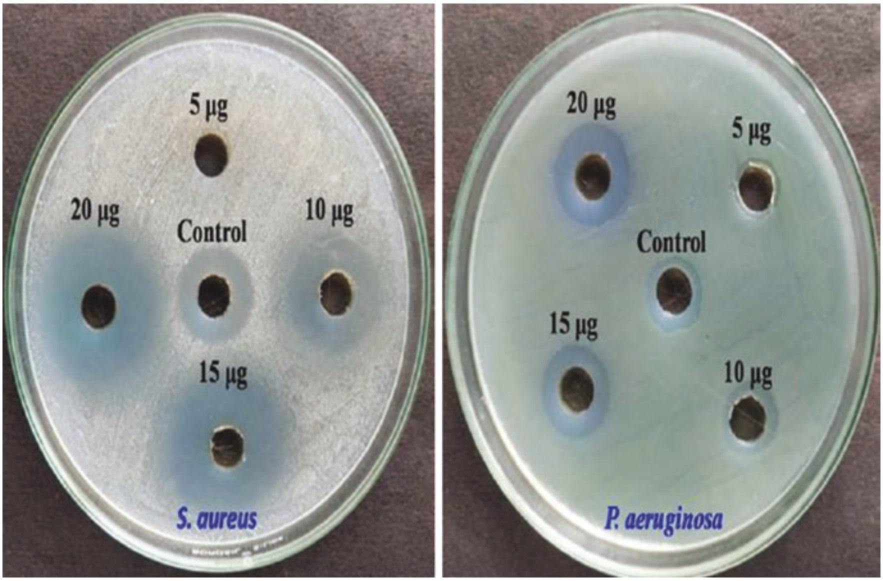

Fig. 19.3 Antibacterial effect of AR-AgNPs against human pathogens. (Reprinted with permission from Gopu M, Kumar P, Selvankumar T, Senthilkumar B, Sudhakar C, Govarthanan M, Selvam K (2021) Green biomimetic silver nanoparticles utilizing the red algae *Amphiroa rigida* and its potent antibacterial, cytotoxicity and larvicidal efficiency. Bioprocess Biosyst Eng 44 (2):217–223. https://doi.org/10.1007/s00449-020-02426-1 Copyright © Springer-Verlag GmbH Germany, part of Springer Nature 2020)

brown color. UV-Vis spectra showed a maximum absorbance peak at 413 nm that is specific to the SPR of AgNPs. The FTIR spectrum for the synthesized AgNPs identified the peaks of OH-stretching vibrations (3440 cm^{-1}) and (NH) = O group stretching (1639 cm^{-1}). The shifting in band from 1631 to 1639 cm^{-1} was reported due to the binding of (NH)C=O group with the particles. The TEM images revealed the spherical shape of the particles, which were 10 nm in size. The antibacterial efficiency of the particles studied through well diffusion assay against *Proteus mirabilis* and *S. aureus* showed the ZOI of 14 mm and 7 mm, respectively.

In another study, Aboelfetoh et al. (2017) fabricated AgNPs by using *Caulerpa serrulata* (green marine algae) that was collected from Red Sea coast, Egypt. The particles were synthesized by adding 5–25 mL of algal extract to 10^{-3} M $AgNO_3$ solution. The change in color of the solution from yellow to reddish brown indicated the formation of AgNPs. The UV-Vis spectral analysis also confirmed the color change of the solution. On increasing the extract concentration from 5% to 25%, the intensity of the SPR band was noted that might be attributed to the aggregation of the particles. Lower pH resulted in the formation of more AgNPs with smaller diameter. FTIR analysis indicated different peaks at 3447, 1730, 2929, 2859, 1613, 1194, 1125, 1023, 2300–2336 cm^{-1} that represents O-H, C=O C-H, C=C, C-O, C ≡ C stretching vibrations. The XRD analysis confirmed the crystallinity of the phycogenic AgNPs with a face-centered cubic structure. The spherical morphology of the AgNPs with an average size of 10 ± 2 nm was observed in the HRTEM images. The antibacterial activity of AgNPs against *E. coli* and *Salmonella typhi* was indicated by ZOI equivalent to 21 mm and 10 mm, respectively. The toxicity of AgNPs toward these organisms was mainly attributed to the disruption of the cell wall and increase in the permeability of the plasma membrane.

González-Ballesteros et al. (2017) demonstrated the synthesis of AuNPs by utilizing marine brown algae *Cystoseria baccata* (CB) collected from the lower intertidal rocky shore in the NW coast of Spain. About 75 μL of 0.01 M $HAuCl_4$ was mixed with 1 mL of CB extract and the mixture was stirred at room temperature for 24 h. The change in pH of the CB extract from 5.4 to 4.5 was noted during formation of the AuNPs. The 0.4 mM concentration of AuNPs was considered optimum. The characteristic peak observed at 532 nm is specific to the SPR of AuNPs. The zeta potential of the particle was −30.7 ± 2.0 mV. The TEM images revealed the spherical AuNPs with 8.4 ± 2.2 nm average size. The HRTEM studies identified the polycrystalline nature of the particles, while the elemental mapping confirmed the presence of carbon in the surrounding of AuNPs. The FTIR spectrum identified the peaks at 3402, 2937, 1662, 1417, 1078, and 1254 cm^{-1} attributing to NH or OH stretching, CH stretching, strong asymmetric stretching of carbonyl, weak symmetric stretching of carbonyl, C-OH vibrations of primary alcohol, and $-SO_3$ stretching, respectively. Treatment with the AuNPs resulted in 48-fold and threefold enhancement in early apoptotic cells in colon cancer cells, Caco-2 and HT-29 cells, respectively. The extrinsic pathway was found to be activated in Caco-2 cells, while both extrinsic and intrinsic apoptotic pathways were activated in the case of HT-29 cells by the AuNPs.

Bhuyar et al. (2020) utilized marine macroalgae *Padina* sp. collected from Mersing, Johor, at Peninsular Malaysia for the synthesis of AgNPs. The aqueous algal extract was prepared by mixing 30 g of algal powder and 300 mL of distilled water in 1:10 ratio. The reaction mixture was heated for 20 min at 60 °C and was further filtered. Reaction between 20 mL of algal extract and 180 mL of $AgNO_3$ (0.01 M) at 60 °C for 48 h under stirring condition resulted in the formation of the AgNPs in dark condition. The color change from yellow-brown to concentrated dark brown indicated the synthesis of the AgNPs. The reaction mixture was harvested after 48 h and centrifuged at 8000 rpm for 30 min at 30 °C followed by recovery of the AgNPs pellet that was further dried at 50 °C for 5 h resulting in a yield of 510 mg. The characteristic absorbance band at 445 nm for the AgNPs in UV-Vis spectra confirmed the successful formation of the AgNPs. The FTIR analysis of the extract revealed peaks of O-H and C=O stretching of hydroxyl and carboxylic acid groups (3350.07, 1637.74, 1106.90 cm^{-1}) and N-H stretching vibrations of peptide linkages and hydroxyl stretch of polyphenols (3350.07 cm^{-1}). Average size of the AgNPs according to the SEM analysis was 33.75 nm. FESEM analysis confirmed that the average size of the AgNPs was 40.45 nm. Polydispersed AgNPs showed spherical, oval, and irregular shapes. EDX spectrum confirmed the presence of 85.02 wt% Ag, 9.04 wt% chloride, and 1.35 wt% carbon in AgNPs. The antibacterial activity of the AgNPs was confirmed from the ZOI of 15.17 ± 0.58 mm, 12.67 ± 0.76 mm, 13.33 ± 0.76 mm, 12.67 ± 0.58 mm against *S. aureus*, *B. subtilis*, *P. aeruginosa*, and *E. coli*, respectively.

In another similar study, Fatima et al. (2020) studied the antibacterial activity of AgNPs synthesized using marine red algae *Portieria hornemannii* collected from Gulf of Mannar, India. The 1 mM $AgNO_3$ (45 mL) was reacted with 5 mL of algal extract and the mixture was stirred continuously at room temperature. Appearance of dark brown color after 48 h indicated the formation of AgNPs that also showed an SPR-associated absorbance peak at 420 nm in the UV-Vis spectra. The FTIR analysis identified the peaks of C-CI stretching, C-N stretching in aliphatic amines, O-C bond (acids), C-O-H bending of carboxylic classes, O-H, C-H, O-H stretching, and N-H groups at 860, 1076 and 1157, 1261, 1380 and 1400, 2862, 2926, 3147, and 3415 cm^{-1}, respectively. SEM analysis showed spherical shape of the AgNPs, which were 70–75 nm in size. The TEM analysis also confirmed the size of the phycogenic AgNPs to be around 60–70 nm. The zeta potential was about −44.5 mV. The antibacterial activity of the AgNPs tested against *Vibrio harveyii*, *Vibrio anguillarum*, *Vibrio parahaemolyticus*, and *Vibrio vulnificus* was confirmed from their MICs, which were 7.81, 3.9, 7.81, and 15.62 μg/mL, respectively.

Namvar et al. (2015) explored the synthesis of AuNPs by using the marine microalgae *Sargassum muticum* collected from the coastal areas of Persian Gulf. The algal powder was mixed with water and heated at 100 °C followed by filtration. The aqueous extract (50 mL) was reacted with identical volume of 0.1 mM $HAuCl_4$ and stirred at 45 °C till light purple color appeared. The AuNPs pellet was collected after centrifuging the solution at 6000 rpm for 10 min. The absorbance peak of the AuNPs in UV-Vis spectra was at 520 nm. The particles were stable in a pH range of

5–9. TEM images showed that the spherical AuNPs were 5.42 ± 1.8 nm in size. The zeta potential was −35.8 mV.

Ramakrishna et al. (2016) evaluated the catalytic activity of the AuNPs synthesized from two marine brown algae *Sargassum tenerrimum* and *Turbinaria conoides*. The 5 mL aqueous extracts of the algae were mixed with 45 mL $HAuCl_4$ (1 mM). The reaction mixture was stirred and the appearance of ruby red color of the solution indicated the formation of AuNPs. The AuNPs were washed by repeated centrifugation for 15 min at 10,000 rpm and redispersion of the pellet. The intense absorbance peak at 540 nm and broader peaks between 700 and 800 nm were observed for AuNPs. FTIR analysis of the synthesized particle revealed the involvement of amine and hydroxyl functional groups in the synthesis and stabilization of AuNPs. The TEM analysis revealed the anisotropic and polydispersed AuNPs with average diameter of 27.5 nm and 35 nm when synthesized by *T. conoides* and *S. tenerrimum* extracts, respectively. The dynamic light scattering (DLS) analysis confirmed the hydrodynamic radius as 28.60 ± 20.65 nm and 82.30 ± 52.82 nm with a polydispersity index (PDI) of 0.521 and 0.412, when synthesized from *T. conoides* and *S. tenerrimum*, respectively. The zeta potential values of AuNPs derived from *T. conoides* and *S. tenerrium* were −26.3 mV and −29.1 mV, respectively. The catalytic activity of AuNPs was tested on 4-nitrophenol, p-nitroaniline, rhodamine B, and sulforhodamine 101. Catalytic conversion of nitroarene (4-nitrophenol) to its corresponding aminoarenes (4-aminophenol) by AuNPs from *T. conoides* was indicated by the decrease in the absorption peak at 400 nm with concomitant appearance of the peak at 298 nm. The reaction was completed in 300 s with a rate constant (k) of $9.37 \times 10^{-3}\ s^{-1}$ and $10.64 \times 10^{-3}\ s^{-1}$ for AuNPs from *T. conoides* and *S. tenerrimum*, respectively. Catalytic conversion of p-nitroaniline (p-NA) to *p*-phenylenediamine in presence of AuNPs was indicated by the reduction of peak at 380 nm with simultaneous increase in the peak at 238 nm. The catalytic conversion completed within 123 s and 140 s with rate constants (k) of $18.73 \times 10^{-3}\ s^{-1}$ and $16.07 \times 10^{-3}\ s^{-1}$ for AuNPs from *T. conoides* and *S. tenerrimum*, respectively. A dose-dependent catalytic reduction of rhodamine B and sulforhodamine 101 was observed.

Valarmathi et al. (2020) developed a route for biogenic synthesis of AgNPs from the marine red algae *Spyridia filamentosa* collected from coastal area of Kanyakumari District, Tamil Nadu, India. The reaction mixture containing 20 mL of *S. filamentosa* extract and 80 mL of 1 mM $AgNO_3$ was incubated at 37 °C for 2 h. On synthesis of the AgNPs, brown color developed and a peak at 420 nm appeared in the UV-vis spectra. The phycogenic AgNPs were spherical in shape and were 20–30 nm in size. The FTIR spectrum showed bands at 3402, 2922 and 2852, 1652 and 1456, and 1099 and 617 attributing hydroxyl and carbonyl groups, methyl group, carboxyl group stretching, and carboxylic acids, ether, and alcoholic groups, respectively. AgNPs inhibited the growth of *Klebsiella* sp. and *Staphylococcus* sp. up to 63.4% and 44.6%, respectively. The biogenic AgNPs were cytotoxic against MCF-7 cells and induced apoptosis.

19.6 Conclusion and Future Perspectives

More recently, biogenic route for synthesis of metal and metal oxide nanoparticles has gained wide attention due to their ease of synthesis, biocompatibility, and stability (Ghosh et al. 2016a, b). Cellular metabolites like reducing sugars, ascorbic acid, citric acid, starch, proteins, enzymes, and others play a significant role in reducing the metal ions to their corresponding nanoparticles and their further stabilization (Bloch et al. 2021; Ghosh 2018). Recent studies have identified the marine microflora as untapped "nanofactories" for the production of metallic nanoparticles due to their rich diversity in metabolites. A major drawback in biogenic nanoparticles is the control of the size and shape. Careful optimization of reaction parameters like time, temperature, pH, metal salt concentration, and cell extract concentration can help to get monodispersed nanoparticles with desired physical and chemical properties (Shende et al. 2017, 2018). At present, very limited reports are available on the therapeutic applications of the nanoparticles synthesized by the marine microorganisms. Hence, antimicrobial, antibiofilm, anticancer, antidiabetic, and antioxidant activities should be evaluated thoroughly to develop more biocompatible nanomedicine (Shinde et al. 2018; Bhagwat et al. 2018). Further, these biogenic nanoparticles can be used for functionalization of drugs for targeted delivery and sustained release that would synergistically enhance the therapeutic index of the drugs (Ghosh 2019; Ranpariya et al. 2021). Bimetallic and alloy nanocomposites would be more potent candidate nanocatalysts due to their enhanced dye-degrading properties that can be employed to treat industrial effluents (Bloch et al. 2022; Tawre et al. 2022; Gami et al. 2022).

In conclusion, marine microbes can be used as promising candidates for synthesis of novel metal nanoparticles with multivariate applications in therapeutics, agriculture, environment, and food industries. Integrated approach using metabolomics, proteomics, and genomics, would help to identify the exact mechanism of synthesis of the nanoparticles by the microbial cell factories.

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20 Bioactive Compounds from Marine Water Ecosystem

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Abstract

Marine ecosystem contains a wide variety of biocompound sources ready to be exploited. Octopuses are a group of at least 200 species that represents one of the main fishing industries around the world. *Octopus maya* is the main product captured in the Gulf of Mexico and the Caribbean Sea and is the principal source of income of the fishing population in the Yucatan Peninsula. Besides its color, size, and characteristic flavor, *O. maya* exploitation is limited to commercialization of fresh or frozen arms, discarding the head and tips. These wastes are a source of protein, vitamins, lipids, amino acids, polysaccharides, and minerals. Collagen is the main protein related to the connective tissue in cephalopods, up to 32% in *O. maya*. Gelatin and collagen peptides are obtained from hydrolysis, enzymatic digestion, or microbial fermentation of collagen. All these biocompounds are considered a potential biomaterial used in Food and Beverage, Healthcare, Cosmetics, and other Industries. In this chapter, we will revise generalities of Octopus as a source of marine biocompounds and their exploitation, characteristics, sources, and extraction methods for collagen, gelatin, and collagen peptides, trends, and uses of these compounds in food industry.

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20.1 By-Products, World Market, and Economic Impact

Currently, 845 species of cephalopods have been found (Hoving et al. 2014), including nautiloids, sepioids, squids, and octopods. About 300 species belong to the eight-armed cephalopods that comprise the orders Cirroctopoda and Octopoda (Boletzky 1999; Nixon and Young 2003). Octopuses of the Order Octopoda are a group of around 200 described species that comprise the Family Octopodidae (Jereb et al. 2013). This family includes most of the known species of octopus. Most of the octopus's species that sustain the fishery are shallow-water species, distributed mainly in offshore reefs and shelf areas, with an estimated lifespan of 1–2 years (Leporati et al. 2009; Leporati and Hart 2015; Herwig et al. 2012). The last global landings of cephalopods (2005–2014) reached 4.8 million tons in 2014 (FAO 2016) of which squid represented approximately 80%, while 10% was due to cuttlefish and another 10% to the octopuses (Sauer et al. 2019).

The octopus's market is one of the main fishing industries globally, with a value of 1.15 billion dollars in exports and 1.24 billion dollars in imports in 2021. In the same year, Mexico ranked seventh place worldwide by export, trading 2.5 million metric tons (MTs) with a value of 20.2 million dollars (Tridge Market Intelligence 2022). In addition, approximately 57% of the octopus's production in Mexico is exported (SAGARPA-CONAPESCA 2009): Portugal, Spain, and Italy are the main exporting countries (Tridge Market Intelligence 2022). In the Gulf of Mexico and the Caribbean Sea, the octopus fishery occupies the first place in generated volume and second place in value, with the species *Octopus maya* (74%) being caught mainly, as well as *Octopus vulgaris* (26%) (CONAPESCA 2018). On the other hand, the governments of Yucatan, Campeche, and Quintana Roo (all three represent the Yucatan Peninsula) intend to obtain the designation of origin of the Mayan octopus, to be internationally classified in the market and distinguished by its color, size, flavor, and characteristics providing added value. Yucatán is the state of Mexico with the highest production of octopus, capturing 74% of the total catch, followed by Campeche (29%). Due to the increase in demand for octopus, the aquaculture of this species is growing in different regions as a consequence of representative characteristics, such as its rapid growth, short useful life, and high capacity to transform food into body mass, which ranges from 30% to 50% (Almeida et al. 2022). In the fishing season, approximately 90% of the region's fishing population is dedicated to this activity, with the participation of around 18,000 fishermen (SAGARPA 2014). However, much of the modern octopus catch is only sold frozen, usually whole or with the arms separated from the head and body (Gullian-Klanian et al. 2017).

The wide chemical and biological diversity of octopuses has led to an intense trend for processing to meet global demand as a rich source of high-added value biomolecules that can be used in many applications. This resource's minerals, lipids, amino acids, polysaccharides, and proteins have unique characteristics. The octopus contains about 80% protein, and is also a rich source of vitamins B3 and B12, with high contents of potassium, iodine, selenium, calcium, sodium, and phosphorus that primarily help maintain good skin, muscles, hair, and nails (CONAPESCA 2018). These are biocompounds with high added value that could be used for applications in diet and human health.

The worldwide seafood market is expected to maintain an annual growth rate of 4.9% through 2030 (Goldstein Research 2020). The growth in the exploitation of cephalopods has resulted in an expansion of the processing industries and the generation of large amounts of solid and liquid waste/by-products, which can cause significant environmental problems.

For example, solid waste from the cuttlefish processing industry can represent 35% of the original material after processing. In comparison, waste from squid fishing can reach 75% of the total weight in the form of skin, head, bone, ink, and viscera (Shahidi 2006; Le Bihan 2006; Balti et al. 2010). On the other hand, in eviscerated Mayan octopus (*O. maya*), the head and the tips of the arms (by-products) represent approximately 20.5% of the total weight. By-products, such as the head, the viscera, and the skin, have compounds that can add value (Ferraro et al. 2010). These by-products are an essential source of proteins, lipids, and other bioactive molecules of high commercial value (Koueta et al. 2014), and are used in the food, cosmetics, and pharmaceutical industries.

On the other hand, according to various surveys and interviews carried out in octopus' studies (Rivera et al. 2019) in the Mayan octopus' market, the necessary consumer requirements to purchase octopus' products have been identified under 4 perspectives: consumer, characteristics (intrinsic and/or extrinsic attributes) of quality, the importance of the features, and relationship between the characteristics. The requirements considered to cover consumer needs were: (1) freshness, (2) price, (3) variety (species), (4) practicality of the product, (5) availability to the consumer. Based on these requirements, the identified attributes that consumers demand were freshness, meat texture, color, size, smell, and flavor to determine their purchase and satisfaction.

With the above, it is sought that the elaboration of octopus-based products meets the characteristics that the consumer requires to acquire them regardless of whether they are made with products (arms) or by-products (mantle, points, viscera).

Currently, physical (color, humidity, pH, water activity, titratable acidity), texture (texture profiles), chemical (antioxidant activity, minerals), chromatographic (polyphenol profile), technological (water retention capacity, gelling temperature, collagen ratio), and functional (bioactive peptides) properties, as well as rheological behavior (viscosity, mechanical spectra), morphometry (size and shape), and sensory attributes (aroma, flavor), of various processed products with high added value as inlay products (ham, sausages, chorizo, surimi); traditional products (preserved octopus in oil); regional products from the Mexican southeast (pickled octopus, black stuffing, pibil); and nontraditional (chicharrón, extruded) have been measured.

20.1.1 Sterilized Octopus' Arms

In sterilization, temperatures ranging from 110 °C to 120 °C are applied with times ranging between 20 and 40 min; thus, the products subjected to this process can be then kept at room temperature for several weeks and even months. However, the heat of these treatments causes changes in nutritional, functional, and sensory properties (taste, smell, color, texture, appearance, among others) (Aït-Kaddour et al. 2021). Industrial processing is designed to reduce microbial deterioration (long shelf life), to be transportable over long distances and with high organoleptic quality (Monteiro et al. 2010). In the sterilization process, food is packaged in pouches (flexible sterilizable bags) for subsequent sterilization with a standardized time and temperature, depending on the product. The use of pouches plus sterilization makes it easy to heat, easy to open, with reduced weight and slight space needs, and does not require refrigeration nor freezing for storage, obtaining long periods at room temperature for the conservation of the processed food. In addition, food treatments such as high hydrostatic pressure inactivate microorganisms and enzymes, improving quality during storage and distribution. When used with octopus, the pressure reduces proteolysis of myofibrillar proteins, implying that this technology may effectively control octopus muscle softening (Hurtado et al. 2006). Some works have been carried out, including preparing octopus in pibil sauce and black sauce (*relleno negro*), two typical dishes from the southeast of Mexico commonly elaborated with pork and turkey, respectively (Olivares-Morales et al. 2022; Guevara-Serrano et al. 2022). Negative behavior in color development, hardness, titratable acidity, and humidity was found in both works during the storage period (5 or 12 months, respectively); all are important physicochemical parameters in determining the shelf life of the product. Additionally, both salt and acid play a significant role in influencing the speed at which meat proteins are denatured in octopus' products. Acid conditions not only confer hardness to muscle proteins but also promote collagen breakdown and cause gelatin to leach into cooking water. On the other hand, salt has the opposite effect, although it may potentially have a negative impact on the taste (McGee 2008); this confirms the importance of quantifying the effect of these additives on new octopus' products.

20.1.2 Sausages Made with Octopus By-Products

The preparation of octopus-based sausages represents a huge technical and scientific challenge due to the nutrient's uptake by the skin of the octopus tentacles. In addition, the body structure of the octopus lacks a skeleton, so when heat treated, the proteins are considerably reduced, causing size reduction and inconveniences for the production of sausages. For this reason, it is necessary to look for alternatives to prepare this raw material through a blanching pretreatment, which eliminates the air and provides optimal conditions to the octopus for the subsequent process toward the production of sausages (Barroso-Ramírez et al. 2022). Moreover, two types of blanching (immersion in water and wet steam) in Mayan octopus tentacles have been evaluated, finding that there is no significant difference in physicochemical properties (color, pH, and percentage of humidity, TPA and cutting force); however, blanching using wet steam uses shorter times to obtain similar results in colored and texture to traditional water immersion blanching. On the other hand, other authors (Souissi et al. 2016) carried out a study on sausages made with 60% octopus by-products that consisted of low-quality octopus and the pieces of meat obtained during the deep-freezing stage, in whose formula other added additives (27% water, 8% starch, 2% NaCl, 0.05% carrageenan, 0.045% ascorbic acid and 2% vegetable oil) were used with cuttlefish skin gelatin; significant positive effects were found on the physicochemical properties of the sausage: an increase in protein content, increase in emulsion stability, higher water-holding capacity, better toughness and chewiness of the formulated sausage, as well as a contribution to the lightness of the final product (sensory). The results suggest that octopus by-products can be used as raw material to produce new products such as octopus' sausage.

20.1.3 Dried Octopus

Currently, there are various final products of octopus on the market. In general, the water content should be reduced to prevent microbial growth and seek size reduction for better handling and longer shelf life. Additionally, mollusks are an important natural source for many novel active biocompounds. Due to this, a recent work sought to elaborate dehydrated octopus to provide added value, analyzing its antioxidant activity and total polyphenol content. The data (b. s.) showed that the content of the antioxidant activity of the Mayan octopus decreased significantly from 71.36% in raw octopus to 21.9 4% after drying for 8 h (Benítez-Noguerón et al. 2022), while the content of total polyphenols was of 63.43 and 64.90 μg GAE/100 g for raw and dried octopus, respectively. Another study (Cruz-Ramírez et al. 2015) presented similar percentage inhibition values for samples of another specie of octopus (*P. limaculatus*) (76.72%). In another work, methanolic extracts from cephalopods (*Sepia pharaonis*, *S. intermis*, and *Octopus vulgaris*) exhibited significant total antioxidant activity, total reducing power, DPPH, NO removal, and hydrogen peroxide removal activity (74.32%, 62.71%, 81.25%, 84.02% and 82.19%, respectively), concluding that these products developed novel antioxidant potential (Ponnusamy et al. 2016). All these extracts from cephalopods must be characterized to verify the active pharmacological biocompounds better.

20.1.4 Octopus Cooking (Heat Treatment)

Heat treatment is the most widely used procedure to soften cephalopods. Since collagen and muscle protein of the tissue have different ways of responding to heat, the combined temperature-time application is complex. One of the most important aspects of handling octopus is the gelling temperature that is directly related to its cooking temperature that compromises the consumer's acceptance. The cooking temperature of mollusks is just 55–60 °C. For example, the mantle of decapods, such as *Sepia officinalis* and *Loligo forbesii*, needs short-term heating at low temperatures (50–60 °C) to become softer and more attractive to the consumer in terms of texture (Mouritsen and Styrbæk 2018). On the other hand, a recent study showed that octopus from three landing ports in Yucatán presented similar gelling temperatures of 51.1 and 68.9 °C, where the viscosity started to rise and stabilize, respectively, leading to structural changes (Elizondo-de la Fuente et al. 2021). The above represents important results to define the heat treatments in products of octopus to confer high added value.

20.1.5 Octopus Collagens

Cooked octopus has a very firm and resistant texture due to the content, structure, and stability of octopus collagen (Morales et al. 2000). Collagen is one of the main protein components of connective tissue in multicellular animals. It is also a constituent of foods and is important in developing the texture of edible tissues and their processed products. Several studies have shown that the collagen present in many aquatic animals is an important determinant of the texture of processed meat. Cephalopods are rich in collagen, containing 3–11% in the mantle of Illex and Loligo squids (Sikorski and Kolodziejska 1986), 18.33% in the mantle of *Dosidicus gigas* (Torres-Arreola et al. 2008), and up to 32% in *Octopus maya*. In addition, the skins are rich in collagen: 70–80% of the dry matter of squid skin is collagen (Lin and Li 2012). Therefore, cephalopod by-products are rich in various biomolecules that have immense potential uses, including protein enrichment by collagen.

20.2 Collagen: Characteristics, Sources and Extraction Methods

20.2.1 Characteristics

Collagen is a principal structural protein in connective tissue, essential for the muscle, skin, and tendons in multicellular animals. The content of collagen molecules includes a specific amino acid sequence, glycine, proline, and hydroxyproline, that forms a triple helical helix composed of three intertwined polypeptide α chains and several triple helical helixes form a collagen fibril and a set of these include a collagen fiber as showed (Fig. 20.1) (Schmidt et al. 2016; Voet et al. 2006). The helixes are responsible for the resistance and rigidity of collagen fibers and are stabilized by numerous hydrogen bonds, covalent bonds, and pyrinoline, a molecule widely related to the crosslinking of collagen fibers. Also, it is known that the older the organism, the greater the resistance and cross-linking of collagen fibers (Ando et al. 2006; Ramirez-Guerra et al. 2015; Tapia-Vasquez et al. 2019).

On the other hand, the variation of amino acids in α chains generates three types of chains called α_1, α_2, and $\alpha_{3,}$ these are associated with different molecules

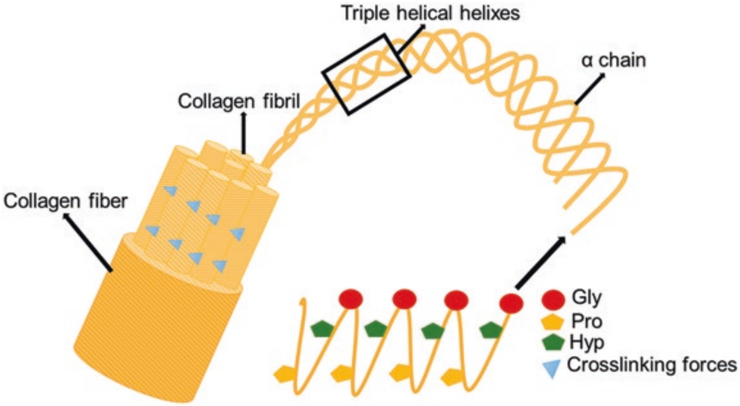

Fig. 20.1 Molecular composition of collagen fibers

generating up to 28 types of collagen, according to some authors, which are differentiated by their size, function, and location (Herrera Batista et al. 2017; Schmidt et al. 2016; Voet et al. 2006; Meisenberg and Simmons 2018). Type I of collagen is the most prevalent form in both human and animal bodies. It consists of two α_1 chains and one α_2 chain; its principal function is to provide the body with flexibility and strength. As a result, it is abundant in structures like skin, bone, tendon, and bone matrix (Meisenberg and Simmons 2018; Silva et al. 2014; Voet et al. 2006).

The polypeptide structure of α chains is responsible for rendering collagen insoluble in water, yet highly soluble in acidic solutions. However, if it is heated, physical changes are generated that increase its solubility; this protein contracts and increases its solubility around 80 °C, collagen is completely hydrolyzed and forms jelly (GELITA 2022; Meisenberg and Simmons 2018; Zuidema et al. 2014). The decrease in meat tenderness as the animal ages is largely related to the changes present in the connective tissue; with the aging or maturation of the animal, the number of intermolecular crosslinking forces in collagen fibers increases, generally covalent bonds, pyridinoline content, etc., between fibril molecules; this, in turn, leads to a decrease in collagen solubility and an increase in the hardness of the meat or tissue (Ando et al. 2006; Ramirez-Guerra et al. 2015; Tapia-Vasquez et al. 2019).

For multiple applications in industry and its abundance in animals, collagen is considered a potential biomaterial used in cosmetics, food, pharmaceutics, bioplastics, medicine, etc. Also, research is growing to find alternatives to this protein due to its biocompatibility, no toxic agent, rheological characteristics, and thermostability that can replace synthetic agents in industrial and medical processes.

20.2.2 Sources

The primary sources for extracting collagen include skin, muscles, tendons, bones, cartilage and, by-products from various animal species. Porcine and bovine collagen are among the most prevalent products. Nevertheless, multiple studies concur on exploring alternative collagen extraction methods due concerns such as spongiform encephalopathy (BSE) and foot-and-mouth disease. However (FMD), and some religious practices that bovine and porcine collagen possess (Schmidt et al. 2016; Singh et al. 2011).

Over the last 15 years, alternative sources for collagen extraction have been sought, presented in Tables 20.1 and 20.2, mainly based on the idea of by-products revaluation, where fish waste such as skin and bones turn out to be a material with a wide potential for extracting type I collagen from the skin and type II collagen from fish cartilage with a wide range of industrial applications (Kim et al. 2013; Singh et al. 2011; Solari and Córdova 2015; Song et al. 2021a; Venugopal 2016). On the other hand, chicken by-products are used in industry as material to obtain collagen and gelatin with yields of approximately 12–62%; however, this source, as well as fish by-products, needs previous conditioning to eliminate the high-fat content of the connective tissue and others impurities as has been reported. Cephalopods such octopus, cuttlefish, jellyfish, and squid represent a great source of collagen, since the muscles of these marine animals use this structural protein for important

Table 20.1 Collagen sources and conventional extraction methods

Source	CT	Extraction method	Y (%)	Applications	Reference
Gallus gallus domesticus (by-products)	I	NaClO, NaOH, CH_3COOH, and pepsin. 36 h	62	Pharmaceutical, cosmetology, biomaterial, and food	Buñay (2017)
Engraulis ringens (by-products)	I	NaOH and CH_3COOH solution. 48 h at 4 °C	5.2	N.R.	
Stomolophus meleagris	N.R.	Urea, HCl, CH_3COOH solution, and pepsin 9 h collagen and chitosan mix	>25	Food and bioplastics	
Dosidicus gigas	I	Urea, $C_2H_3NaO^2$, CH_3COOH solution, and pepsin. 36 h at 4 °C	20–70	Food and bioplastics	Torres-Arreola et al. (2008)
Rattus sp. (tendon)	I	PBS 1×, HCl solution. 168 h at 4 °C	N.R.	Biopolymers	Lopez et al. (2018)
Oreochromis spp. (skin)	I	3% protease in TRIS buffer. 2–20 h at 35 °C, followed with CH_3COOH hydrolysis for 5 h at 35 °C	56.6	N.R.	
Bovinae (tendon)	I	Acetone cleaning. CH_3COOH solution and pepsin. 24 h at 4 °C	54.3	N.R.	

CT collagen type, *N.R.* not reportedm, *Y* yield

Table 20.2 UAE methods to extract collagen

Source	CT	Extraction method	Y (%)	Application	Reference
Lateolabrax japonicus (skin)	I	NaCl solution, distilled water, and ethanol. CH_3COOH and ultrasound treatment with 80% amplitude (20 kHz) for 3 h at 4 °C	33–90	Cosmetology, pharmaceutical, and food	Kim et al. (2013)
Lateolabrax japonicus (skin)	I	NaCl solution, distilled water, and ethanol. CH_3COOH solution and ultrasound treatment with 80% amplitude (20 kHz) from 0 to 24 h at 4 °C	45–85	Food, pharmaceutical, and biofabrics	
Paralichthys olivaceus (skin)	I	Distilled water, ethanol, and NaCl solution. CH_3COOH solution and ultrasound treatment with 60% amplitude (20 kHz) from 1.5 to 3 h at 4 °C	31.3–46.2	Food, cosmetics, and health care	Song et al. (2018)
Pelodiscus sinensis (cartilage calipash)	N.R.	NaCl solution and TRIS for 12 h. Na_2CO_3 solution for 24 h. CH_3COOH solution for 24 h in 200 W (24 kHz) ultrasound at 4 °C	<16.3	Food, medicine, cosmetics, and biomedical materials	Zou et al. (2017)

CT collagen type, *N.R.* not reported and *Y* yield

biological activities to movement and growth. From giant squid, common octopus, and cannonball jellyfish, type 1 collagen has been obtained with applications in the production of bioplastics, food, and cosmetics. All reported sources present differences in the characteristics of the extracted collagen, possibly attributed to the extraction method, the degree of crosslinking, water temperature, age, etc. related to thermal resistance, amino acid composition, molecular weight, and rheological properties (Silva et al. 2014; Tapia-Vasquez et al. 2021).

20.2.3 Extraction Methods

Yield collagen extraction depends on the raw material's method and collagen fibers crosslinking level. Commonly extraction methods are based on collagen acid solubility; alkaline solutions are used to separate the myofibrillar protein of connective tissue and after the first hydrolysis, connective tissue gets into a second hydrolysis using acid to break collagen fibers. Also, in acid treatment enzyme is added to increase collagen solubility, breaking crosslinking forces and separating protein triple helix. The latest research showed that ultrasonic-assisted extraction (UAE) in combination with enzymatic processes increases extraction yield and decreases time extraction and acid concentration due to the cavitation effect (Li et al. 2009; Schmidt et al. 2016; Song et al. 2018; Zou et al. 2017).

The raw material is prepared by eliminating fats using alcohols and, in some cases, pigments with the use of sodium hypochlorite, in such a way that components are discarded to perform an effective extraction.

Conventional extraction involves a series of steps consisting of first treatment of the homogenized raw material (cut into small pieces or ground) salts as NaOH in common concentrations of 0.5 M to 0.1 M are generally used to eliminate the myofibrillar proteins by solubilizing it in alkaline solution for 12 to 72 h (Buñay 2017; Lopez et al. 2018; Schmidt et al. 2016; Torres-Arreola et al. 2008). This solution is separated by centrifugation, retaining only the precipitate containing the connective tissue. The connective tissue is added to an acid solution, especially acetic acid or hydrochloric acid, in concentrations of 0.01 M to 0.5 M for 12 to 72 h. This step includes collagen solubilization, breaking some of the intermolecular forces of the collagen fibers but preserving the triple helix characteristic of the native collagen (Fig. 20.1a). The hydrolysate is commonly separated by centrifugation, obtaining 2 fractions: the soluble fraction found in supernatant, which corresponds to triple helix separated from the collagen fibril and the insoluble fraction present in the precipitate, which consists in crosslinked collagen that is not solubilized in acid as shown (Fig. 20.2a).

When collagen solubility increases, consequently the insoluble fraction decreases, and enzymatic hydrolysis is used for this purpose. Thus, once the connective tissue (or the insoluble fraction of collagen obtained by previous chemical hydrolysis) is added to the acid solution with enzymes such as pepsin or other proteases to hydrolyze separating the collagen triple helix from the complete fiber and obtaining some low molecular weight peptides (Fig. 20.2b). The enzyme breaks some covalent bonds, Vander Waals forces or pyridinoline eliminating the crosslinking of collagen; this extraction method has higher yields than chemical extraction but causes some changes in the rheological properties of the extracts, because contrary to the first case where gels are obtained in this type of extraction obtains solubilized collagen in the liquid medium (Jridi et al. 2013; Schmidt et al. 2016; Song et al. 2021b).

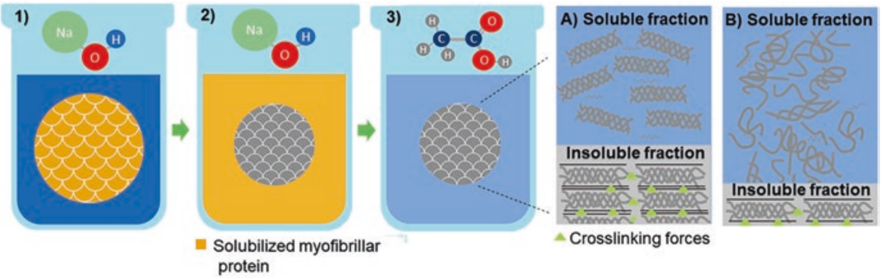

Fig. 20.2 Collagen extraction process. (1) Raw material with NaOH solution. (2) Myofibrillar protein solubilized in NaOH solution and separated connective tissue. (3) Connective tissue in acetic acid solution. (**a**) Native collagen and (**b**) Hydrolyzed collagen

On the other hand, UAE extractions shorten extraction time and increase yields by generating small bubbles for sound energy which tend to collapse, thus increasing the temperature and pressure. In this type of extraction, the triple helix structure of collagen is not at risk of obtaining native collagen (Fig. 20.2a), however, the temperature is an important factor to consider since cavitation produces a considerable temperature increase and this could cause collagen denaturation. Some studies suggest that an ultrasound treatment combined with enzyme obtains the best yields, cavitation increases the activity and dispersion of pepsin in the connective tissue (Schmidt et al. 2016; Song et al. 2018; Zou et al. 2017). Finally, after obtaining the collagenous extracts, the soluble and insoluble fractions are commonly separated by centrifugation, dialyzed and lyophilized for preservation.

20.3 Gelatin

Gelatin is obtained by hydrolysis of collagen. Generally, the hydrolysis process is obtained in hot water, with a strong or weak acid or base, or by enzymatic means, under controlled temperature, pH, and ionic strength conditions. Collagen is a ubiquitous fibrous protein in mammalian animals. It is obtained from bovine bone and pig skin, although obtaining it from marine sources such as fish has also been explored. Especially from cod by-products, pollock salmon, octopus, tilapia, Nile perch, subtropical water catfish and frog (Aksun Tümerkan et al. 2019; Jellouli et al. 2011). Globally, commercial gelatin comes from pig skin, bovine, pork and bovine bone and fish, distributed at 42%, 29%, 23.1% and 1.5% from other sources, respectively (Aksun Tümerkan et al. 2019; Al-Hassan et al. 2021; Gómez-Guillén et al. 2021; Milovanovic and Hayes 2018; Nitsuwat et al. 2021). Namely, 95.6% of the gelatin is obtained from porcine and bovine by-products. Although, there is controversy about its obtaining from by-products of bovine origin due to the appearance of diseases such as bovine spongiform encephalopathy or aphthous fever (Mrázek et al. 2022; Nitsuwat et al. 2021). Due to this, chicken skin, camel bone and recombinant systems for obtaining gelatin have been proposed as alternative sources (Aksun Tümerkan et al. 2019; Al-Hassan et al. 2021; Avila Rodríguez et al. 2018). The partial process of acid hydrolysis breaks the unmodified collagen triple helix in the polypeptide chain (collagen derived from pigskin) produces type A gelatin with isoelectric point between 7.0 to 9.0, due to side residues of asparagine and glutamine (Karim and Bhat 2009). While type B gelatin is the hydrolysis product of collagen derived from bovine bone and calfskin, the isoelectric point varies between 4.0 to 5.0 a 5.0 (Gomez-Guillen et al. 2011). The alkaline treatment produces residues of polypeptide chains with carboxylic groups of aspartic and glutamic acid (Lee and Mooney 2001). Gelatin is a protein that, depending on the extraction method, can obtain different isoelectric points. The isoelectric point is a property where a protein carries a net charge of zero and becomes insoluble at a particular pH. Variations in isoelectric points depend on the relative molar fractions of amino acids within the protein. In an acidic solution, gelatin has a positive charge, while in an alkaline solution, it produces a negative charge (Gómez-Guillén et al. 2002).

Gelatin is considered a protein not found in nature and is called a derived protein. Industrial, food, and pharmaceutical grade gelatin can be obtained, with a lower or higher molecular weight distribution; 100, 200, and 300 g/mol, corresponding to alpha, beta, and gamma polypeptide chains, respectively (Ji et al. 2022) and different physicochemical properties such as thermal stability, melting point, gelatin, and gel strength. Properties depend on the hydrolysis level and proline and hydroxyproline content in the collagen triple helix (Al-Hassan et al. 2021). These two amino acids, often found in greater abundance in collagen, are responsible for both gel strength and the melting point. In this sence, it is also mentioned that an intact polypeptide chain, higher molecular weight, and a greater proportion of alpha polypeptide chains contribute to higher gel strength (Al-Hassan et al. 2021; Nitsuwat et al. 2021). Gelatin applications are based on its physicochemical properties; protective colloid; insoluble in cold water, but soluble in hot water; stabilization, gelatin, texturing, emulsification, settling, clarification, thickener, viscosity improver, clear and flexible coatings, tackiness and foaming (Al-Hassan et al. 2021; Mrázek et al. 2022). The preparation of gelatin gels for coating applications in food or controlled release in medicines requires low concentrations of gelatin, while the preparation of gels with high concentrations of gelatin is used in tissue engineering, due to their mechanical properties (Abdullah et al. 2018; Mrázek et al. 2022).

20.3.1 Chemical Composition and Structure

Gelatin is a complex macromolecule, made up of different fragments of polypeptides, different size, molecular weight, and amino acids that follow a repetitive pattern, like the composition of the original collagen. Gelatin solution above 25 °C is levorotatory, possibly due to the amino acid composition resulting from the sequences Gly-Pro-Pro and Gly-Pro-Hypro (Abdalbasit Adam and Hadia Fadol 2013; Shoulders and Raines 2009; Karim and Bhat 2009; Raja Nhari et al. 2011). Eighteen different amino acids are found in collagen and form polypeptide chains that consist of two identical chains, called α1, a slightly different one called α2 intertwined chains that resemble a bar formed by the three polypeptide chains and form a triple helix (Shoulders and Raines 2009). The gel strength and melting point of gelatin depend on the molecular weight, and ratio of α and β chains. Nitsuwat et al. (2021) describe that a greater proportion of alpha chain will have greater gel strength.

The amino acids from lowest to highest concentration in the gelatin analysis range from 0.2% tyrosine to 30.05% glycine. The amino acids in the highest proportion in gelatin are glycine (26.4 to 30.5%), proline (14.8 to 18%), hydroxyproline 13.3 to 14.5%, glutamic acid 11.1 to 11.7% and alanine 8.6 to 11.3% (Gómez-Guillén et al. 2002). While the minor amino acids in decreasing order are arginine, aspartic acid, lysine, serine, leucine, valine, phenylalanine, threonine, isoleucine, hydroxylysine, histidine, methionine, and tyrosine. The concentration of these 13 amino acids is approximately 20% (Abdalbasit Adam and Hadia Fadol 2013; Uriarte-Montoya et al. 2011). Pure gelatin contains 85% protein, a supplement that provides 17 amino acids, eight of which are essential amino acids (isoleucine, leucine, valine, lysine, methionine, phenylalanine, threonine, and histidine). Essential amino acids are necessary because the human body cannot synthesize them. Amino acids are part of proteins, which fulfill various physiological functions such as growth, immune response, as well as the transport of lipids and glucose. For example, L-histidine and isoleucine control both the formation and degradation of proteins. Histidine is part of the carnosine dipeptide, which controls physiological pH and, neutralizes and transports metal ions, has anti-inflammatory and antioxidant effects (Blachier et al. 2021; Moro et al. 2020; Solís-Ortiz et al. 2021). While leucine prevents protein degradation in humans. Methionine and lysine produce an amino acid called L-carnitine, and it is essential in the reduction of fatty acids, controlling the accumulation of these in the human body (Nair et al. 1992).

20.3.2 General Process for Obtaining Gelatin

The process for obtaining type A gelatin: start with washing to remove grease and foreign material. After washing, the raw material is subjected to acid treatment, controlling the temperature between 5 and 10 °C, sometimes at 25 °C, from 12 to 48 h. Acids frequently used are mineral acids; hydrochloric, phosphoric, or sulfuric, or organic acids; citric, formic, propionic, or lactic. The acid solutions used are dilute, the concentration often being between 0.05 and 0.5 moles per liter. After the acid treatment, washing reduces the acid used in the process. Consecutively, the pH is controlled (1.5 to 3.0), depending on the collagen source (amount of intermolecular and intramolecular crosslinking), at least 5 extractions are performed, water at different temperatures (45 to 55 °C) is frequently used, for at least 10 h (Gómez-Guillén et al. 2021; Mokrejš et al. 2019; Nitsuwat et al. 2021). The solutions obtained are treated by suitable means to remove fat. Finally, the obtained solution is purified using filtration or elimination of minerals by ion exchange; the average yield varies between 5.5 to 66.0% (Al-Hassan et al. 2021).

20.3.3 Applications

Gelatin is soluble in water and polyhydric alcohols, it forms gels, it is innocuous and practically does not trigger allergies, it is a multipurpose protein. The solutions are used as solvents for candies, marshmallows, desserts (fruit snacks, gummies, cakes). In the dairy industry and frozen foods, gelatin prevents the formation of water and sugar crystals. Gelatin can be used as an ingredient with functional properties, due to its antioxidant and cryoprotective effects. Gelatin with an isoelectric point between 4 and 5 and with repeating fragments of Gly-Pro-X, and Gly-X-Y has been reported to reduce crystal growth by a factor of 10 in ice cream formulations. The combination of peptides derived from the gelatin (Pro-Ala-Gly-Tyr) and caffeic acid have a cryoprotective effect and slow the oxidation of proteins and lipids in meat products, especially pieces of meat (Liu et al. 2015). In food formulations, gelatin has a binding effect and improves texture (Abdalbasit Adam and Hadia Fadol 2013). Gelatin is used in the pharmaceutical industry to obtain hard capsules, soft gels, ointments, and encapsulants to administer medications. To obtain soft gels, type A gelatin with Bloom values between 170 to 180 g is used, or type B gelatin with Bloom values between 150 to 175 g. The hard capsules are manufactured with type A gelatin with Bloom between 250 to 280 g, or type B gelatin from 225 to 250 g, or mixtures of both gelatins, in which vitamins A, D and E can be microencapsulated (Abdalbasit Adam and Hadia Fadol 2013). Gelatin is also used as a protective vehicle for capsules, suppositories, and ovules. In medical applications they are used as a sponge to retain bleeding and stabilizer for vaccines, the cosmetics industry uses them in shampoo and masks (Abdalbasit Adam and Hadia Fadol 2013).

20.4 Peptides with Biological Activity

Numerous peptides with biological activities (PBA) have recently been reported in recent years as naturally hidden in the complex protein food matrix and released by intestinal digestion of nutrients or/and generated by hydrolysis with endogenous and exogenous enzymes during processing or storage. These peptides can be obtained from various sources through a different kind of process, in most cases consisting of fewer than 50 amino acid residues most of the peptides include acid proline, arginine, and lysine. PBA biological activity is determined by structural characteristics like molecular weight, charge, and hydrophobicity (Gong et al. 2016). PBAs have been considered the new generation of biologically active compounds that can show antioxidant and antimicrobial activity and a variety of physiological functions like anticancer that make them latent nutraceutical compounds for pharmaceutical and food industries (Wang et al. 2022).

New research has been focused on producing and studying PBA generated from by-products and processing waste of different industries due to potentially reduce pollution, increase added value and reduce economic cost as advantages over other raw materials, for example, the marine and aquaculture industries (Harnedy and FitzGerald 2012). The wide range and quantity of by-products and processing waste derived from these industries, such as animals out of specifications (whole animal), skin, fins, mantle, viscera, or blood not only represents a potential source of minerals, vitamins, polyunsaturated fatty acids, polysaccharides, antioxidants, enzymes, and proteins as a nutritional compound, but also these proteins, that are one of the major constituents, can be used as a raw material for an unlimited and unexplored resource of new peptides with potential use as bioactive compounds in food, pharmaceutical, and feed industry applications, among others (Gong et al. 2016; Sierra Lopera et al. 2018). This section focuses on the PBA obtaining of the microbial and enzymatic processes.

20.4.1 Methodologies for Obtaining PBA: A Strategic Approach

Protein hydrolysates (PHs) are unpurified peptides consisting of a complex mixture of proteins and peptides of different sizes produced by the hydrolysis of integral proteins. Firstly, in commercial products of food and feed products, PH was commonly used only as an alternative protein source, and recently depending on their biological characteristics, their proposal was used as the main component to exert functional and nutraceutical activity (Sierra Lopera et al. 2018). On the other hand, purified peptides are commonly found in experimental applications or pharmacy groups due to the unavailability of large-scale technologies and the high cost of purification techniques that permit the commercialization of PBA. In this context, the strategic processing steps are crucial to obtaining pure peptides with biological activities. In this framework, considering the high potential for commercial exploitation of marine and aquaculture by-products and processing waste-based proteins as raw material, this section aims to review the available methods for the production and recovery of peptides. A wide range of methods is commonly employed by academic and industry researchers. The trends indicate the development of new strategic methodologies for PBA extraction, with an emphasis on reducing time and costs (Wang et al. 2022). According to the current literature, different methods have been conducted to break down protein, including chemical, enzymatic hydrolysis, and microbial fermentation.

20.4.2 Enzymatic Digestion By In Vitro Enzyme Hydrolysis and Microbial Fermentation

Firstly, chemical acid-alkaline methods were the most used method to produce protein hydrolysate and peptides due to low cost and easy processing conditions, and the lack of control over the hydrolysis, which results in a heterogeneous yield of peptides (Ghaly et al. 2013). On the other hand, enzymatic digestion by in vitro enzyme hydrolysis promotes protein breakdown in a precise way to produce short chains of polypeptides. This is the most extensively used method for the production of protein hydrolysates because it is the most efficient, easily controlled, and reliable method to produce peptides with well-defined characteristics, and the final peptide products lack toxic compounds such as residual organic solvents (Peighambardoust et al. 2021). Enzymatic hydrolysis involves substrate (whole food matrix or protein extract raw material) preparation such as homogenization and inactivation of endogenous enzymes by heating, followed by adding enzyme or an enzyme mix, constantly measuring the enzymatic hydrolysis products, and inactivating endogenous enzymes to stop the reaction. Trypsin, quimotripsyn, alcalase, pancreatin, and papain, among others, are the most commonly employed in the enzymatic hydrolysis process (Ghaly et al. 2013).

Nevertheless, enzymatic modifications of the protein's food matrix are carried out under specific temperature conditions, enzyme concentrations (0.01–5.00%, w/w), and optimal pH (1.5–11), which necessitates a consideration of process costs.

In this context, proteolytic enzymes derived from by-products of the meat industry or microorganisms emerge as the most promising sources. Alternatively, the utilization of immobilized enzymes through resins can extend the duration of modifications on the food matrix (Basso and Serban 2019).

Microbial fermentation is one of the oldest and most applied technologies used in food processing and preservation. Still, this method has recently been carried out on the generation of small peptides with biological activity by the action of both microorganisms and endogenous proteolytic enzymes that have high proteolytic potency (Cruz-Casas et al. 2021; Vázquez et al. 2020). The method consists of the use of continuous processes rather than conventional batch methods. Microbial hydrolysis was rigorously explored by researchers and defined the optimal conditions for the enzymatic hydrolysis with several strains, including the medium percentage, pretreatments of raw material like dehydration process and sterilization conditions, storage, processing time, and temperature, among others, to obtain higher yields and lower operating costs. Microbial hydrolysis can be carried out in a reactor or a simple Erlenmeyer flask.

In vitro enzymatic hydrolysis and microbial fermentation can result in mixtures of small peptides that can enhance both the quantity and diversity of their biological activities (Peighambardoust et al. 2021). Both methods use a whole complex food matrix, but only enzyme hydrolysis can use protein extract to produce efficient quantity and a wide range of PBA due to the limited energetic components and micronutrients in microbial fermentation that play a crucial role in the homeostasis of microbial growth and release of proteinases from the cell wall and several intracellular proteins. The method using endopeptidases, effectively enables continuous hydrolysis to extract PBA from various protein sources, yielding low molecular weight peptides that exhibit the highest biological activities. Still, recently, studies have shown that using both methods increases the production PBA (Vázquez et al. 2020). However, the complexity of by-products and processing waste matrix as a raw material makes a complicated process protocol.

As an advantage over other methods, microbial fermentation costs are relatively low due to continuous culture strains; another advantage is using some microorganisms like *Lactobacillus* culture so that their products are considered safe (Cruz-Casas et al. 2021). Nonetheless, the challenge for this method is to define the minimal nutritional requirements for medium (by-products and processing waste characteristics) and type of microorganism. The most critical parameters are the strategic planning methodology and the raw material matrix to develop the maximal yield, scalability, reproducible effects, and sustainability approach of PBA production.

20.4.3 Extraction and Isolation of PBAs

Although marine and aquaculture protein hydrolysates could exert biological activities, after standard protocol isolation, their biological activities will be increased and the potential risk could be minimized (Cheung et al. 2015). In this sense, precipitation of unhydrolyzed protein, fractionation, and purification stages is highly recommended after the raw material hydrolyzation. For the fractionation stage, a common method is ion exchange chromatography, a method based on the net charge of peptides. Then, fractioned peptides are purified using technologies like reversed-phase HPLC used to separate peptides based on their characteristics of hydrophobicity and hydrophilicity. Common technologies for the purification stage are based on molecular weight, like ultrafiltration, gel filtration, and nanofiltration. From active fractions, peptides are frequently identified using mass spectrometry methods like MALDI-TOF and MALDI-MS, among others. However, new technologies have recently proposed the attention of characterizing the enzymatic hydrolysis of marine by-products in real time, one example is benchtop nuclear magnetic resonance spectroscopy (Anderssen and McCarney 2020).

20.4.4 Functional and Nutraceutical PBAs

Several studies have been showing that PBA derived from marine and aquaculture sources can exert functional properties such as solubility, emulsifying, foaming, water-holding, and fat-binding capacity and biological activity such as antioxidant, antimicrobial, antihypertensive, antiproliferative, antihypercholesterolemic, and immunomodulating, among others, which highlight their current interest for the pharmaceutical, food, and feed industries (Uhlig et al. 2014). Among the numerous relevant activities attributed to PBA, noteworthy the most studied are antioxidant and antimicrobial, but there is a lack of studies focused on health promotion such as antihypertensive, antithrombotic, and immunomodulatory activities.

20.4.5 Mode of Action

Many biological activities are proposed for PBA, and even if the mechanism of action is not completely elucidated, antioxidant, antimicrobial, and ECA activity are the most studied. In this regard, peptides containing methionine have been demonstrated to be an antioxidant peptide, and their mechanism is attributed to the sulfide of methionine’s thioester group that transfers two electrons. In the same way, their metal chelators’ activity is ascribed to amino acids like threonine, glutamic acid, aspartic acid, phosphorylated serine, and histidine.

Antimicrobial peptides are among the most extensively studied bioactive peptides. Biophysical analysis have revealed that microbial growth inhibition is achieved through factors such as selectivity in interacting with cell membranes. This interaction leads to permeabilization through the electrostatic interface between peptides and the fatty acid membrane composition of the bacteria strain; this process, encourages the formation of pores on cell membranes, and allows the efflux of essential nutrients and ions (Tan et al. 2019).

The antihypertensive peptides mechanism modulates endothelial function by increasing vasodilatory factors like enhancing nitric oxide (NO) and upregulating

ACE2 expression. Moreover, recent academics proposed a relationship between gut microbiota and lowering blood pressure through the use of purified peptides derived from marine resources (Song et al. 2021a).

Several studies have been conducted on identifying and characterizing peptide structures and functions; nevertheless, further studies are required to determine the mechanisms that may promote one or several biological effects simultaneously.

20.4.6 Potential Risks of Protein Hydrolysates and PBA

Even though bioactive peptides have been shown that are easily metabolized, on the face of it, the toxicity must be considered a critical point during the processing steps of PBA production, and for the final product, adverse consequences may occur in the body from intake, dosage, and time supplementation. The first potential problem of this activity is that PBA is not selective on membranes; in other words, PBA can act on eukaryotic cells as well, increasing the potential toxicity (Cruz-Casas et al. 2021; Ruiz-Capillas and Herrero 2019). Secondly, another potential risk in using whole protein hydrolysate-derived (unpurified small peptides with large polypeptides) from microbial fermentation is the origin of potential biogenic amine production. These are harmful nitrogenous compounds precursors of amino acids from protein-rich raw material such as marine and aquaculture by-products and processing waste produced mainly by amino acid-decarboxylating microorganisms that may cause intoxication in humans. Naturally, they are detoxified by human intestinal amine oxidases and detoxification system, but moderately to high dosage has become one of the most important processing issues for preventing intoxications; putrescine and cadaverine are common examples (Ruiz-Capillas and Herrero 2019). In this sense, the approach of whole protein hydrolysate as a nutritional product and enriched-PBA additive is necessary as a control strategy for reducing biogenic amines. However, a few studies have demonstrated that some culture strains like *Lactobacillus plantarum* and *Bacillus* sp. can reduce the number of biogenic amines biologically active (Park and Joo 2017; Zhang et al. 2022).

Studies suggested that purified PBAs are mainly toxicologically safe, have a wide spectrum of biological activities, and are better absorbed in the intestinal tract. Compared to synthetic drugs, PBA from in vitro enzymatic and microbial hydrolysis side effects are much less serious (Cebrián et al. 2019).

20.5 World Industry Trends: Collagen, Gelatin and Peptides

20.5.1 Collagen

The global collagen market comprises a wide range of companies; some of the key players include Gelita AG, Croda International Plc., Collagen Solutions Plc., Beyond Biopharma Co. Ltd., Weishardt Holding S.A., Titan Biotech, Ashland, and Rousselot. The worldwide collagen market in 2020 was assessed up to 8000 million dollars and thanks to its multiple uses in food, beverage, cosmetics, and health-care industry, an annual increase of 9.3% is predicted until 2028. From all collagen sources, marine sources are envisaged to increase at an annual growth rate of 10% until 2028 due to its high quality and bioavailability (Collagen Market Size, Growth & Analysis Report, 2021-2028 (grandviewresearch.com)). Industrial applications of collagen can be divided into at least 3 categories: Food and Beverages, Healthcare, and Cosmetics.

Food and Beverage category includes products such as

- Functional (Food and Beverages).
- Dietary supplements.
- Dairy.
- Confectionery.
- Desserts.
- Meat and fish processing.
- Nutraceuticals (Sport nutrition and Weight management).

The Health-care category includes products related to ameliorate wellness:

- Health supplements.
- Wound dressing.
- Tissue regeneration.
- Medical implants.
- Cardiology.
- Drug delivery.
- Research (cell culture and behavior).

In the Cosmetics category, there are products related to beauty:

- Nutricosmetics (supplements for inner beauty).
- Topical products.

Food products containing collagen are as diverse and innovative as the market growth. Table 20.3 summarizes some examples of the novel uses of collagen in Food & Beverage Industry. It is possible to find protein bars, energy drinks, coffee, desserts, water, and tortilla wraps containing collagen as a functional ingredient in stores. These products are some examples of the possibilities to be explored for collagen in the food and beverage industry.

20.5.2 Gelatin

The key companies in the global gelatin market include Gelita, Darling Ingredients, Nitta Gelatin, and PB Gelatins. These companies produce a wide range of gelatin products for use in various applications, including food, pharmaceuticals

Table 20.3 Global trends of food and beverage products added with collagen

Product type	Food product	Origin	Enterprise
Functional beverages	Chocolate and coffee	United Kingdom	Planet Paleo® https://planetpaleo.co/
Sport nutrition	Protein bars	USA	Caveman Foods® www.cavemanfoods.com
Functional beverages	Energy drink	USA	PepsiCo®[a]
Desserts/Dairy	Ice cream	USA	Re:Think Ice Cream® www.rethinkicecream.us
Functional beverages/sport nutrition	Protein drink	Germany	Black Labels Company® www.black-labels.de
Functional beverages	Protein water	England	Protein Water Co® www.proteinwater.co
Functional beverages	Beer	Japan	Suntory® www.suntory.com
Weight management	Wrap (tortilla)	U. S.	Cali'flour Foods® www.califlourfoods.com

[a] PepsiCo launches new Rockstar energy drink flavors with added collagen—FoodBev Media

(manufacturing of pills and capsules), and other industries like photographic films, adhesives, cosmetics as a thickening agent, or textile industry as sizing agent. In addition to these larger, global players, many smaller, regional companies produce and sell gelatin products. The global market for gelatin is expected to continue growing as more people become interested in the potential health benefits of collagen and as the demand for natural and organic products increases. The world market for gelatin between 2016 and 2021 was estimated at between 3710 and 5800 million dollars, respectively (https://www.grandviewresearch.com/industry-analysis/gelatin-market-analysis). The world market for collagen between 2016 and 2021 was estimated at between 3710 and 5800 million dollars, respectively. Gelatin's demand is extremely important and an annual production increase equivalent to 9.5% is forecast from 2022 to 2030. It is expected to reach 6630 million dollars by 2025. A similar market growth is a collagen biomaterial in tissue engineering, a compound annual growth rate of 10.4% is forecast by the end of 2025 (Avila Rodríguez et al. 2018). The global gelatin market volume reached 447,300 tons in 2021 and expected to reach 496,470 tons by 2027, a compound annual growth of 1.66% from 2022 to 2027. The United States occupies 23.04% of the global market, that is, approximately 553 million dollars for this concept. The second market for gelatin is China, it is expected that by 2027, the annual consumption, equivalent in dollars, will reach 375.9 million and become one of the most important markets for gelatin consumption.

20.5.3 Collagen Peptides

Collagen peptides are short chains of amino acids derived from collagen. They are typically obtained through hydrolysis, breaking the collagen protein into smaller peptides. These peptides are then used in various products, including dietary supplements, skin care products, and food products, as a source of protein and to improve the texture and consistency of the product. The global collagen peptides market is expected to grow as long as the collagen and gelatin market does. Besides powders, pills, and other supplement presentations, collagen peptides can be found as a novel ingredient in food and beverage products. Creamers, butter, and coffee alternatives are some examples of the trends (Table 20.4).

20.6 Conclusions and Perspectives

Collagen, gelatin, and collagen peptides are biocompounds with multiple applications in Food and Beverage, Healthcare, Cosmetics, and other Industries. These biocompounds derived from marine and biotechnological sources could replace the current sources and the drawbacks mentioned in this chapter. Interests in these compounds grow to find alternatives with these proteins due to their biocompatibility, no toxic agent, rheological characteristics, and thermostability that can replace synthetic agents in industrial and medical processes. They may be an important supplement alternative for developing nonencapsulated and microencapsulated systems for better nutrition. They provide practically all the amino acids and can be used to supplement balanced diets to obtain better health well-being. In combination with other additives, novel products with improved physicochemical properties can be manufactured.

Efforts are currently being made to develop endemic octopus' new products to reach the freshness, price, variety, practicality, and availability sought by the consumer. Also, a wide variety of products have been conducted to promote octopus' diet. Despite the main limitations to industrializing developed products, such as excessive logistics cost, competition with raw materials such as vegetables, algae,

Table 20.4 Global trends of food and beverage products added with collagen peptides

Compound	Food product	Origin	Enterprise
Collagen peptides	Creamer	U. S.	Vital Proteins® www.vitalproteins.com
Collagen peptides	Nut butter	U. S.	Vital Proteins® www.vitalproteins.com
Collagen peptides	Matcha latte	U. S.	Great Lake Wellness® www.greatlakeswellness.com
Collagen peptides	Kombucha drink	Australia	Utonic® www.utonic.com
Collagen peptides	Gummies	U. S.	Havasu Nutririon® www.havasunutrition.com

etc., temporary supply of raw materials, population sensitivity and fluctuation, the freshness of by-products, and regulatory requirements. Moreover, several studies are conducted to characterize the biomolecules of this cephalopod to enhance its acceptance worldwide. On the other hand, octopuses have been developed in Mexico farms, implying less impact on the ecosystem and encouraging a circular economy. It is necessary to take the opportunity to create new products by processing cephalopod by-products after studying and characterizing their properties for use in the food, pharmaceutical, or cosmetic industries.

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21

Marine Fish Microbiome: Current Status and Future Perspectives

Rajesh Pamanji and Joseph Selvin

Abstract

The microbiome of fish is home to a diverse collection of creatures, including Protoctista, fungi, yeasts, viruses, and individuals belonging to the bacterial and archaeal kingdoms. Bacteria, on the other hand, make up the bulk of the microbiota that may be found in the digestive tract of fish. The Firmicutes, Proteobacteria, Bacteroidetes, and Fusobacteria are some of the most important phyla. The microbiota that lives in marine fish guts plays an important part in a variety of important processes, including adaptation to new environments, the development of social behavior in fish, research on carbon cycling, the production of probiotics, multidrug resistance, bioindicators of environmental pollution, and the evolution of hosts. There are 33,700 different kinds of fish that can be found in the world. Because it is difficult to provide the full microbiome of marine fishes, we will try in this chapter to present the marine fishes and their microbiomes that have been examined and researched the most. In addition to this, a significant number of edible and ornamental fishes that have not been investigated for their gut microbiomes were included.

Keywords

Microbiome · Marine fish · Environment · Social behavior

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21.1 Introduction

The microbiome of fish contains a wide variety of organisms, such as Protoctista, fungi, yeasts, viruses, and members of the bacterial and archaeal kingdoms. Bacteria, on the other hand, are the microbiota that predominate in the fish intestine. Fish and surrounding microorganism might have a pathogenic or mutualistic connection. Like humans and other mammals, fishes' symbiotic gut microbiota plays a role in immunological defense, metabolic equilibrium, and nutritional supply. Fish gut microbiota studies vary in many ways, such as the types of fish studied, and the ways samples are collected and analyzed. Different workers may get different results about the composition of the microbiome, because they use different methods to figure out what microbes are in the sample. This can make it hard to compare results and figure out how diverse the world really is.

Different microbial species, both harmful and beneficial, can alter various immune and physiological processes in a fish by adhering to, colonizing, and multiplying within the fish's digestive tract. There are developmental shifts in the gut microbiome of fish. Within a species, there may be a correlation between the size of fish and the make-up of their gut microbiota. A fish's intestine may store between 107 and 1011 germs per gram of food. The diversity and abundance of bacteria in a fish's digestive tract may change with the seasons. Bacteria in the intestines can be either autochthonous (originating from the host's local environment) and so adhered to the intestinal mucosa, or allochthonous (originating from elsewhere) and hence unable to attach. The community structure of the microbiota in the gut is primarily influenced by three factors: environmental conditions, trophic level and/or feeding behavior, and host-specific traits (Ofek et al. 2021). 7Microbiome can vary depending upon different stages of development.

The world's aquatic ecosystems are under peril because of human activity and natural disasters alike. Microbe-host interactions in aquatic organisms have been altered by climate change, ocean acidification, eutrophication, and pollution. The smallest inhabitants of our freshwater and oceanic ecosystems are sensitive to environmental elements including light, temperature, and oxygen levels. The symbiotic link between microbial communities and vertebrates is complex and easily disrupted. It is possible that the health of aquatic vertebrates is affected by these changes to the microbiota. The anthropogenic activities that are changing the conditions of the ocean may have a negative influence on fisheries by making the fish more stressed and prone to disease. The Census of Marine Life (CoML) is a project that brings together top marine scientists from all over the world to work together on a project that has never been done before. On average, 160 new marine fish species are discovered every year, or about three new species every week since 2000. The experts at CoML think that the final number of marine fish species will be around 20,000 (Census of Marine Life 2003). There are 33,700 species of fish in the world. This is almost half of all vertebrate species and includes a wide range of physiologies, ecosystems, and life cycles. In this chapter, we attempt to highlight the microbiomes of marine fishes that have been extensively studied and researched, as it is challenging to cover the entire microbiome of these organisms.

21.2 Importance of Marine Fish as Food and Their Microbiome

Fish is an important food source for people. According to the Food and Agriculture Organization (FAO) of the United Nations, it is the most important source of high-quality protein for humans. About 16% of the animal protein eaten by people around the world comes from it (1997). Fish is also very important to society and the economy. The FAO says that international trade in fish is worth US$ 51 billion per year (FAO 2000). FAO says that fishing and aquaculture directly employ more than 36 million people and that up to 200 million people get direct or indirect income from fish (Garcia and Newton 1997).

The microbial communities that live in fish guts have the potential to boost the host's metabolic capacity by having a positive effect on the digestion and assimilation of nutrients, as well as protect the host from invasive diseases (Nayak 2010). For example, fish intestinal microbiomes have the ability to create a wide variety of digestive enzymes, such as lipase, protease, and cellulase, which aid in the fish's ability to digest their meal (Ray et al. 2012).

21.3 Marine Fishes and Microbiome

Marine fish gut microbiome is diversified with different bacterial phyla families, genera, and species (Fig. 21.1). Major phyla include Firmicutes, Proteobacteria, Bacteroidetes, and Fusobacteria. The diversification is unique and combinational. Each type of fish has its own combination of bacterial genera that makes it unique. Even among Atlantic cod taken in the same area, there is a wide variety in the

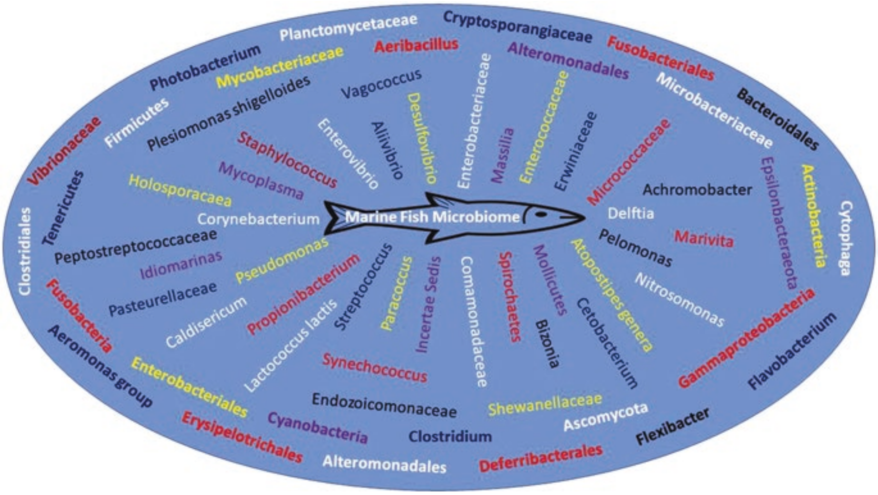

Fig. 21.1 Marine fish microbiome with different bacterial phyla, families, genera, and species

make-up of their gut microbes. Quantitatively, the Atlantic cod gut microbiota is dominated by the order Vibrionales, which is also represented among the shared OTUs. Other orders represented include Bacteroidales, Erysipelotrichales, Clostridiales, Alteromonadales, and Deferribacterales, but to a lesser extent (Star et al. 2013). Some of the fishes, which were characterized for their gut microbiome, are explored in this chapter.

21.3.1 Atlantic Bluefin Tuna (*Thunnus thynnus*)

One of the most valued fish in danger of overfishing is the Atlantic bluefin tuna, *Thunnus thynnus*, also known as blue fin tuna, blue-fin tunny, bluefin tuna, horse mackerels, northern bluefin tuna, and squid hounds. The upper, or dorsal, surface of an Atlantic bluefin has a deep blue to black color, while the underside is a silvery grey (underneath). Finlets extend from the bluefin's dorsal (back) and ventral (side) sides all the way down to its anal fin. The gut microbiome of *Thunnus thynnus* is diversified and consists of bacterial species like Tenericutes and Spirochaetes, Cyanobacteria, Firmicutes, and Bacteroidetes, Vibrionales, Pseudomonadales (Minich et al. 2020) and Pasteurella and Moraxella genera (Kapetanović et al. 2017).

21.3.2 Atlantic Cod (*Gadus morhua*)

Atlantic cod (*Gadus morhua*) is an ecologically valuable species native to the North Atlantic Ocean. Atlantic cods have a broad, rounded head and a prominent barbel (a whisker-like organ, like a catfish's) under the lower jaw, making them stocky fish. The steep, rocky slopes and ledges near the ocean floor are home to Atlantic cod. They prefer bottoms with coarse sediments over finer mud and silt, and they can live in cold waters between 30 and 500 ft deep. Researchers have reported a wide variety of bacterial communities in Atlantic cod. These communities include, but are not limited to, Fusobacteriales, Clostridiales, Photobacterium, Vibrionales, Bacteroidales, Erysipelotrichales, Alteromonadales, and Deferribacterales (Riiser et al. 2018, 2019; Le Doujet et al. 2019; Star et al. 2013).

21.3.3 Atlantic Halibut (*Hippoglossus hippoglossus*)

Atlantic halibut are flatfish belonging to the Pleuronectidae family. They are demersal fish, which means they live between 50 and 2000 m deep on or near sand, gravel, or clay bottoms. The halibut is in danger of extinction, because its slow growth rate and overfishing have made it an endangered species despite being one of the largest teleost fish in the world. Different bacterial species has been reported from the gut of Atlantic halibut, which includes *Photobacterium phosphoreum* and Pseudomonas spp., (Hovda et al. 2007; Verner-Jeffreys et al. 2003) Cytophaga, Flexibacter, Flavobacterium group, Vibrio, Aeromonas group (Bergh et al. 1994).

21.3.4 Atlantic Mackerel (*Scomber scombrus*)

The Atlantic mackerel (*Scombrus scombrus*), also known as the Boston mackerel, Norwegian mackerel, Scottish mackerel, or simply mackerel, is a species of mackerel found in the temperate waters of the Mediterranean Sea, Black Sea, and northern Atlantic Ocean, where it is extremely common and occurs in massive shoals in the epipelagic zone down to about 200 m. (660 ft). The body of the Atlantic mackerel is elongate, steel-blue with wavy black lines dorsally and silvery-white ventrally, and its snout is long and pointed. Svanevik and Lunestad (2011) reported that the gut of Atlantic mackerel is hub of different bacterial species like Psychrobacter, Proteus, Photobacterium, Vibrio, Shewanella, Synechococcus, Oceanisphaerae, Bizonia, Pseudoalteromonas, Flavobacteriaceae, Vagococcus, *Bacillus, Mycobacterium, Staphylococcus, Mycoplasma,* and *Clostridia.*

21.3.5 Atlantic Salmon (*Salmo salar*)

The Atlantic salmon, commonly called the "King of Fish," is an anadromous species, which means it can survive in both saltwater and freshwater environments. The body of an Atlantic salmon is elongated and spindle shaped, meaning that it is spherical, broadest in the middle, and tapers at both ends. Salmon species almost often have a morphology that is somewhat flattened toward the sides. They live between 50 m and 100 m depth and sometimes deeper. The gut microbiota that was explored by the people includes Mycobacteriaceae, Cryptosporangiaceae, Microbacteriaceae, and Planctomycetaceae (Skrodenytė-Arbačiauskienė et al. 2021) and Mycoplasmataceae, Proteobacteria, Firmicutes, Bacteriodetes and Actinobacteria, Tenericutes (Llewellyn et al. 2017).

21.3.6 Atlantic Striped Bass (*Morone saxatilis*)

Atlantic striped bass are anadromous, have thick bodies with seven to eight stripes that run horizontally from their gills to their tails. The general life expectancy is 30 years. Striped bass lives along the Atlantic coast of North America from the St. Lawrence River to Louisiana, where the Gulf of Mexico meets the Atlantic Ocean. A recent study reported by Fowler et al. (2021) describes the distribution of different bacterial communities in the gut of Atlantic striped bass. Most bacterial species include Peptostreptococcaceae, *Lactococcus lactis, Plesiomonas shigelloides,* or *Ralstonia pickettii.*

21.3.7 Baltic Herring (*Clupea harengus*)

One of the fish species with the greatest global abundance is this one. Both sides of the Atlantic Ocean have Atlantic herrings. The body of an Atlantic herring is fusiform. Incoming water is filtered by gill rakers in their lips, which also catch any phytoplankton and zooplankton. Proteobacteria, Firmicutes, Actinobacteria, Shewanella, Pseudomonas, Aeromonas, Shewanella, Psychrobacter, and Epsilonbacteraeota are some of the bacterial communities found in Baltic herring (Huotari et al. 2022).

21.3.8 Black Rock Cod Fish (*Notothenia coriiceps*) and Blackfin Icefish (*Chaenocephalus aceratu*)

Black rock cod fish (*Notothenia coriiceps*) live in less than 200 m depth. Scales are usually brown or grey in color. It has a tooth plate with several rows of teeth and canine-shaped teeth on the outside of its jaw. Blackfin icefish (*Chaenocephalus aceratus*), which lives in the coldest marine habitat on Earth, is home to Antarctic icefishes. These extraordinary creatures are the only vertebrates with nonfunctional hemoglobin genes and nonfunctional red blood cells; they are white blooded (Kim et al. 2019). To better understand the microbiome of *Notothenia coriiceps* and *Chaenocephalus a*ceratus, which have different pelagic distributions and eating habits, Ward et al. (2009) investigated bacterial 16S rRNA gene sequences from their digestive tracts and found that *Photobacterium* and *Vibrio* species are predominant.

21.3.9 Bluefish (*Pomatomus saltatrix*)

Bluefish have a greenish blue upper body, silvery sides, and a white belly. At the base of each pectoral fin is a single dark spot. The bluefish is the only living member of the Pomatomidae family. It is a marine pelagic fish that may be found all over the world in areas that are either temperate or subtropical, apart from the northern part of the Pacific Ocean. Newman et al. (1972) reported the incidence of *Vibrio, Pseudomonas*, Enterobacteriaceae, and *Achromobacter* in the gut of bluefish.

21.3.10 Brown Trout (*Salmo trutta*)

Salmo trutta, more commonly known as the brown trout, is a member of the salmonidae family that is widespread and popular. It derives its name from the yellow-orange-olive-brown coloring on its sides. Brown trout thrive in cold streams with plenty of cover and a gravel or cobble substrate. Brown trout originated from Europe and Asia. The gut microbiota of brown trout is diversified, which includes Firmicutes, Planctomycetaceae, Enterobacteriaceae, Gammaproteobacteria (Giang et al. 2018) and Proteobacteria, Fusobacteria, Firmicutes, and Bacteroidetes (Michl et al. 2019).

21.3.11 Cardinalfishe (Apogonidae) and Damselfish (Pomacentridae)

The Pomacentridae (damselfish) and Apogonidae (cardinalfish) families are two of the most common fish families found on coral reefs. Both families go through a pelagic larvae phase before settling on the reef, where adults play important roles in benthic habitat structuring and trophic interactions. Whole gut microbiomes from ten species of damselfish and two species of cardinalfish were analyzed and found to be Endozoicomonaceae, Shewanellaceae, and Fusobacteriaceae, Vibrionaceae and Pasteurellaceae (Parris et al. 2016).

21.3.12 *Chlorophthalmus albatrossis, Helicolenus hilgendorfi*, and *Glossanodon semifasciatus*

Fish of three different deep-sea species (*Chlorophthalmus albatrossis*, *Glossanodon semifasciatus*, and *Helicolenus hilgendorfi*) were hauled in from a trawl net at a depth of about 300 m in Suruga Bay, Japan. Proteobacteria dominated the gut microbiota of *C. albatrossis* and *H. hilgendorfi*, with relative abundances ranging from 66.4% to 98.7%. Photobacterium (Proteobacteria) was the most common genus, followed by *Vibrio, Enterovibrio, Aliivibrio* (Proteobacteria), and *Clostridium* (Firmicutes). The phyla Spirochaetes (genus Brevinema) and Tenericutes predominated in the gut microbiota of *G. semifasciatus* (Iwatsuki et al. 2021).

21.3.13 European Bass (*Dicentrarchus labrax*)

The European bass (*Dicentrarchus labrax*) is an ocean-going fish that is native to the waters off Europe's western and southern coasts, as well as Africa's northern coast. Found in the littoral zone on a variety of bottoms in estuaries, lagoons, and rivers. In the summer, they enter coastal waters and river mouths, but in colder weather, they migrate offshore and spend the winter in deep water. Recent studies found that European bass gut is diversified with bacterial communities like Bacillaceae, Clostridiaceae (Serra et al. 2021), aecalibacterium, Filifactor, Butyricicoccus, and Erysipelotrichaceae (Liu et al. 2022), and Bacillaceae, Enterococcaceae, and Lachnospiraceae, Actinomycetaceae (Rangel et al. 2022).

21.3.14 Gilthead Seabream (*Sparus aurata*)

Found in sandy bottoms and seagrass beds as well as in the surf zone frequently to depths of around 30 m, but adults can be found as deep as 150 m. A fish that lives in sedentary environments, either alone or in small groups. In the spring, you can frequently see them in the estuaries and lagoons that have brackish water. Mostly carnivorous, with some herbivory as an accessory diet. Different studies have been conducted on Gilthead Seabream to found out the gut microbiota at different time periods. Few studies identified the bacteria at phylum level includes Proteobacteria, Firmicutes, Cyanobacteria, Actinobacteria (Cerezo-Ortega et al. 2021) and some at family level like Bacillaceae, Bacillales Family XII. Incertae Sedis, Comamonadaceae, Enterobacteriaceae, Enterococcaceae, Erwiniaceae, Micrococcaceae, Pseudomonadaceae, and Staphylococcaceae families. Enterobacter spp. (Salgueiro et al. 2020) and few at genus level *Vibrio* and *Pseudomonas* (Rabelo-Ruiz et al. 2022), *Actinobacillus, Streptococcus, Massilia, Paracoccus*, and *Pseudomonas* (Califano et al. 2017).

21.3.15 Grass Puffer (*Takifugu niphobles*)

Grass puffer *(Takifugu niphobles*) is a marine fish species that frequently migrate to the rivers. The grass puffer, *Takifugu niphobles*, has an unusual pattern of behavior when it spawns. During the spring tide, large groups of fish congregate on coastal spawning grounds several hours before high tide (Motohashi et al. 2010). The Grass puffer gut microbiome consists of bacterial communities like *Vibrio*, Cyanobacteria (Li et al. 2020) and *Actinobacteria, Bacilli, Clostridia, Gammaproteobacteria, Mollicutes,*and *Spirochaetes* (Shiina et al. 2006).

21.3.16 Gulf Pipefishe (*Syngnathus scovelli*)

The Gulf Pipefish is a bony fish that is long and slender. Its primary habitat is the Gulf of Mexico, but it can also be found in certain places of South America. Proteobacteria is widely distributed bacteria in the gut of Gulf Pipefish (Ransom 2008).

21.3.17 Long-Jawed Mudsucker (*Gillichthys mirabilis*)

The tidal flats, bays, and coastal sloughs are home to this species. Prefers a seabed consisting of muck in quite shallow water. Facultative air-breather, known for its extraordinarily wide mouth and the capacity to remain alive for brief periods of time apart from water. Fish were gathered from five different locations across northern and southern California, in the United States. Mycoplasmas predominated in the hindguts of the animals (Bano et al. 2007).

21.3.18 Mariana Trench (*Pseudoliparis swirei*) and Kermadec Trench (*Notoliparis kermadecensis*)

Hadal snailfishes, found in trenches at depths of 6000 to 8000 m, are the deepest-dwelling fishes on Earth. While researchers have begun characterizing the microbial communities of trench habitats, little is known about the microorganisms associated with hadal megafauna. Here, using 16S rRNA gene amplicon sequencing, we characterize the gut microbiomes of two hadal snailfishes: *Pseudoliparis swirei* and Notoliparis kermadecensis and found that Mycoplasmataceae and Desulfovibrionaceae are present in their gut (Blanton et al. 2022).

21.3.19 Mugil cephalus

The *Mugil cephalus* is found in the coastal waters of the subtropical and tropical oceans. Including the Gulf of Mexico, striped mullet can be found throughout the western Atlantic Ocean all the way from Nova Scotia, Canada, to Brazil. *Mugil cephalus* inhabit warm, salty, or fresh waters between 8 and 24 °C. The striped mullet's body is subcylindrical and compressed in the front. Their noses are broad, and their small, terminal mouths have few, barely visible teeth. The lower lip has a slight hump at the tip, and the lips are thin overall. The fat eyelid is very noticeable, leaving just a little slit above the pupil. The head is more flattened than it is tall, and the body is long and lean. The gut microbiome is dominated by Proteobacteria, Firmicutes, and Actinobacteria (Le and Wang 2020).

21.3.20 Orange-Spotted Grouper (*Epinephelus coioides*)

The orange-spotted grouper, scientifically known as Epinephelus coioides, is a highly edible fish species. The head of the orange-spotted grouper is either flat or slightly convex between the eyes. Its body is long and elongated. The orange-spotted grouper can be found across the Indo-Pacific region. The unfortunate reality is that orange-spotted groupers have been fished and exploited to an unsustainable level. The gut microbiome of Orange-spotted grouper consists of Proteobacteria, Vibrionaceae as reported by Sun et al. (2022) and Proteobacteria, *Firmicutes, Exiguobacterium, Acinetobacter, Propionibacterium, Marivita*, unidentified-Holosporacaea, *Idiomarinas, Halomonas, Caldisericum*, and *Nitrosomonas* by Xiao Joe et al. (2019).

21.3.21 Parrot Fish (*Chlorurus sordidus*)

One of the most common parrotfishes, but it has a lot of different forms, some of which are probably subspecies. Live in coral-rich and open pavement areas of shallow reef flats, lagoon and seaward reefs, and drop-offs, where they act differently in different places. Males in their final phase are green with pinkish to purple scale edges, a pale yellowish to pinkish cheek, a bluish to pale purple snout, and a pale green caudal peduncle. In their initial phase, females are pale reddish-brown on the head and front of the body and get darker as they move back. They have 3–4 vertical pairs of small white spots and often a large white area on the caudal peduncle that surrounds a large black spot (Bray 2020). The bacterial communities Vibrio, Photobacterium (Smriga et al. 2010) and Gammaproteobacteria, Fusobacteria (Roeselers et al. 2011) are found to be the prominent gut microbiota of parrot fish.

21.3.22 Pinfish (*Lagodon rhomboides*)

The saltwater fish genus Lagodon is part of the bream and porgies family, Sparidae. Only one species, Lagodon rhomboides (pinfish), exists in this genus. Its preferred habitats are the warm, shallow coastal waters of the subtropical Atlantic and Gulf of Mexico. Young pinfish are more likely to be found in areas with some shelter, such as seagrass beds, rocky bottoms, jetties, pilings, and mangroves. The gut microbiome of pinfish in diversified with Proteobacteria, Firmicutes, Actinobacteria, Aeribacillus (Larsen et al. 2013), Clostridium, Mycoplasma (Ransom 2008) and Photobacterium, Propionibacterium, Staphylococcus, Pseudomonas, Corynebacterium (Givens et al. 2015).

21.3.23 Red Drum (*Sciaenops ocellatus*)

Red drum is tall, slender, near-shore fish ranges in color from dark copper to silver, and it has an obvious eye spot on its tail. Because of its adaptability to a wide range of environmental conditions, it can be found anywhere from surf zones and seagrass meadows to estuaries and river mouths. The swim bladder and abdominal muscles let it to generate a drumming or croaking sound, hence the name. The red drum, a euryhaline species, can be found in the waters from Cape Cod, Massachusetts, to Tuxpan, Mexico. Cetobacterium is the most dominant bacterial population compared to Proteobacteria, Vibrio in *Sciaenops ocellatus* (Ofek et al. 2021).

21.3.24 Red Snapper (*Lutjanus campechanus*)

Lutjanus campechanus, often known as the northern red snapper, is a ray-finned fish native to maritime environments. Its natural habitats include reefs, which are found in the western Atlantic, the Caribbean, and the Gulf of Mexico, where it was first discovered. The northern red snapper has a sloping profile, medium-to-large scales, a spiny dorsal fin, and a laterally compressed body. Different studies conducted on gut microbiome of *Lutjanus campechanus* found that *Vibrio, Pseudomonas, Photobacterium, Pseudoalteromonas*, Gammaproteobacteria (Tarnecki et al. 2016) and Proteobacteria, Firmicutes and the Actinobacteria, *Vibrio* spp. and *Photobacterium* spp. (Arias et al. 2013) are predominant bacterial species.

21.3.25 Sardines (*Sardinella longiceps*)

The Indian oil sardine, or *Sardinella longiceps*, is a ray-finned fish species. The Indian oil sardine is found only in the northern parts of the Indian Ocean and is one of the more restricted *Sardinella* species. Plankton and zooplankton make up the bulk of these fishes' diets. In fact, the bodies of these *Sardinella* are so stretched out that they hardly resemble cylinders. They have a moderately round stomach and eight rays on their pelvic fins. The gut microbiota of *Sardinella longiceps* composes bacterial communities like Proteobacteria, *Photobacterium*, *Vibrio*, and *Shewanella* spp. (Johny et al. 2021).

21.3.26 Pacific Chub Mackerel (*Scomber japonicus*)

The chub mackerel, or *Scomber japonicus*, is one of the most important fish for trade in the East China Sea countries. It is a Pelagic species lives, near the coast, and epipelagic to mesopelagic species on the continental slope to a lesser extent. The chub mackerel has a swim bladder that is well developed and connected to its esophagus. The gut microbiota of *Scomber japonicus* majorly constitutes bacterial species *Photobacterium damselae* (Minich et al. 2020).

21.3.27 Siberian Sturgeon (*Acipenser baerii*)

The Siberian sturgeon (*Acipenser baerii*) belongs to the family Acipenseridae and is one of the most valuable species for aquaculture, because it gives us caviar and high-quality meat. It is found most often in the big river basins in Siberia. Found in both deep and shallow parts of rivers, usually at depths of 1 to 8 m with moderate to fast current. The predominant bacterial colonies, invading in the gut of *Acipenser baerii*, include Proteobacteria, Firmicutes, and Fusobacteria (Xu et al. 2019; Geraylou et al. 2013).

21.3.28 Southern Flounder (*Paralichthys lethostigma*)

The southern flounder, or *Paralichthys lethostigma*, is a large-toothed flounder endemic to the waters of the eastern United States and the northern Gulf of Mexico. A stealthy species that can survive in fresh water or brackish water, as it does so regularly in estuaries and bays. The adult population predominates over muddy bottoms in estuaries and coastal seas up to around 40 m deep. More than 25 species of flatfishes call the coastal waters of Texas home, with the southern flounder being the largest. Because the left side is pigmented and considered the "upside," this flounder is referred to as a "left-eyed flounder." Givens et al. (2015) found that Photobacterium and Clostridiaceae are the major constituents of *Southern flounder* gut microbiome.

21.3.29 Speckled Trout (*Cynoscion nebulosus*)

These fish are not trout at all but rather belong to the family of drum fish, so called because of the croaking, drumming sounds they may make. Spotted seatrout are distinguished by their elongated, silvery bodies that are covered in irregular black patches over the upper half. They like sandy and grassy sea floors but can be found in brackish estuaries and even freshwater rivers when the weather gets chilly. As a demersal species, the spotted seatrout can be found in both saltwater and freshwater environments. Over sandy bottoms and seagrass at depths of 33 feet, it has been spotted in shallow coastal and estuarine areas. Proteobacteria, Firmicutes, Actinobacteria, and Aeribacillus are natural inhabitants of Speckled trout (Larsen et al. 2013).

21.3.30 Striped Eel Catfish *(Plotosus lineatus)*

Plotosus lineatus, often known as the striped eel catfish, is a type of eeltail catfish found in the genus Plotosus and family Plotosidae. The second dorsal, caudal, and anal fins of this species are fused together like those of eels, making them the most distinctive aspect of this animal. The rest of its body is quite similar to that of a freshwater catfish; for example, it has four sets of barbels, two on each side of its mouth. Each of the pectoral fins and the first dorsal fin are tipped with a poisonous spine. The common bacterial species found in the gut *of Plotosus lineatus* are *Serratia* sp. and *Aeromonas salmonicida* (Jammal et al. 2017).

21.3.31 Surgeon Fish (*Acanthurus nigricans*)

Reefs from the central Indo-Pacific to the eastern Pacific coast are home to the whitecheek surgeonfish, sometimes known as the goldenrim surgeonfish or yellow-spotted surgeonfish. The adult lives in the lower surge zone to at least 67 m, feeding on filamentous algae in hard substrate parts of clear lagoon and seaward reefs. The metagenomic analysis of gut microbiota found that Proteobacteria, Bacteroidetes, and Firmicutes are predominant in gut of *Acanthurus nigricans* (Smriga et al. 2010).

21.3.32 Turbot (*Scophthalmus maximus*)

Scophthalmus maximus, or turbot, is an economically valuable flatfish that has attracted a lot of interest in fishing and farming from the Arctic Circle to the north-east Atlantic. It is also prevalent in the Baltic and the northern Mediterranean, including the Marmara and the Black Sea. Adults like brackish environments and can be found on sandy, rocky, or mixed bottoms. The turbot gastrointestinal (GI) microbiome was predominated by proteobacteria and firmicutes, which were present in both GI content and mucus. The metagenomic dataset of the adult turbot GI tract was dominated by proteobacteria and firmicutes, with the remaining species mostly consisting of tenericutes, bacteroidetes, actinobacteria, cyanobacteria, ascomycota, and fusobacteria. Most common bacterial orders included Vibrionales, Alteromonadales, and Enterobacteriales (Xing et al. 2013). A study reported by Zhang et al. (2020) found the variation of gut microbiome *Lactococcus piscium, Pseudomonas brenneri, Carnobacterium maltaromaticum, Streptococcus agalactiae, Pseudomonas fragi and Lactococcus piscium, Solibacillus isronensis, Bacillus cereus, Lactococcus lactis,* and *Arthrobacter psychrolactophilus* raised in different farms.

21.3.33 Zebraperch (*Hermosilla azurea*)

Zebraperch is a kind of marine ray-finned fish that lives in shallow inshore environments up to 8 m deep, in stony reefs along the coast, and reef flats with algae development. Herbivorous in nature and is indigenous to the shores of the Pacific Ocean to the east in North America. The bacterial species like *Enterovibrio* spp., Bacteroides, *Faecalibacterium*, and *Desulfovibrio* are common inhabitants (Fidopiastis et al. 2006).

21.4 Unexplored Marine Fish Species

The marine fish microbiome of many fish is still not explored, though they are easily accessible, edible, and ornamental in nature (Table 21.1).

21.5 Future Perspectives

21.5.1 Microbiome in Adopting to Different Environments

The hypothesis that the microbiome might play a role in ecological adaptation has recently been backed up by several studies. This can become apparent because of a variety of processes, such as an association with novel bacterial taxa, differences in the functional capabilities of associated microbial strains, or the acquisition of novel genes from the surrounding environment through the mechanisms of horizontal gene transfer (Voolstra and Ziegler 2020).

21.5.2 Microbiome Extends Host Evolutionary Potential

The microbiome shapes many host traits, yet the biology of microbiomes challenges traditional evolutionary models. In his book “Extended Phenotype,” Richard Dawkins acknowledged the ways in which organisms alter the habitats and ecological systems around them. An organism’s phenotypic impacts can be extended

Table 21.1 Unexplored marine fish

S. no.	Marine fish species
1	*Acanthurus mata*
2	*Alepes djedaba*
3	*Argyrosomus regius*
4	*Ariosoma albimaculata*
5	*Atule mate*
6	*Centrolabrus exoletus*
7	*Cephalopholis formosa*
8	*Diplodus puntazzo*
9	*Dussumieria acuta*
10	*Dussumieria modakandai*
11	*Gerres erythrourus*
12	*Gerres filamentosus*
13	*Lutjanus Lutjanus*
14	*Melanogrammus aeglefinus*
15	*Molva molva*
16	*Monodactylus kottelati*
17	*Pagrus pagrus*
18	*Pellona ditchela*
19	*Pollachius pollachius*
20	*Pollachius virens*
21	*Pomadasys maculates*
22	*Psettodes erumei*
23	*Rastrelliger kanagurta*
24	*Sardinella albella*
25	*Sardinella fimbriata*
26	*Secutor insidiator*
27	*Secutor ruconius*
28	*Siganus argenteus*
29	*Sillago sihama*
30	*Sphyraena obtusata*
31	*Sphyraena putnamae*
32	*Squalus acanthias*
33	*Stolephorus indicus*
34	*Symphodus melops*
35	*Synaptura commersonii*
36	*Synodus dermatogenys*
37	*Terapon jarbua*
38	*Terapon puta*
39	*Trachinocephalus myops*
40	*Trichiurus lepturus*
41	*Tripterodon orbis*
42	*Tylosurus crocodilus crocodiles*
43	*Upeneus vittatus*

beyond the boundaries of its own genome when the environment in which it lives is modified, which suggests that evolution is influenced by the interactions between ecological groups. This hypothesis, which was created for free-living ecosystems, can also be applied to the interactions between hosts and their microbiomes. Because the host incorporates the extended effects of the microbiome into its phenotype, the microbiome, with its consortium of genomes, extends the genetic repertoire of the host to form what is now being referred to by some as the "Extended Genotype." This occurs because the microbiome forms what is known as the "Extended Genotype." This expanded genetic repertoire may influence the way host phenotypes are distributed among a population and, as a consequence, may have an effect on the host's capacity for evolutionary change (Henry et al. 2021).

21.5.3 Bioindicators of Environmental Pollution

Microorganisms are frequently used within marine and coastal habitats for the purpose of testing ecosystem pollution. This is because due to their rapid growth, microorganisms always respond even when exposed to low pollutant rates and exhibit essential signs of changes in the ecosystem. Because they are so common in a wide variety of ecosystems, analyzing them for the presence of pollutants is simple and does not need much effort. Pollutants in the environment are becoming significant and decisive contributors to the formation of an individual's gastrointestinal microbiotype.

The microbiome of Atlantic cod in the gut is changed by exposure to crude oil, even at sublethal concentrations. Despite the small sample size, we were able to identify 8 OTUs that were present in either lower or higher relative abundances in the low- and high-exposure groups. In particular, the relative abundance of OTUs belonging to the families Porphyromonadaceae, Rikenella, Ruminococcaceae, Alistipes, and Clostridiales declined. It was determined that Deferribacterales was the only OTU whose relative abundance increased with increasing oil exposure. This suggests that Deferribacterales could serve as a valuable microbial biomarker of oil exposure in Atlantic cod (Bagi et al. 2018).

The intestinal microbiota plays a dominant role in both the methylation and demethylation of mercury, and the biotransformation of mercury in the digestive tract has the potential to have a significant impact on the overall accumulation and distribution of mercury in fish bodies. The significance of intestinal microbiota in the interaction between mercury and fish, as well as the possibility that mild modification of the gut microbiome could be a way to reduce mercury contamination in fish (Yang et al. 2021).

21.5.4 Marine Fish Microbiome as Probiotics

In their study on Atlantic salmon, Chen et al. (2015) discovered that certain bacterial species, such as *Vibrio alginolyticus*, have the ability to act as probiotics and protect the fish from the harmful effects of other bacterial species, such as viz. *Aeromonas salmonicida, Vibrio anguillarum,* and *Vibrio ordalii.* According to the findings of Merrifield et al. (2010), there is a significant opportunity for the creation of probiotics from the presence of such a huge number of endogenous lactic acid bacterial strains.

21.5.5 Antibiotic Resistance Study in Microbes Isolated from Marine Fishes

When compared to microorganisms obtained from freshwater fish, marine fish microorganisms were more resistant to antibiotics. Isolates of *Salmonella* spp. from the ocean were resistant to six different antibiotics, while those from freshwater fish were only resistant to three. Isolates of *Shigella* that were obtained from marine fish showed resistance to the antibiotics tetracycline, gentamicin, and ciprofloxacin (Marijani 2022). The spread of bacteria resistant to antibiotics is an increasing problem, and it is not limited to hospitals or laboratories; for example, marine organisms can be reservoirs for antibiotic-resistance genetic determinants if they host antibiotic-resistant bacteria. High levels of erythromycin and tetracycline resistance were observed in the enterococci strains isolated from Gilthead seabream (Barros et al. 2011).

21.5.6 Microbe-Driven Marine Carbon Cycling Research

The Aquatic Microbiology Section has contributed significantly to the advancement of this subject through studies of marine primary production, organic matter biodegradation and biotransformation, and microbial responses to natural and manmade environmental gradients and stressors.

21.5.7 Gut Bacteria Are Essential for Development of Social Behavior in Fish

Microglia are needed for pruning of neural connections in zebrafish larvae, and a normal pruning process and social behavior in fish are both dependent on a healthy microbiota. The results also show that there is a crucial period of development during the first week of a larva's life, when the microbiota causes microglia to move to the forebrain, where they cut neural connections (Bruckner et al. 2022).

21.6 Conclusion

From this study, it was concluded that marine fish microbiome paly vital role in different aspects of environment. Only a part of marine fish microbiome was explored till date and much more research is needed to better understand Microbiome role in adopting to different environments, development of social behavior in fish, Carbon Cycling Research, as probiotics, multidrug resistance, bioindicators of Environmental Pollution, and in host evolutionary processes.

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