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Reservoir Ecotoxicology

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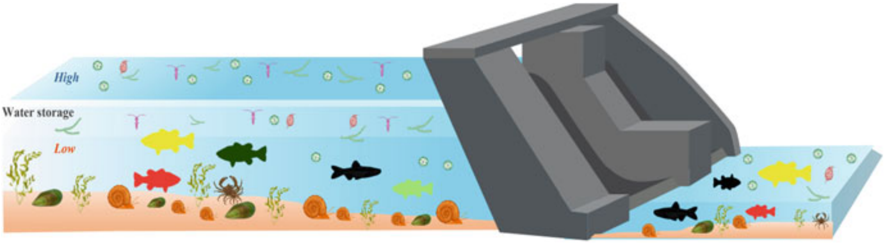

Pictorial Illustration of the reservoir ecotoxicology

De-Sheng Pei • Naima Hamid • Marriya Sultan •
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This book is in memory of my parents (Mr. Chao Pei and Ms. Jinhua Wang), who passed away on October 22, 2020 and February 14, 2014, respectively.
—De-Sheng Pei

I write this book in loving memory of my beloved parents, Mr. Hamid Javeed Awan, Ms. Shasita Hamid, and husband, Mohsin Khubaib Ahmed.
—Naima Hamid

I would like to dedicate this book to my beloved parents, Mr. Muhammad Sultan, Ms. Tahira Sultan, and my siblings, Iqra Sultan, Tayyab Sultan, and Abdul Basit Sultan.
—Marriya Sultan

I would like to dedicate this book to my child S. Krishiv and the support of my family, which helped me for contributing to the book.
—Suman Thodhal Yoganandham

Foreword

This book, written by De-Sheng Pei, Naima Hamid, Marriya Sultan, and Suman Thodhal Yoganandham, introduces a modern scientific concept of reservoir ecotoxicology, which plays a vital role in water resource management. The reservoir as a specific geographic area is important in irrigation, hydroelectric power, flood control, and shipping. Reservoir ecotoxicology has noticeable and different features, compared to other types of ecotoxicology. The chapters on the reservoir's environmental characteristics, study methods for identifying pollutants, and its ecotoxicological effects on aquatic species demonstrated that national and international collaborative effort is required to resolve the problems. Furthermore, it is of great concern that, in many countries, the majority of reservoir water is also used for drinking purposes. Moreover, reservoirs protect native flora and fauna at the population, community, and ecosystem levels, ensuring the ecosystem's integrity and resilience.

Therefore, I believe this book will be a valuable resource for students, scientists, policymakers, and regulatory authorities working to protect precious freshwater resources.

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Jun Xia

Preface

Reservoir construction is the key strategy for water resource management. In recent years, the migration of pollutants and their transformation into the reservoir ecosystem has become a global problem. Intensified anthropogenic activities and unsustainable practices have amplified the pollutant levels in the reservoir. Therefore, this book intends to highlight the environmental characteristics of the reservoir, study methods of ecotoxicology, and toxicological mechanism pathways of pollutants in the reservoir.

Introductory Chap. 1 proposes the term reservoir ecotoxicology and highlights the important role of reservoirs in irrigation, hydroelectric power, flood control, and shipping. Moreover, the reservoirs protect the native flora and fauna at the population, community, and ecosystem levels, and support ecosystem integrity and resilience. Chapter 2 describes the ecosystem characterization of the reservoir and understands the effect of various environmental stressors on the species diversity of various communities. Hydrologic changes, sedimentation flux, nutrient loading, and erosion are all major environmental stressors that affect water quality and disturb the species composition of flora and fauna in the reservoir.

Chapter 3 describes the major sources of pollution and the trend of pollution distribution in the Three Gorges Reservoir Area (TGRA), which serves as an example for understanding pollutant dynamics in the reservoir ecosystem. Heavy metals (HMs) were the most prevalent class of pollutants found in the TGRA from 2008 to 2016. Phthalate esters (PAEs) mainly DEHP showed higher health risks to the aquatic species. Moreover, intensified pyrogenic and petrogenic combustion are considered as the major sources of polycyclic aromatic hydrocarbons (PAHs). Similarly, agricultural activities, hospital waste, and industrial effluent increase the fingerprints of pharmaceutical and personal care products (PPCPs) in the TGRA.

Chapter 4 highlights the important factors involved in the migration and transformation of the pollutants. HMs were prevalent in the TGRA but found within the safety limits prescribed by the US EPA. The hydro-fluctuation (HFB) belt greatly affects the HMs migration from the inner layers of soil to the upper layers. Seasonal trends show a lower pollution load in summer, autumn, and spring than in winter due to the varied impoundment levels. Overall, the TGRA's ecological health has been relatively stable. Chapter 5 summarizes that excessive nutrients and algal growth degrade the reservoir water quality. The abundant release of phosphates and nitrates in the reservoir from natural and anthropogenic sources causes eutrophication and accelerates the process of algal blooms.

Chapter 6 highlights the increasing sources and abundance of microplastics (MPs) contamination in the reservoir ecosystem. Moreover, this chapter introduces different strategies and techniques used for MPs sampling, pre-treatment, and characterization. Chapter 7 describes the microorganism distribution in the TGRA. The authors review the microorganism species contribution before the dam construction. The ecological risk was assessed using the bacteria-based index of biotic integrity (Ba-IBI), which revealed that 25% of all sampling sites were Excellent, while 50% and 25% were Good and Fair, respectively.

Chapter 8 highlights the primary producers that play a vital role in the transfer of pollutants through food chain. Major primary producers including *Vibrio fischeri* (bacterium), *Raphidocelis subcapitata* (microalgae), *Spirodela polyrhiza* (floating macrophytes), and *Myriophyllum* (submerged macrophytes) are widely used as the ecotoxicity assessment species. Chapter 9 introduces various ecotoxicological methods using invertebrates for the assessment of the reservoir's water quality. The most commonly used standard species in ecotoxicity testing are Daphnids (*Daphnia magna*, *Daphnia pulex*, and *Ceriodaphnia dubia*). Previous studies also reported the use of crustaceans, such as amphipods, branchiopods, insect species, and rotifers. Furthermore, numerous acute and chronic toxicity tests that are widely used for toxicity screening in water bodies have been approved by international organizations.

Chapter 10 reviews the model fish species that are involved in ecotoxicological studies. Water quality and fish health are interrelated and can be used as the pollution indicator for monitoring reservoir pollution. Based on the contaminant type and toxicity endpoints, zebrafish, rainbow trout, Japanese medaka, and fathead minnow are the most recommended model fishes. Chapter 11 covers the topic of the ecotoxicology methodology of sediment toxicity in the reservoir. The reservoir sediment serves as a sink as well as the contaminant source. When the pollutant is released into the water column, it can be dangerous for biotic species. Moreover, aquatic species including algae, amphipods, and bivalves are commonly used for measuring aquatic toxicity.

Chapter 12 discusses the significance of mesocosm in the reservoir ecosystem. Moreover, mesocosm have broadly assessed the toxic effects of water-borne contaminants on various biological levels of organizations, as well as their impact on the overall ecosystem. Several studies have been conducted to assess the impact of contaminants, such as pesticides, microplastics, and persistent organic pollutants on macroinvertebrates and fish species with endpoints including species richness, diversity, and morphological & physicochemical parameters. Moreover, these systems mimic the natural environment to generate real-world exposure scenarios based on the study objectives, available cost, and time.

Chapter 13 presents information on the molecular toxicity mechanism of HMs in the reservoir. Heavy metal bioaccumulation may have toxic effects on various tissues and organs. Heavy metal exposure inhibits growth, proliferation, differentiation, and damage repair. The molecular pathways of heavy metal toxicity in the reservoir include reactive oxygen species (ROS) formation, enzyme inactivation, DNA damage, cell death, etc. Chap. 14 explains the intricate mechanistic toxicity pathway caused by persistent organic pollutants (POPs) exposure. POPs mainly disrupt the aryl hydrocarbon receptor (AhR) signaling pathway. Furthermore, when polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and organochlorinated pesticides (OCPs) were exposed to mice and zebrafish, they may induce neurodevelopmental disorders (NDD), oxidative stress, DNA damage, endocrine disruption, etc.

Chapter 15 discusses the molecular toxicity pathway caused by MPs. MPs in the reservoir can adsorb environmental pollutants, including heavy metals and organic pollutants, implying combined toxicity effects on organisms. MPs exposure may generate reactive oxygen species (ROS) and cause developmental and reproductive failure. Chapter 16 describes that plasticizers (PAEs) and bisphenol A (BPA) exposure can affect growth development by altering the thyroid and estrogen axis, leading to infertility. It also decreases egg production *via* reducing steroidogenesis, activating peroxisome proliferator-activated receptors (PPARs), and enhancing oxidative stress levels. Chapter 17 highlights toxicity effects caused by PPCPs exposure in the reservoir. These emerging chemicals may disturb the reproductive cycle and decrease sperm production. Particularly, sulfonamides (SAs) and tetracycline (TC) can affect the detoxification metabolism pathway. Even exposure to a low level of PPCP exhibits cytotoxicity and genetic toxicity with increased levels of apoptosis and DNA damage.

Chapter 18 describes the importance of the adverse outcome pathway (AOP) of the pollutants in the reservoir. The AOP framework aids to decipher molecular or biochemical data from field-sampled organisms exposed to a mixture of contaminants. The resulting endpoints are then used to infer the potential ecological risk. A more advanced AOP, known as quantitative adverse outcome pathway (qAOP), includes biology-based computational models delineating key event (KE) correlations and combining a molecular initiating event (MIE) with an adverse outcome (AO). Chapter 19 highlights the problem of invasive alien species caused in the reservoir. Many exotic plant and fish species made their way to the reservoir region after the reservoir construction. The majority of the invasive species were the result of factors such as impoundments, international trade via water, road access, and recreational activities, such as aquaculture. Chapter 20 provides final thoughts and concluding remarks of this book. It highlights reservoir ecotoxicology as an emerging academic discipline is a multidisciplinary study, including ecology, ecotoxicology, environmental science, environmental hygiene, reservoir management, water resources engineering, etc.

This book presents reservoir ecotoxicology as a modern scientific concept, and there are numerous serious ecotoxicological issues in the reservoir that must be addressed thoroughly. It also provides the recent advances in the theoretical background of legacy and emerging pollutants in the reservoir. Furthermore, detailed mechanistic toxicity trends via model species will help academic scientists and government officials develop background knowledge and formulate mitigation plans based on multidisciplinary research.

Chongqing, China De-Sheng Pei
Lahore, Pakistan Naima Hamid

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De-Sheng Pei is a professor at Chongqing Medical University, China. Dr. Pei got his Ph.D. degree from the Institute of Hydrobiology (IHB), Chinese Academy of Sciences (CAS) in 2006. From June 2006 to February 2009, Dr. Pei worked at IHB (CAS) as an assistant professor. From February 2009 to February 2011, as a senior postdoctoral research associate, Dr. Pei concentrated his study on the mechanisms of DNA damage and repair at Northeastern University (USA). Thereafter, Dr. Pei worked as a Research Fellow at Dana-Farber Cancer Institute/Harvard Medical School (USA) to study the mechanism of carcinogenesis of neuroblastoma from February 2011 to February 2013. In August 2012, Dr. Pei got the professor position at the Chongqing Institute of Green and Intelligent Technology (CIGIT), Chinese Academy of Sciences (CAS). From February 2012 to September 2021, he led the Research Center for Environment and Health (RCEH) at CIGIT. From September 2021 till now, he was introduced to Chongqing Medical University as a distinguished professor of excellent high-level talent. His discoveries have been published in more than 120 journals, including *Cancer Cell*, *Nucleic Acids Research*, *Brain*, *PLOS Genetics*, *Biomaterials*, *Journal of Hazardous Materials*, *Environmental Pollution*, *etc*.

Naima Hamid joined Lahore College for Women University as an assistant professor in November 2021. She completed doctoral studies at the University of Chinese Academy of Sciences (UCAS), China, which ranked 84th in the World University rankings for 2020–2021. She was awarded the prestigious Chinese Academy of Sciences (CAS)-The World Academy of Sciences (TWAS) presidential scholarship for three years (2017–2020). Her research work was focused on the combined toxicity of phthalates or pharmaceuticals and personal care products (PPCPs) with endocrine-disrupting perspectives. She published more than 25 international SCI articles, including *Journal of Hazardous Materials*, *Science of the Total Environment*, *Environmental Pollution*, *Aquatic toxicology*, *Chemosphere*, *etc*. She remains associated with the Ministry of Climate Change, Pakistan, and worked on the National Climate Change Policy. As a research scholar, she joined Pakistan Meteorological Department with the climate change and modeling research group. Her research interests are focused on ecotoxicology and ecological risk assessment of hazardous chemicals in environmental matrices. Presently, she is involved in the development of integrated experimental and computational monitoring methods of environmental pollutants with special reference to their toxicological mechanisms.

Marriya Sultan is currently enrolled in a Ph.D. program in environmental sciences at the Chongqing Institute of Green and Intelligent Technology, Chinese Academy of Sciences under a CAS-TWAS fellowship 2019. Previously, she obtained her M.Phil. degree in environmental sciences from Quaid-I-Azam University, Islamabad, Pakistan. Currently, she is working on a Ph. D. project, entitled "*Co-existing characteristics of microplastics and DEHP in the Three Gorges Reservoir Area and mechanism of compound reproductive toxicity on zebrafish model,*" under the supervision of Prof. & Dr. De-Sheng Pei. Her expertise includes the characterization of organic and inorganic environmental pollutants (exposure routes, dose–response, and mode of action), rigorous evaluations of contamination using novel techniques, and analysis of environmental pollutants data.

Suman Thodhal Yoganandham got his Ph.D. degree from Sathyabama University in 2017. From June 2014 to December 2018, Dr. S. Thodhal Yoganandham worked at the Center of Ocean Research in Sathyabama University as a senior scientific assistant. From December 2018 to March 2021, as a postdoctoral research fellow, Dr. S. Thodhal Yoganandham concentrated his study on the characterization of marine plastic debris and the effects of microplastics on aquatic organisms at Henan Normal University & Chongqing Institute of Green and Intelligent Technology, Chinese Academy of Sciences (China). He currently works as a postdoctoral research fellow for environment engineering at Changwon National University, South Korea. His research mainly focuses on micropollutants and their environmental impacts.

Abbreviations

ABS	Absorbance Spectrum
AFT	Acute Fish Toxicity
AgNPs	Silver Nanoparticles
AHRR	Aryl hydrocarbon receptor repressor
AhRs	Aryl hydrocarbon receptors
Al	Aluminum
AO	Adverse outcome
AOP	Adverse Outcome Pathway
AOX	Alternative oxidase
APEs	Alkylphenol ethoxylates
APHA	American Public Health Association
APs	Alkylphenols
As	Arsenic
ASD	Autism Spectrum Disorder
ASTM	American Society for Testing and Materials
ATR	Attenuated Total Reflectance
Ba-IBI	Bacteria-based Index of Biotic Integrity
BBP	Benzyl-butyl phthalate
BCF	Bioconcentration Factor
BCFK	Bioconcentration Factor Kinetics
BF	Bezafibrate
BPA	Bisphenol A
CAT	Catalase
CBZ	Carbamazepine
CCA	Canonical Correspondence Analysis
Cd	Cadmium
CFB	*Cytophaga Flavobacterium Bacteroides*
CI	Chemical index
CI	Combination index
Co	Cobalt
COD	Chemical Oxygen Demand

CP	*Chlorella pyrenoidosa*
Cr	Chromium
CYP1A, CYP11A1	Cytochrome P450 family
DBP	Di-butyl phthalate
DDT	Dichlorodiphenyltrichloroethane
DEGs	Differentially Expressed Genes
DEHP	Di-(2-ethylhexyl) phthalate
DEP	Di-ethyl phthalate
DIC	Diclofenac
DMP	Dimethyl phthalate
DNA	Deoxyribonucleic acid
DNOP	Di-n-octyl phthalate
DO	Dissolved Oxygen
EC_{50}	Half maximal effective concentration
ECCC	Environment and Climate Change Canada
EDCs	Endocrine-Disrupting Chemicals
EDS	Energy Dispersive Spectroscopy
EE	Ethinyl estradiol
EE2	Ethynylestradiol
ELISA	Enzyme-linked immunoassay
EPA	Environmental Protection Agency
ER	Estrogen Receptor
ERCs	Environment Relevant Concentrations
ERα	Estrogen Receptor alpha
Erβ	Estrogen Receptor beta
ESEM	Environmental Scanning Electron Microscopy
EU	European Union
F1	First generation
F2	Second generation
FA	Factor Analysis
FAO	Food and Agriculture Organization
Fe	Iron
FET	Fish Embryo Toxicity
FTIR	Fourier transform infrared
GI	Germination Index
GMO	Glycol monostearate
GMS	Glycerin monostearate
GO	Gene Ontology
GPx	Glutathione peroxidase
GST	Glutathione S-transferase
HA	Humic acid
HAB	Harmful algal bloom
HFB	Hydro-fluctuation Belt
Hg	Mercury

HM	Heavy Metals
HPF	Hours post fertilization
HPF	Hours per fertilization
HPG	Hypothalamus pituitary gonad
HPT	Hypothalamus pituitary thyroid
IA	Independent addition
Igeo	Geoaccumulation Index
IM	Indomethacin
KE	Key Events
KERs	Key Event Relationships
LC_{50}	Lethal Concentration that kills 50% population
LC-MS	Liquid chromatography mass spectroscopic analysis
LH	Luteinizing hormone
LOEC	Low Observed Effect Concentration
LPO	Lipid peroxidation
MAPK	Mitogen-activated protein kinase
MDA	Malondialdehyde
MDR	Molecular Diagnostic Ratios
MET	Methionine
MIE	Molecular Initiating Event
MLR	Multiple Linear Regression
Mn	Manganese
MO	Microorganisms
MPs	Microplastics
NDD	Neurodevelopmental Disorders
Ni	Nickel
NMR	Nuclear magnetic resonance spectroscopy
NOEC	No Observed Effect Concentration
NOEL	No Observed Effect Limit
NPs	Nanoparticles
NSAIDs	Non-steroidal anti-inflammatory drugs
OCPs	Organochlorine Pesticides
OECD	Organization for Economic Co-operation and Development
OM	Organic Matter
PA	Polyamide
PAEs	Phthalate Esters
PAHs	Polycyclic Aromatic Hydrocarbons
Pb	Lead
PBBs	Polybrominated Biphenyls
PBDEs	Polybrominated Diphenyl Ethers
PCA	Principle Component Analysis
PCBs	Polychlorinated Biphenyls
PCDFs	Polychlorinated Dibenzofurans
PCNs	Polychlorinated Naphthalenes

PCPs	Personal care products
PE	Polyethylene
PECs	Predicted Effect Concentrations
PET	Polyethylene Terephthalate
PFOS	Perfluorooctane Sulfonates
PKs	Pharmaco-kinetics
POPs	Persistent Organic Pollutants
PPARs	Peroxisome proliferator receptors
PPCPs	Pharmaceutical and Personal care products
PS	Polystyrene
PVA	Polyvinyl alcohol
PVC	Polyvinyl chloride
qAOP	Quantitative Adverse Outcome Pathway
RA	Redundancy Analysis
RNA	Ribonucleic acid
ROS	Reactive Oxygen Species
RXR	Retinoid X receptor
SAs	Sulfonamides
SDZ	Sulfadiazine
SEM	Scanning Electron Microscopy
SEPA	State Environmental Policy Act
SMR	Sulfamerazine
SMX	Sulfamethoxazole
SO	*S. obliquus*
SOD	Superoxide dismutase
SPY	Sulfapyridine
ST	Sulfameter
SULTs	Sulfotransferases
T_2	Testosterone
T_3	Triiodothyronine
T_4	Thyroxine
TBT	Tributyltin
TC	Tetracycline
TCDD	Tetrachlorodibenzo-p-dioxin
TGD	Three Gorges Dam
TGDR	Three Gorges Dam Region
TGR	Three Gorges Reservoir
TGRA	Three Gorges Reservoir Area
TH	Tetracycline hydrochloride
TH	Thyroid axis
TMP	Trimethoprim
TOC	Total Organic Carbon
TOX_{21}	Toxicity testing in the 21st century
TPs	Transmembrane proteins

TRH	Thyrotropin-releasing hormone
TSCA	Toxic Substances Control Act
TSH	Thyroid-stimulating hormone
UGTs	Glucuronosyltransferases
US EPA	United States Environmental Protection Agency
VTG	Vitellogenin
VTN	Vitellin
WLFZ	Water-Level Fluctuation Zone
XRE	Xenobiotic Response Element
Zn	Zinc

Part I
Introduction

Chapter 1
An Introduction to Reservoir Ecotoxicology

De-Sheng Pei

Abstract Reservoir ecotoxicology is a new term proposed by us for the description of ecology and toxicology in the reservoir, focusing on the effects of toxic substances and especially pollutants in the reservoir environment. As we know, reservoir construction is the key strategy for water resource production and management, such as irrigation, hydroelectric power, flood control, and shipping. Different types of reservoirs possess distinct pollution characteristics, and the toxicological impacts of contaminants on wildlife in the reservoirs deserve in-depth studies. Reservoir ecotoxicology aims to protect reservoir native flora and fauna at the population, community, and ecosystem levels and support their ecosystem integrity and resilience. This chapter provides an overview of reservoir ecotoxicology with an emphasis on topics and perspectives that are different from other fields of ecotoxicology.

Keywords Reservoir ecotoxicology · Dams · Ecosystem · Water resource · Environmental impacts

1 Definition of Reservoir Ecotoxicology

The term reservoir ecotoxicology is derived from ecotoxicology focusing on ecology and toxicology in the reservoir, which is a new term proposed as a modern scientific subdiscipline of environmental toxicology. Reservoirs as open-air storage areas of many water supply systems around the world possess their own environmental characteristics [1]. Thus, reservoir ecotoxicology has noticeable and different features, compared to other ecotoxicology. To well address reservoir ecotoxicology, we should clarify the environmental characteristics of reservoirs, including the source, distribution, and migration of pollutants in the reservoir. Moreover, we need to figure out the diversity of species composition (such as benthos, plankton, and nekton), food chain, and reservoir ecosystem in the hydro-fluctuation belt, backwater zone, reservoir bank, riparian zone, open water belt, and deep-water belt. Thus, reservoir ecotoxicology can be broadly defined as the scientific study of interactions among organisms including humans and their reservoir environment, typically focusing on protecting reservoir native flora and fauna at the population, community, and ecosystem levels. Besides, the ecotoxicological effect and molecular mechanisms

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of toxicity in the reservoir should be different because of the specific surrounding environment, compared to other water bodies [2].

2 Types of Reservoirs

Reservoirs are large open storage structures for collecting and storing water, and water bodies contained by embankments or a dam. Reservoirs play important roles in irrigation, flood control, hydroelectric power, water supply, and leisure activities [3, 4]. Reservoirs can be classified into different types according to construction purposes and dam location [5, 6]. Based on the terrain trait, reservoirs can be divided into four types: plain reservoirs [7], valley reservoirs [8], underground reservoirs [9], and coastal reservoirs [10]. According to use features, reservoirs are also classified as follows: storage reservoirs [11], flood control reservoirs [12], distribution reservoirs [13], and multipurpose reservoirs [14]. Based on their function, reservoirs are also classified into three types: bank-side reservoirs [15], valley-dammed reservoirs [16], and service reservoirs [17]. Thus, types of reservoirs may be named differently in terms of multiple classification criteria. Here, we summarized the major types of reservoirs based on different classifications (Table 1.1).

3 Distribution of Reservoirs in the World

Although there are many types of reservoirs, the first and usually largest type of reservoir is valley-dammed reservoirs. Indeed, around 30–40% of irrigation water was supplied by dammed reservoirs globally [18]. Hydropower generated 16.6% of the world's electricity by 2015 and 71% of all renewable electricity in 2016 [18, 19]. The Jawa Dam in Jordan is the world's oldest dam, which was built around 3000 BCE to store irrigation water [20]. Globally, 36,222 dams were identified and they are spatially concentrated along major river basins in Asia, North America, South America, and Europe (Fig. 1.1) [21]. As shown in Fig. 1.1, dammed reservoirs are mainly used for irrigation (34%) and hydroelectricity (25%). Although many developed countries in North America, Europe, and Oceania reduce the construction number of dammed reservoirs since the 1970s, developing countries in Africa, Asia, and South America accelerate the progress of the construction of dammed reservoirs. To date, Asia has the highest number (10,138) of dammed reservoirs completed and possesses 28% of worldwide dam construction. India's first dam is the Kallanai built on the Cauvery River. India alone has over 5200 dams for various purposes, among which Indira Sagar Dam Reservoir is the largest reservoir in India followed by Nagarjuna Sagar Reservoir.

Table 1.1 Classification of reservoirs in the world

Classification criteria	Types	Features	Subcategories	Examples
Terrain	Plain reservoirs	Shallow, low dams, and widespread water surfaces	River-type and dam-type	Qianmu Dang, Haiyan, Zhejiang, China
	Valley-dammed reservoirs	Located in narrow valley areas where tremendous amounts of water can be held in by the valley's sides and a dam	Large-scale, medium-sized, and small	Owen Falls Dam, Jinja, Uganda
	Underground reservoirs	Stored water in the gap between soil and rock or cave	Dam-type, non-dam-type, and funnel filling type	Mihuaishun, Beijing, China; Montsouris Reservoir, Paris, France
	Coastal reservoirs	A water body is enclosed by a barrier or barriers inside a large waterbody for specific purposes	Inner river, mouth, beyond river mouth, and beside river mouth	Punggol Reservoir, Singapore
Construction purpose	Storage reservoirs	To maintain minimum supplies of water for irrigation, hydroelectric generation, etc.	Large-scale, medium-sized, and small	Owen Falls Dam, Jinja, Uganda; Kariba's reservoir, Zambezi
	Flood control reservoirs	Temporarily store the flood water and release it slowly at a safe rate after the floods	Detention basins and retarding basins	Three Gorges Reservoir, China
	Distribution reservoirs	Small storage reservoir to distribute and manage water in a city	Surface reservoirs, elevated reservoirs, and standpipes	Location in water supply systems
	Multipurpose reservoirs	Protect the downstream areas from floods and provide irrigation, water supply, hydroelectric purpose, etc.	Irrigation, power, flood control, municipal and industrial, recreation, and fish and wildlife benefits	Bhakra Dam, Nangal, India; Nagarjuna Sagar Dam, Andhra Pradesh, India
Function	Bank-side reservoirs	Made by diverting water from rivers and streams to an existing reservoir	By excavation, encircling bund, and embankment	JhongJhuang Bank-Side Reservoir, Taiwan, China
	Valley-dammed reservoirs	Water is contained by the walls of a valley in mountain ranges	Large-scale, medium-sized, and small	Lake Mead Reservoir, Nevada, USA

(continued)

Table 1.1 (continued)

Classification criteria	Types	Features	Subcategories	Examples
	Service reservoirs	Reservoirs located above the ground or below the ground	Water towers and other elevated structures	Honor Oak Service Reservoir, London, UK

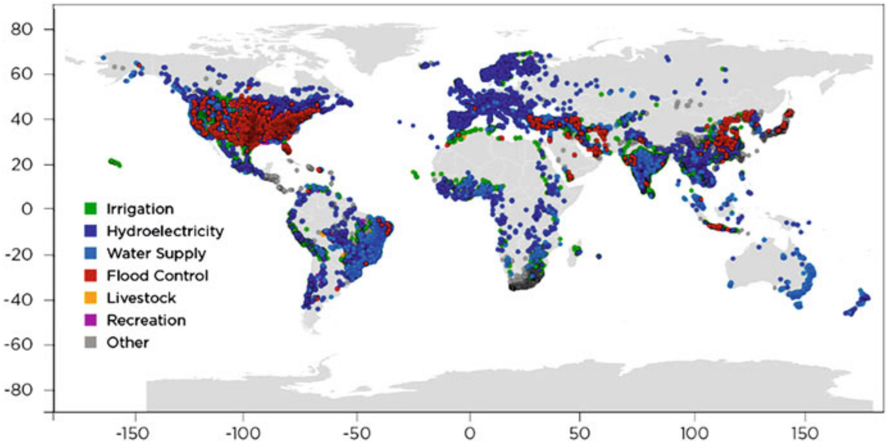

Fig. 1.1 The spatial location of world dams based on their functions. Data were retrieved from AQUASTAT, GRanD, WRI, and GDAT and modified by Zhang TB et al. [21]. Irrigation (Green), Hydroelectricity (Violet blue), Water Supply (Blue), Flood Control (Red), Livestock (Yellow), Recreation (Magenta), and Other (Gray)

4 Environmental Impacts of Reservoirs

Reservoirs were established for generating hydroelectric power, controlling floods, improving water quality for irrigation and drinking, and offering recreational opportunities. However, the construction of reservoirs potentially may impede the flow of essential nutrients, affect aquatic life, and release greenhouse gas [18, 22]. Due to anti-seasonal hydrological regulation for most flood control reservoirs, dynamic change in a water-level fluctuation zone (WLFZ) also raises critical environmental concerns in the riparian zone [23].

4.1 *Water Temperature Change*

As we know, water released from deep outlets below the surface of large dams can significantly disturb water temperature in the downstream river, consequently affecting aquatic biota and river health. A previous study showed that cold-water pollution occurred in the downstream reaches of 11 major Murray–Darling Basin (Australia) dams, where water temperature varied up to 11.1–16.7 °C [24, 25]. Although the adverse consequences of water temperature pollution on river health were widely recognized, little was done to correct this problem in Australia. Thus, species of native warm water fish may be extinct in cold-water pollution-affected rivers. The ecosystem downstream can be adversely affected, and reduced temperatures can be observed in the 250–350 km downstream [25]. Thermal suppression is considered by the operators of Burrendong Dam on the Macquarie River (Eastern Australia) via hanging a geotextile curtain on a new outlet turret to selectively release the surface water [26].

4.2 Reservoir Sedimentation Risk

Sedimentation is one of the most important threats to reservoir ecosystems around the world. Reservoir sedimentation is the filling of the reservoir behind a dam with sediment carried into the reservoir by streams. The water-storing capacity of the reservoir is automatically reduced after the deposition of sediment. When the process of sediment deposition lasts longer, the whole reservoir will be silted up and lose function [27, 28]. The accumulation of sediment limits the storage and lifespan of the reservoir [29], and the reservoir's life can be divided into three stages: continuous and rapidly occurring sediment accumulation, partial sediment balance, and full sediment balance. Aquatic organisms are sensitive to changes in sediment supply and flow regimes [30]. Moreover, reservoir sediment is a primary carrier of suspended pollutants including nitrogen, phosphorous, and heavy metals [31], which greatly affects the reservoir environment.

4.3 Greenhouse Gas Pollution and Climate Change

Dammed reservoirs are thought to be an important source of greenhouse gases (GHGs) in the atmosphere [32]. In the reservoir, carbon dioxide is emitted into the atmosphere through the deforestation process, contributing to 20% of the GHGs emissions [33]. GHGs emissions from reservoir water surfaces were estimated to account for 0.8 (0.5–1.2) Pg CO_2 equivalents per year, with the majority of this forcing due to CH_4 [34]. The terrestrial organic matter stocked in the reservoir may fuel microbial decomposition and convert organic matter to CO_2, CH_4, and N_2O [35]. The climate impact of CH_4 was up to 25 times greater than CO_2 on a 100-year scale [36]. Currently, atmospheric CH_4 concentrations in the last 650 ky are the highest. Moreover, the GHG footprint of reservoirs deserves to quantify based on whether they have generated new fluxes visible to the atmosphere [37]. Of note, China firstly announced "dual carbon goals: carbon peaking and carbon neutrality" at the 75th session of the United Nations General Assembly on September 22, 2020

[38]. China will aim to achieve peak CO_2 emissions before 2030 and carbon neutrality before 2060 [39], demonstrating China's determination in pursuing green and low-carbon development and its responsibility as a major and responsible country to actively tackle climate change and safeguard a bright future for humanity. Thus, reducing greenhouse gas emissions from reservoirs should be an important tache, contributing to realizing this goal.

4.4 Water Pollution

Water quality in reservoirs is deteriorating due to population growth, industrialization, and urbanization. Reservoirs have a more vulnerable and complex ecosystem than rivers because they do not have a weak self-purifying ability. Reservoirs as environmental sinks collect not only sediments but also most of the pollutants that are washed into them. Nutrients (nitrogen and phosphorus) are extracted from flooded plants and soil. Reservoir eutrophication may occur due to large influxes of organic loads and/or nutrients [18, 40]. Moreover, heavy metals have recently been identified as major pollutants in sediments of the reservoir, such as As, Cd, Cr, Cu, Ni, Pb, and Zn [41].

4.5 Effects of Water-Level Fluctuation Zone

Reservoir water-level fluctuation zone (WLFZ) is a new and fragile ecosystem and attracts high attention, which refers to the drawdown area formed between the highest and the lowest water level due to seasonal water-level drawdown and periodic water storage. In the Three Gorges Reservoir (China), the height of WLFZ is 30 m because of the discharge–storage cycle between 145 m and 175 m at the Three Gorges Dam. The WLFZ usually contains three belts: the littoral zone (wooded wetland, wet meadow, marsh, and aquatic vegetation), the riparian zone, and the riparian ecotone zone [42]. Due to the annually cyclic variation of the water level in the Three Gorges Reservoir, frequent alternation of erosion and deposition profoundly disturbs the geochemical and biological processes and affect the water quality and ecosystem of the reservoir. Consequently, nutrients (typically P and N) and heavy and trace metals may be gradually deposited in the WLFZ at the low-water level, but released into the water at the high-water level, potentially triggering eutrophication incidents or water pollution [43]. Besides, many original vegetation species failed to survive under prolonged submergence conditions during the inundation stage [44], resulting in the reduction of vegetation diversity in the reservoir. Therefore, to well understand the complex geomorphological, geochemical, and biological processes of reservoir WLFZ needs more in-depth studies.

5 Conclusion

The main aim of reservoir ecotoxicology is to protect the reservoir's native flora and fauna at the population, community, and ecosystem levels. There are different types of reservoirs in the world. Thus, we should figure out the environmental characteristics of reservoirs, including creature types, the composition of reservoir ecological systems, major pollutants in the reservoir, and their migration & conversion. The toxicological impact of contaminants on reservoir organisms should be considered at the population and community levels based on adverse outcome pathways (AOPs). Contaminants exposure is complicated and should reflect bioavailability and other factors. Acute and chronic exposure to combined pollutants, especially because of bioconcentration, bioaccumulation, and biomagnification, will be helpful to elucidate the mechanistic effects at the biochemical level and the biological pathways. Advanced research approaches should be introduced to assess reservoir environmental impacts from both prospective and retrospective views.

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Conflicts of Interest The author declares no conflict of interest.

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Part II
Environmental Characteristics in Reservoir Ecosystems

Chapter 2
Characterization of Reservoir Ecosystem

Marriya Sultan and De-Sheng Pei

Abstract This chapter intends to describe the ecosystem characterization of the reservoir and understands the effect of various environmental stressors on the species diversity of various communities. The ecological trait of reservoirs is subjective to their association with the watershed and functional dynamics utilities of the impounded water. Various temporal and spatial studies related to fish assemblage, microbial diversity, and the composition of macroinvertebrates & vegetation revealed that the ecosystem of the reservoir has been changed in terms of species richness and composition due to the stressors like hydrologic alterations, nutrient loading, sedimentation flux, and erosion, resulting in the modification of ecological characteristics. Moreover, anthropogenic activities have affected the water quality, which is also the major cause of changes in species composition of flora and fauna in the reservoir.

Keywords Ecosystem · Ecological characterization · Diversity · Species composition · Water quality

1 Introduction

Reservoir characterization is the quantitative description of reservoir information gathered from different sources. According to the report by Kelkar [1], reservoir characterization is the elucidation of reservoir properties that reliably incorporates data of different qualities and capacities. In the process of reservoir characterization, information composed at several levels needs to be combined into a single, ample, and coherent depiction of the reservoir [2].

Characterization of the reservoir ecosystem provides a precise description of the present ecological tenets of the site and assists in the identification of ecological resources of interest, such as critical habitats, wetlands, and endangered species that are affected by remedial actions. Moreover, it also aids to ascertain areas that require further sampling or monitoring and provides ecological information for exposure and toxicity assessments [3].

The ecological subtleties of the reservoirs are influenced by their association with the watershed, functional dynamics, and manifold utilities of the impounded water.

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Reservoirs pose various challenges for ecosystem management. Since different associations exist between different operational processes in the reservoir that impact the ecosystem directly or indirectly, the integration of ecologically changing aspects of the reservoirs with management is necessary. For this reason, integrated basin-scale analysis and a proper understanding of reservoir engineering and operational techniques are required [4].

Figure 2.1a explains the different components on the basis of which the ecology of the reservoir is characterized. It includes specific characteristics (location, existing resources, and environmental conditions geology of an area) and physical transport mechanisms, including hydrology, erosion, sedimentation, and the description of prevalent natural communities in habitats around the reservoir area. The services that the reservoir provides become diversified by the passage of time. Moreover, the water quality parameter is also vital and if it is well-upheld, then the overall degradation could be prevented [5]. Figure 2.1b represents the influence of various drivers, such as climate, geology, and hydrological alterations on ecosystem components, such as changes in the diversity of various natural communities.

2 Site-Specific Characters

2.1 Biophysical Setting

For example, the Three Gorges Reservoir Area ranges 663 km from the west of Chongqing to the east of Yichang City (Hubei province) alongside the upper end of the Yangtze River (Changjiang) and lies about 44 km upstream from the Yichang gauging station [6]. The overall area is around 58,000 km^2, together with the 19 administrative units on both edges of the river. The average depth of the reservoir is about 70 m whereas the maximum depth in the anterior is around 170 m [7]. The overall storage capacity of the reservoir is 3.93×10^{10} m^3 with an installed capacity of 18,200 MW and a rated power of 4990 MW, whereas the lowest and highest water discharges are 1580 and 98,800 m^3/s, respectively. The reservoir executes services of flood regulation, power generation, irrigation, and course-plotting [8].

2.2 Climate

The region has a southeast subtropical monsoon with a mean yearly temperature of 16.5 °C and annual precipitation of almost 1100 mm [9]. During summer subtropical high pressure from the western pacific drifts over this region and forms a drought climate and increases the temperature. It also brings humid air from the sea in the south, which meets the airstream of the southwest (Guizhou and Hubei), which offers abundant humidity for precipitation. Rainfall frequently occurs between May

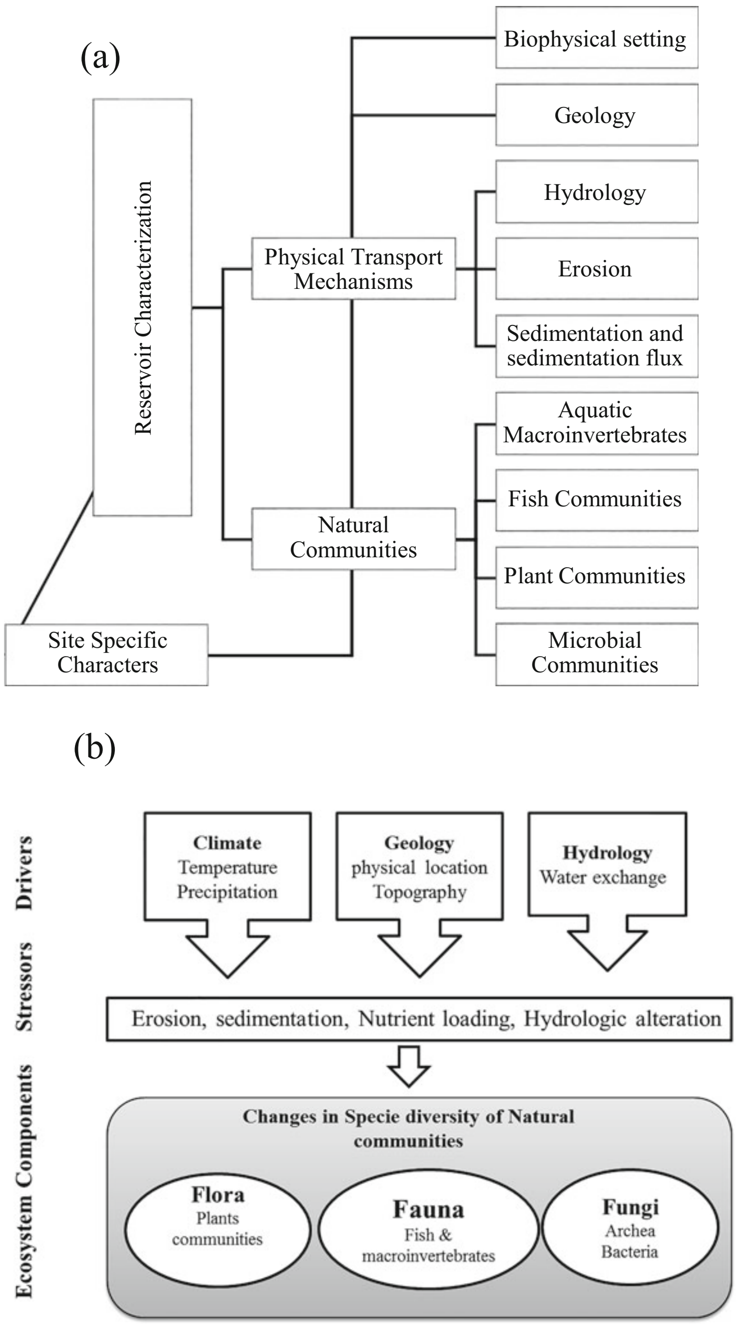

Fig. 2.1 General ecological characterization of reservoir systems. (**a**) Components of ecological characterization; (**b**) Influence of drivers and stressors on ecosystem components

and September in the form of the rainstorm and accounts for 60–80% of annual rainfall [10].

2.3 Geology

The topography of the TGR region is uneven and intricate. 74% of the landscape is composed of mountainous areas and 21.7% comprises hills [11], whereas the plains along the river valleys cover 4.3% of the remaining area in the reservoir region. The geology of the region entails a translucent basement composed of metamorphic and magmatic rocks and Jurassic sedimentary cover comprised of inter-embedded limestone, sandstone, and shale [11, 12]. Based on the age and rock type, the TGR area could be categorized into three geological sections. The initial one extends from Fengjie to Badong, interbedded with dolomite, carbonates, and shale; the next is Jurassic extending from Badong to Zigui, and the third is a granitic area that ranges from Zigui to Yichang [13].

Karst landform is widespread all over the TGR region as it is popular in a temperate and subtropical climate in southwest China [14]. Cave mines development is also swift and many caves are being quarried. Water from reservoir seeping into the regions's limestone caves, mines and underground rivers causes the caves to become unstable and eventually collapse. Reservoir water could discharge into underground rivers, mines, and limestone caves, which are distributed in the region due to caves become unstable and ultimately collapse [15].

3 Physical Transport Mechanisms

3.1 Hydrology

The hydrological conditions of water systems are disturbed by anthropogenic activities and climate. Since 2003, after the impoundment of the TGR, this area's ecosystem has been transformed, altering the water and sediment discharges downstream [16]. Dam operations can lead to substantial alterations in the water flow regime, particularly concerning monthly flow patterns and the magnitude of the flood [17, 18]. Dam operations usually disrupt the sequence of sediment pathways and modify the river hydrology, morphology, and ecology [19]. The TGD has strongly affected the downstream hydrological regime of the Yangtze River.

The first dam impoundment was commenced in 2003 with defiance of gravity to 139 m. Following another impoundment in 2006, which raised the water level to 156 m. The third elevation in the year raised the level to 172.8 m. Later in 2010, a remarkable water level of 175 m was experienced [20]. Human activities substantially influence the total runoff, which could result in rainfall erosion in agricultural land [21]. Moreover, according to the description by Mei et al. [22], the minimum and maximum water levels downstream of the TGD decreased significantly in retort to erosion, despite the fact the yearly maximum water level was raised because of other anthropological activities in the pre-dam era. Modifications in hydrological processes may considerably distress spatial and time-based circulation, availability, and quality of water, resulting in reshaping the biota of the region [23].

3.2 Erosion

Soil permeability, resistance to erosion, thickness, anti-eroding ability, etc., have strong effects on the erosive process. In the TGR area, soils vary from yellow earth to yellow-brown earth and brown earth, whereas non-regionalized soil varies from purple, chalky, and paddy soil. Yellow, yellow-brown, and chalky soils create surface runoff because of their sticky nature and poor infiltration. The purple soil is the derivative of mudstone, which is thinner in texture and has good infiltration during a rainstorm. In riverine valleys, the dominant soil types are yellow, purple, and rendzina alluvial soil [11]. Because of the presence of these soil types, soil erosion may occur and cause the loss of nutrients [24]. During the mid-1990s, the problem of soil erosion was reported [25]. By the year 1997, the land area experiencing soil erosion comprised 82.9% of the overall reservoir area. Due to the clearing of vegetation for rebuilding road networks and urban development, a considerable quantity of soil organic material was lost, causing the degradation of soil [11].

The soil erosion emerges in the central portion of the TGR region, Chongqing Municipality, which accounted for 48.6% of the entire area [26]. According to previous studies [27], the risk of soil erosion will increase in the future. Moreover, sedimentation and erosion in the Yangtze River is a probable risk to the protection of the TGR [27]. The soil erosion in the TGR area has been promoted since 2000, due to the resettlement of urban and rural people near the reservoir region. Occurrence of geological hazards prompted by increasing the storage of the reservoir [28]. These factors might also aggravate the problem of soil erosion, whereas the existing ecological projects could help to reduce the extent and intensity of soil erosion in the reservoir region [29]. Other factors impelling soil erosion encompass urban expansion and reconstruction [11]. According to a previous report [26], the discrepancy in soil erosion in 2001 and 2006 illustrates the possible correlation between soil erosion and the precipitation intensity in the TGRA.

4 Natural Communities

Reservoirs are dynamic bodies with vertical zonation on the horizontal axis. Along the reservoir region, spatial heterogeneity of various regions and transference of nutrients to a downstream region takes place. The concentrations of various nutrients, trophic states, and operational processes in the upstream area affect metabolic

Table 2.1 Summary of various groups of natural communities in the TGR region

Community	Dominant species	Reference
Fish	*Aristichthys nobilis, Coreius heterodon, Coreius guichenoti, Hypophthalmichthys molitrix, Pelteobagrus vachelli, Rhinogobio cylindricus, Hemiculter bleekeri, Cyprinus carpio in abundance. Protosalanx hyalocranius, Ictalurus punctatus, Megalobrama amblycephala*	[7]
Microbiota	*Alpha-, Beta-, Gamma Proteobacteria, Verrucomicrobia, and Planctomycetes, Spirochaetes, Nitrospirae, Chloroflexi,* and *Acidobacteria*	[31]
	Firmicutes, Proteobacteria, and *Actinobacteria*	[32]
	Chironomidae, Heptageniidae, and *Baetidae*	[33]
	Diatoma vulgare Melosira varians, Cocconeis placentula, Gyrosigma scalproides, and Oscillatoria tenuis, M. varians, Cymbella affinis, D. vulgare, Eucapsis alpina, and M. granulata, M. varians, C. affinis, and *C. placentula,*	[34]
Macroinvertebrates	*Nais–Polypedilum, Limnodrilus*	[35]
	Branchiura sowerbyi, Bothrioneurum vejdovskyanum, Nematoda spp, Polypedilum scalaenum, Limnodrilus hoffmeisteri, Stictochironomus sp., *Teneridrilus mastix, Nais variabilis, Paranais frici, Procladius* sp., and *Polypedilum scalaenum*	[36]
Plants	*Pinus massoniana Lamb., Cupressus funebris Endl.*	[37]
	Bidens tripartita, Cynodon dactylon, *Cyperus rotundus*, *Digitaria* sp., *Echinochloa crus-galli*, *Setaria viridis*, *Polygonum lapathifolium*, and *Xanthium sibiricum*	[38]

progressions of a downstream reservoir and modify the overall water quality [4]. The altered watercourses, the segregating effect of the dam, and the transformed sediment composition have modified the habitations and associated vegetation, vertebrates, and invertebrate species in the riparian and reservoir ecosystems. Besides, variation in water level due to changes in hydrological patterns has resulted in the loss of land-dwelling and riparian flora fauna [30]. A summary of different natural communities found in the TGR region is shown in Table 2.1.

4.1 Fish Communities

Reservoirs being important artificial ecosystems alter the ecological and hydrological physiognomies of a river [39]. The ecological and hydrological regimes of the river are modified by artificial ecosystems like dams due to changes in physical, biological, and chemical variables [40]. For physical gradients, reservoir region is distributed into riverine, transitional, and lacustrine zones in the longitudinal direction [41]. In the riverine zone, surface and deep-water sediments are well mixed and the environment is lotic. The ecotone between the upstream and the lacustrine zone is called a transitional zone, whereas the lacustrine zone is the stratified lake-like area

[40]. The flow rate decreases from upstream to downstream in the TGRA, due to different niches for organisms were created and gradients of different nutrients were found [42]. The zonation configuration is very useful to study the time- and space-based pattern of fish accumulations. To manage fish diversity, it is important to understand the assemblage of fish along the gradient [7] Information related to the characteristics of fish congregations in dams is also very vital for the development of operative conservation policies [43].

In a study by Lin et al. [7], fish assemblage patterns in river dam gradient were assessed in the TGR region. The reservoir area was defined as the riverine zone, transitional zone, and lacustrine zone. Three fish zones were reported in the TGR. The riverine zones were subjugated by rheophilic species, such as *Coreius guichenoti* and *Pelteobagrus vachelli*. The transitional zones were found the fish species, such as *Coreius heterodon* and *Rhinogobio cylindricus*. The lacustrine zone was dominated by eurytopic species including *Aristichthys nobilis*, *Cyprinus carpio*, *Hypophthalmichthys molitrix*, and *Hemiculter bleekeri*. Moreover, the lacustrine zone also contained 18 alien species like *Protosalanx hyalocranius, Ictalurus punctatus, Megalobrama amblycephala, and Tilapia.*

4.2 Microbial Diversity

The microbial populations are mostly dominated by the homogeneous selection, ecological drift, and limitation of dispersal in the sediments of deep-water reservoirs [42]. In the previous studies [44, 45], Proteobacteria dominated in the bacterial communities; whereas among Protistan communities, *Ochrophyta*, Fungi, *Ciliophora*, and *Chlorophyta* were dominant species in major hotspot areas of the TGR region [46]. *In a similar study, it was observed that in bacterial communities, Proteobacteria*, such as *Alphaproteobacteria* and *Betaproteobacteria*, were the most diverse among all samples, followed by *Bacteroidetes (Bacteroidia* and *Flavobacteriia), Chloroflexi* (*Anaerolineae*), and other Phyla including *Planctomycetes, Acidobacteria, Spirochaetes, Verrucomicrobia*, *Nitrospirae*, *Actinobacteria, Firmicutes, Chlorobi*, and *Euryarchaeota*. In a previous study [46] utilizing the metabarcoding approach, 6217 non-singleton protistan and 26 protistan phyla were identified, and *Ochrophyta* (*Bacillariophyta*), *Fungi* (*Ascomycota*), *Ciliophora* (*Litostomatea* and *Spirotrichea*), *Chlorophyta* (*Chlorophyceae*) were dominant, compared to others.

Among algae communities, almost 103 species from 45 genera and 4 families were identified in River Daxi, Caotang, and Meixi in the Fengjie district of Chongqing, in which *Diatoma vulgare*, *Melosira varians*, *Cocconeis placentula*, *Gyrosigma scalproides*, *Oscillatoria tenuis*, *Cymbella affinis*, *D. vulgare*, *Eucapsis alpina*, and *M. granulata* were common and their composition in the region was influenced by environmental factors, like pH, temperature, total nitrogen, and total phosphorus [34].

Another study investigated the taxonomic groups of bacteria in the TGR region and reported that *Firmicutes* instead of *Proteobacteria* and *Actinobacteria* are the predominant group at the phyla level. However, *Proteobacteria* and *Actinobacteria* can contribute to more than 50% of total bacteria in surface water [32], which are indicators of freshwater bacteria. The high levels of *Firmicutes* are usually detected in wastewater [47] where it is involved in solid waste biodegradation [47, 48], implying that surface water of the TGR might be contaminated with it.

In freshwater bacterial clusters like alpha-, beta-, gamma-, and deltaproteobacteria, *Actinobacteria*, *Cyanobacteria*, *Cytophaga-Flavobacterium-Bacteroides (CFB)*, *Firmicutes*, *Planctomycete*s, and *Verrucomicrobia* are usually present. Alpha-, Beta-, Gammaproteobacteria, Verrucomicrobia, and Planctomycetes were found in the TGR according to the previous study [31]. Besides, *Spirochaetes*, *Nitrospirae*, *Chloroflexi*, and *Acidobacteria* were also detected.

4.3 Macroinvertebrate Community Composition

Macroinvertebrates are ubiquitous and diverse and are utilized as biological indicators in ecological assessments. Their populations are affected by anthropological stress and natural factors like temperature, light penetration, water chemistry, food resources, and habitat diversity [33]. Time-based variations in macroinvertebrate composition could be associated with life-history patterns in the community, in response to food availability and sporadic alterations in physicochemical properties. Seasonal variation leads to food abundance and impacts the life cycles of the aquatic community [49].

In a previous study [33], 27 main tributaries of the TGRC were evaluated for species richness during the four seasons. 87 taxa were recorded and the overall richness of the taxa varied in different seasons (61 families in spring, 40 families in summer, 47 families in autumn, and 52 families in winter). The most abundant taxa in all seasons were *Chironomidae*, *Heptageniidae*, and *Baetidae*.

Previous data acquired before the damming revealed that the benthic community has drastically altered in the reservoir. Xie reported that before the construction of the reservoir [50], mayflies and caddisflies were common macroinvertebrates among benthic fauna. However, the occasional presence of caddisflies and no occurrence of mayflies have been detected. Later, *Oligochaetes* and *Chironomids* with morphological adaptations dominated the taxon composition and are still presently dominating [51, 52]. *Nais–Polypedilum* community type was more common, which occurred in the low-water discharge inflow during winter and spring after the second impounding year. *Limnodrilus* community occurred in the high discharge inflow during autumn and summer [35]. Many studies showed that the community of macroinvertebrates in the TGR is regulated by the subtropical monsoon climate with seasonal cycles of destruction followed by reestablishment [36].

In a 10-year survey study [36], 49 taxa were collected, which are classified into three groups, *Chironomidae*, *Tubificidae*, and *Naididae*. During the three impoundment stages, 10 taxa were found, which include *Polypedilum scalaenum*, *Branchiura sowerbyi*, *Limnodrilus hoffmeisteri*, *Stictochironomus* sp., *Teneridrilus mastix*, *Nais variabilis*, *Paranais frici*, *Procladius* sp., *Bothrioneurum vejdovskyanum*, and *Nematoda* spp. *Polypedilum scalaenum* was the most frequent taxon, occurring in 70% of all 40 surveys. Other frequent taxa included *B. sowerbyi*, *Procladius* sp., *N. inflata*, *Nematoda* spp., *T. mastix*, *Stictochironomus* sp., and *L. hoffmeisteri*.

4.4 Plant Communities and Distribution Patterns

The TGR region is known to be one of the 25 biodiversity hotspots around the globe [53] and one of the three richest biodiversity centers in China [54]. Factors, such as high temperature in summer and extensive floods in winter, alter the water level and affect aquatic and terrestrial species of the TGR. Because of the distinctive geography and landscape, some of the species present in the TGR region have survived the late Tertiary and Quaternary periods, and this area is well known for the presence of a high level of rare, endemic, and ancient species [30]. Previously, the region was dominated by original subtropical vegetation *Castanopsis* spp. and *Phoebe* spp. [55], but it has been substituted by secondary forests composed of *Pinus massoniana Lamb*. and *Cupressus funebris Endl.*), shrublands, grasslands, and croplands [37]. Various studies have been carried out to assess the impact of the TGD on local biodiversity [31, 37, 56–58]. A total of 22 plant communities (four woody, nine shrubs, and nine kinds of grasses) were vulnerable to the submergence [37].

Before the closing of the TGR, the vascular plants of the downward areas were studied thoroughly by different research scholars. Wang et al. [59] collected 377 vascular plants in the downstream area of the TGR, whereas in another study [58], 392 different plant species were reported around the reservoir region. These studies suggest that the TGR impoundment has reduced the floral diversity of the downstream region. *Bidens tripartita, Cynodon dactylon*, *Cyperus rotundus, Digitaria* sp., *Echinochloa crus-galli*, *Setaria viridis, Polygonum lapathifolium*, and *Xanthium sibiricum* were most widely distributed in the whole TGR [38].

A recent study using quantitative methods found 150 vascular plant species belonging to 130 genera of 56 families in the TGR region [60]. The majority of species were annual herbs, which confirms that the region is ecologically distressed and is at an initial phase of succession. The medium and steep slopes of the riparian zone have comparatively fewer species, whereas the upper zone had more diverse species and community types. At the site scale, the major influence that affects the structuring of communities was the landscape. While at community scale habitat, soil, nitrogen, organic matter, and flooding have severely impacted the structure.

5 Conclusion

The overall aim of this chapter is to understand the characterization of the reservoir ecosystem and to identify the core drivers and stressors that affect the composition and distribution of the various natural communities in the region. Previous studies have revealed that species composition is affected by various stressors, and drivers that are part of ecology and disturbance in ecological entities affects the species richness and type. Over the years, the ecology of the reservoir region has changed gradually because of anthropogenic activities and natural disturbances. The reservoir has also affected the downstream ecology of the Yangtze River and has also reduced the number of some important species especially fish biodiversity. These results of various studies highlight the need of conserving native fish in the TGR region.

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Chapter 3
Main Sources and Distribution of Pollutants in the Reservoir

Naima Hamid and De-Sheng Pei

Abstract This chapter particularly aims to identify the sources and the pollutants distribution trend observed in the Three Gorges Reservoir Area (TGRA). It was found that, among various classes of pollutants, heavy metals (HM) were the most widely reported from 2008 to 2016. The general trend showed that the HM levels were relatively high in this area. Moreover, phthalate esters (PAEs) including DEHP showed higher risks to the aquatic species with concentrations double the prescribed limit by WHO and US EPA. Polycyclic aromatic hydrocarbons (PAHs) enter the TGRA from the pyrogenic and petrogenic combustion sources. Besides, the PPCPs sources could be attributed to agricultural activities, hospital waste, and industrial waste. Higher microplastic pollution loads were particularly found in the upper stream, which also increases the ecological risks. In summary, detailed temporal studies are imperative to foresee the clear pollution trend. Also, regulative authorities should properly implement the rules and restrict the use of hazardous chemicals that enter the TGRA.

Keywords Pollutants · Three Gorges Reservoir Area · Sources · Distribution

1 Introduction

Over the past decades, increased urbanization, industrialization, and exponential population growth have led to the increasing exploitation of natural resources [1]. The continuous release of pollutants into the environment had posed detrimental effects on the environment, particularly on the reservoir ecosystem [2, 3]. Numerous pollutants enter the reservoir through natural processes (runoff, flooding, and transport) and a variety of anthropogenic interventions (industrial activities, agriculture activities, and tourism) [4]. The prominent pollutants that are released in the reservoirs include different classes of organic and inorganic pollutants possessing persistent, toxic, and bioaccumulative properties. Different classes of pollutants are frequently found in the reservoir ecosystem (Fig. 3.1) [5].

Globally, the reservoir ecosystem is manmade that is used for irrigation, water supply, industrial, domestic, flood mitigation, and hydropower generation purpose. Through direct and indirect sources, chemical contaminants pollute the reservoir

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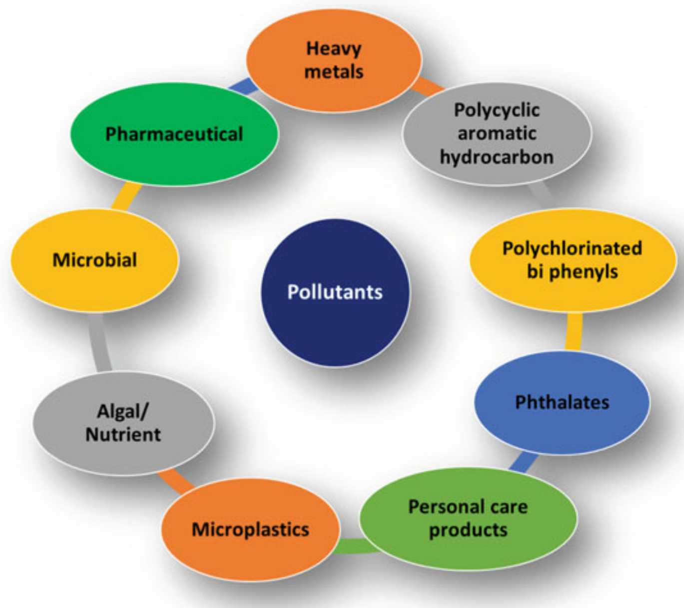

Fig. 3.1 General classification of the pollutants found in the reservoir ecosystem

water and cause human health risks. Previously, a 50-year sedimentary record of heavy metal pollution was reported in the Lot rivers, France [6]. From the geoaccumulation index (Igeo), it was found that Lot river sediments were considered the most polluted reservoirs, particularly with cadmium (Cd) and zinc (Zn) [6]. Similarly, higher concentrations of eight heavy metals including iron (Fe), Zn, chromium (Cr), manganese (Mn), cobalt (Co), lead (Pb), cadmium (Cd), and nickel (Ni) were detected in the Al-Najaf sea depression reservoir, Iraq [7]. The majority of the published studies were focused on heavy metal pollution; therefore, more studies are needed to elucidate more classes of pollutants trends in reservoirs.

2 Sources of Pollutants

Pollution sources can be classified as point and non-point sources. Point source refers to any single identifiable point where pollutants enter the water body. Whereas, non-point source involves various contaminants enter in the water from different sources. Generally, non-point sources are easy to identify but difficult to manage [8, 9]. Previously, various studies have demonstrated the sources of water pollution. Every class of pollutants possesses different sources. For example, PAHs mainly originated from pyrogenic or petrogenic combustion [10, 11]. Phthalates (PAEs) ubiquitous presence in the environment is from industrial or wastewater treatment plant waste [12, 13]. PCBs sources are attributed mainly to agricultural waste [14]. Similarly, heavy metals came from industrial activities and wastewater treatment plants [15]. The major sources of PPCPs are hospital waste, industrial waste, and domestic waste [16].

3 Source Apportionment Techniques

Numerous source apportionment techniques have been used to identify the possible sources and their quantitative contribution to the Three Gorges Reservoir Area (TGRA). The most widely used techniques include the principal component analysis (PCA), molecular diagnostic ratios (MDRs), Monte Carlo source apportionment, regression analysis, geoaccumulation index, enrichment index, and isotopes analysis [2, 17]. Nevertheless, each technique has its own advantages and disadvantages, implying that the use of multiple methods may reduce bias instead of an individual method.

4 Pollution in the TGRA

In 1994, the TGRA was implemented to promote sustainable water resources in China. The TGRA is also recognized as China's Golden Waterway, because it plays a key role in the national economy [18]. It is the longest river in Asia, and flows throughout China [19]. In 2009, the TGRA project was completed and comprises the upper, middle, and lower reaches. However, with the construction and its functional use, the untreated wastewater discharge imposed threats to ecological and public health effects [3, 18]. Similarly, various agricultural and industrial pollutants were reported to be discharged into the river and are responsible for ecological and health risks to freshwater ecosystems [15, 20, 21].

Generally, in the TGRA, pollutants enter mainly from the industrial wastewater, domestic wastewater, and agricultural discharges, which include different organic and inorganic pollutants, such as heavy metals (HM), polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls, phthalates (PCBs), microplastics (MPs), microbial and algal pollutants, organic matter (OM), pharmaceutical and personal care products (PPCPs) [3, 20, 22, 23]. Each pollutant category comprises different points and non-point sources. According to the previous studies from 2003 to 2014, the TGRA holds many pollutants, of which 37.2% and 62.7% accounted for industrial and domestic wastewater, respectively [10, 24]. Moreover, this could be attributed to rapid industrialization, urbanization, and an increase in population. Besides, chemical oxygen demand (COD) is considered the major indicator of water quality and was reported to increase from 2003 to 2014, suggesting 68.2% of the domestic wastewater sources [14, 25, 26].

5 Spatial Distribution of Pollutants in the TGRA

5.1 Heavy Metals

In the TGRA, heavy metals are the most widely studied pollutants. Previously a study quantified Hg, Cr, As, Cu, Cd, Fe, Mn, and Zn in soil and sediments samples from 2008 to 2009 and showed that Cd, As, Cu, Pb, and Zn were higher in the upper and lower reaches of the TRGA. Source apportionment techniques, such as factor analysis (FA) and multiple linear regression (MLR) suggested that As and Cd are the main chemical pollutants with 45% contribution from domestic sewage and 59% from industrial waste [27] (Fig. 3.2). Gao et al. evaluated the HMs status in surface water for the years 2008–2013 in the TGRA [17]. It was found that the highest metal levels were observed in the upper reaches with the concentrations of Cu (10.3 μg/L), Hg (0.05 μg/L), Cd (1.47 μg/L), As (3.06 μg/L), and Pb (15.02 μg/L). Similarly, in 2015–2016, Cu, Zn, Cd, Pb, and Cr were found in the surface water and sediments, and the PCA results implied that the HM levels were lesser in the drinking water and reached the surface water quality standards of China. The HMs were found to be non-polluted in 2015–2016. Cr levels in the sediments were also non-polluted, except for Cu, Zn, Pb, and Cd, which were slightly polluted in the TGRA [3].

Bioaccumulation and ecological risk of main HMs (Cu, Fe, Hg, Zn, Cd, and Pb) were observed in aquatic invertebrates and fish from the TGRA [25]. It was found that, in the impoundment years from 2003 to 2010, all the HM levels were under the criteria of safety guidelines [25]. Moreover, Cu, Zn, Hg, and Fe levels in fish and aquatic invertebrates were relatively higher, compared to the levels before impoundment. Also, the pollution trends of HMs were the same in the upper, middle, and lower reaches [25] (Table 3.1).

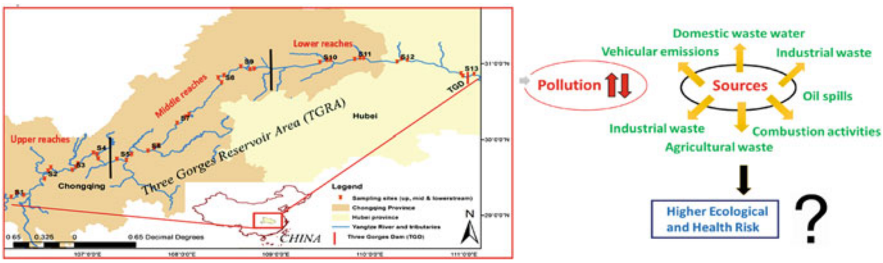

Fig. 3.2 A general illustration of the sources of pollutants in the TGRA

Table 3.1 Summary of pollutants levels in the TGRA environment and biological matrices

Chemical class	Sampling time	Matrix	Concentrations/levels	Reference
Heavy metals	2008	Surface water	Cu: 10.3 μg/L, Hg: 0.05 μg/L, Cd: 1.47 μg/L, As: 3.06 μg/L, Pb: 15.02 μg/L	[17]
Heavy metals	2013	Surface water	Cu: 3.01 μg/L, Hg: 0.016 μg/L, Cd: 0.771 μg/L, As: 1.53 μg/L, Zn: 10.43 μg/L, Pb: 7.89 μg/L	[17]
Heavy metals	2008–2013	Surface water	Cu: 8.94 μg/L, Hg: 0.03 μg/L, Cd: 1.02 μg/L, As: 2.33 μg/L, Zn: 13.03 μg/L, Pb: 11.20 μg/L	[17]
Heavy metals	July 2015	Sediments	As: 0.05–50.90 μg/L, Mo: 0.30–1.63 μg/L, W: 0.01–0.42 μg/L	[28]
Heavy metals	2011–2012	Fish	Hg: 17.8 ng/g, Cd: 18.8 ng/g, Pb: 27.3 ng/g, Cu: 515.0 ng/g, Fe: 6969.7 ng/g, Zn: 6163 ng/g	[25]
Heavy metals	December 2015	Surface water	Cu: 1.21 μg/L, Zn: 12.98 μg/L, Pb: 0.04 μg/L, Cr: 0.47 μg/L, Cd: 0.02 μg/L	[3]
Heavy metals	December 2015	Sediments	Cu: 58.9 mg/kg, Zn: 165.9 mg/kg, Pb: 56.7 mg/kg, Cr: 96.5 mg/kg, Cd: 1.14 mg/kg	[3]
Heavy metals	2014	Sediments	Cd: 0.99 mg/kg, Cr: 94.2 mg/kg, Cu: 69.0 mg/kg, Ni: 40.8 mg/kg, Pb: 56.7 mg/kg, Zn: 161.0 mg/kg	[29]
Heavy metals	2014	Sediments	Cd: 1.18 mg/kg, Cr: 115.8 mg/kg, Cu: 79.14 mg/kg, Ni: 48.70 mg/kg, Pb: 71.17 mg/kg, Zn: 167.47 mg/kg,	[30]
Heavy metals	2016	Sediments	Cd: 1.01 mg/kg, Cr: 86.4 mg/kg, Cu: 49.5 mg/kg, Ni: 38.6 mg/kg, Pb: 54.5 mg/kg, Zn: 185.1 mg/kg	[29]
Heavy metals	2008	Soil	Cd: 0.77 mg/kg, Cr: 44.2 mg/kg, Cu: 32.0 mg/kg	[27]
Heavy metals	September 2008	Soil	Hg: 1.73 mg/kg, As: 3.98 mg/kg, Cr: 0.56 mg/kg, Cd: 3.18 mg/kg, Pb: 1.58 mg/kg, Cu: 1.29 mg/kg, Zn: 1.17 mg/kg, Mn: 1.23 mg/kg	[23]
Heavy metals	June 2009	Soil	Hg: 3.20 mg/kg, As: 1.30 mg/kg, Cr: 0.67 mg/kg, Cd: 4.27 mg/kg, Pb: 2.11 mg/kg, Cu: 1.64 mg/kg, Zn: 1.49 mg/kg, Mn: 1.26 mg/kg	[23]
PAH	2015	Surface water	ΣPAHs mean: 23–1630 ng/L	[10]
PAEs	2015	Surface water	DEHP: upper reaches 6.21 μg/L, middle reaches 1.48 μg/L, lower reaches 0.38 μg/L	[22]
Tetracycline	August 2015	Surface water	OTC: 10.62 ng/L, TC: 22.91 ng/L, CTC: 30.06 ng/L, TCs: 6.6 ng/L	[31]
Sulfonamide	August 2015	Surface water		[31]

(continued)

Table 3.1 (continued)

Chemical class	Sampling time	Matrix	Concentrations/levels	Reference
			SDZ: 95.39 ng/L, TMP: 119.0 ng/L, STZ: 30.12 ng/L, ST: 14.37 ng/L, SMZ: 78.55 ng/L, SAs: 337.5 ng/L	
Quinolones	August 2015	Surface water	OFL: 31.12 ng/L, ENR: 19.32 ng/L, FQs: 50.4 ng/L	[31]
Tetracycline	August 2015	Sediments	OTC: 4.16 ng/g, TC: 31.76 ng/g, CTC:14.68 ng/g, TCs: 51.4 ng/g	[31]
Sulfonamide	August 2015	Sediments	SDZ: 7.02 ng/g, TMP: 11.71 ng/g, STZ: 3.01 ng/g, ST: 35.93 ng/g, SMZ: 24.91 ng/g, SAs: 82.6 ng/g	[31]
Sulfonamide	2011	Surface water	SMX: 7.9 ng/L, SDZ: 1.45 ng/L	[32]
Sulfonamide	2015	Surface water	SMX: 289.3 ng/L, SMR: 6.57 ng/L, SPY: 76.57 ng/L, ST: 6.68 ng/L, SDZ: 1.93 ng/L	[20]
Quinolones	August 2015	Sediments	OFL: 22.46 ng/g, ENR: 42.59 ng/g, FQs: 68.1 ng/g	[31]
Microplastics	July 2017	Sediments	1–5 mm particles (33.24–10^3 particles/m^{-2}) 0.5–1 mm particles (23.78–10^3 particles/m^{-2}) 0.1–0.5 mm particles (34.35–10^3 particles/m^{-2})	[33]
Microplastics	August 2016	Surface water	0.5–5 mm (1597–12,611 n/m^3)	[34]
Microplastics	August 2016	Sediments	0.5–5 mm (25–300 n/kg)	[34]

5.2 *Polycyclic Aromatic Hydrocarbons (PAHs)*

PAHs fingerprints in the TGRA and their toxicological impacts were evaluated at the highest impoundment level of 175 m in 2015. It was found that 16 PAHs congeners ranged in the upper reaches (83–1631 ng/L), middle reaches (354–1159 ng/L), and lower reaches (23–747 ng/L) [10]. Among PAHs structural composition, low molecular weight (LMW) compounds were >85% of high molecular weight compounds (HMW). Diagnostic molecular ratios (DMRs) were used for source apportionment, implying that wood and coal combustion, heavy traffic, industrial emissions, gasoline combustion, and agriculture were the major sources of PAHs contamination in the TGRA. Besides, ecological risks were ranked as the upper reaches > middle reaches > lower reaches [10]. Transgenic Tg(cyp1a:gfp) zebrafish evaluated the toxicological aspects in the form of genetic expression, which revealed the contrary scenario may be due to the higher levels of HMW found in the middle and lower reaches, which is also confirmed by the prediction of Cox hazard proportional model [10].

5.3 Pharmaceuticals and Personal Care Products (PPCPs)

PPCPs involve various human and veterinary antibiotics, cosmetics, and daily-use products when released into the environment, which is also known as emerging pollutants [35]. However, in the TGRA, limited literature is available on PPCPs. A study reported by Yan et al. determine the distribution of antibiotics in the TGRA [31]. Among the detected pharmaceuticals, sulfonamides (SAs) levels were higher than the total concentration of tetracyclines (TCs) and quinolones (FQs). The concentrations of SAs, TCs, and FQs ranged from 21.55–536.86, 3.69–438.76, and 15.78–213.84 ng/L, respectively [31]. Furthermore, the class 1 integron gene (*Int*I1) showed more proliferation and propagation among bacterial-resistant genes [31]. Similarly, a recent study reported the individual and combined toxicogenetic effects of sulfonamides in the TGRA [20]. It was found that, among individual SAs, sulfamethoxazole (SMX: 289.3 ng/L) exhibited the highest levels followed by sulfamerazine (SMR: 6.57 ng/L), sulfapyridine (SPY: 76.57 ng/L), sulfameter (ST: 6.68 ng/L), and sulfadiazine (SDZ: 1.93 ng/L) [20]. The major sources were the emissions from the pharmaceutical industries and hospitals abundantly found in the upper part of the TGRA. However, the lower levels in the middle reach of the TGRA were due to lower anthropogenic pressure and less human intervention/urbanization [20].

5.4 Phthalates (PAEs)

PAEs as plasticizers are ubiquitously released in the TGRA. Among six priority (PAEs), Di-(2-ethylhexyl) phthalate (DEHP) is considered the most toxic compound. The DEHP levels were previously identified in the TGRA and evaluated in vivo or in vitro exposure at environmentally relevant concentrations (ERCs) (Table 3.2) [22]. It was found that DEHP levels were ranked as follows: upper reaches (6.21 μg/L) > middle reaches (1.48 μg/L) > lower reaches (0.38 μg/L) in the TGRA surface water, and also double that of the United States Environmental Protection Agency (US EPA) guidelines, indicating high-level ecological risk [22]. The sources and primary reason for these high concentrations could be related to greater production and consumption rate of PAEs in this area [22]. Moreover, toxicological bioassays revealed that DEHP at ERCs significantly impaired the normal functions of aquatic species in the TGRA.

5.5 Microplastics (MPs)

MPs refer to the small plastic items that can break down into smaller particles size usually <5 mm. Previous studies indicated that various sizes of microplastics existed

Table 3.2 Summary of the distribution of microplastics and DEHP in the TGRA surface water and sediments

River	Abundance/levels in water	Abundance/levels in Sediment	References
Microplastics			
Yangtze River	34.1×10^5 to 136.1×10^5 particles/km^2	–	[36]
Estuaries of Yangtze River tributaries	1.92×10^5 to 118.9×10^5 particles/km^2	–	[36]
Yulin River	2.00×10^{-2} to 7.00×10^{-1} items/L,	–	[37]
Yangtze River	1597–12,611 particles m^{-3}	25–300 particles kg^{-1}w	[38]
Xiangxi Bay TGR	0.55×10^5–342×10^5 particles km^{-2}	80–864 particles m^{-2}	[39]
Xiangxi River (Three Gorges Reservoir)		550–14,580 particles m^{-2}	[33]
Yangtze Estuary	500 n/m^3 to 10,200 n/m^3		[40]
DEHP			
Three Gorges Reservoir	0.38–6.21 μg/L	–	[22]
Yangtze River tributaries	1.7–394.4 ng/L	10.9–1107.1 ng/g	[41]

in the TGRA, which can produce a high ecological risk to aquatic species [33]. Limited studies have been reported regarding the microplastic distribution in the TGRA. A recent study reported by Zhang et al. [33] indicated that microplastic abundance in surface water ranged from 1597 to 12,611 n/m^3. Whereas, in sediments, it was 25–300 n/kg wet weight and urban areas were the most contaminated, compared to the countryside. Among different types of MPs, fibers (polystyrene) with small-sized particles were the most dominant microplastics. Similarly, Mathias et al. [34] reported that, in surface water of the TGRA, microplastic size from <0.5 to 5 mm occupied 1597–12,611 n/m^3 fractions, whereas, in the sediments, it ranged from 25 to 300 n/kg (Table 3.2). The authors mentioned that wastewater from the nearby plant, domestic waste, and the runoff from the river might be the major sources of MPs pollution in the TGRA. Besides, the flourished tourism might contribute to the elevated MPs levels.

5.6 Nutrient or Algal Pollution

Organic matter (OM) is a vital parameter in the terrestrial environment. However, it enters the surface water via leaching, runoff, and flooding, and ultimately affects biogeochemical cycling and disturbs homeostasis. Moreover, many pollutants, such as trace HM, POPs, and PAHs, may enter the surface water. However, very few studies are available regarding the effect of dissolved organic matter (DOM) in the

TGRA. A study reported by Jiang et al. [18] evaluated the DOM extracted from the soils in the TGRA area, especially the water fluctuation zone via various analytical methods. It was found that samples from the TGRA were comprised of OM, polysaccharides, and lignin. Besides, the compositional complexity of soil OM is very crucial, because OM plays an important role in the fate of contaminants in the TGRA [18].

A study reported that phosphate waste was discarded in the Xiangxi river bay [11]. Phosphorous leaching was determined under both neutral and acidic conditions. It was found that phosphorous release was totally dependent on solubility, which increased as the pH decreased [11]. The authors further recommended that phosphate rock's weathering or leaching should be monitored timely, because it could be the point source of pollution for the aquatic species and harmful to public health [11].

5.7 Microbial Diversity and Pollution

In 2015, microbial abundance and water quality were detected along the upper, middle, and lower reaches of the TGRA by Niu et al. [42]. Results indicated that, at the highest chemical oxygen demand (COD) level, the highest number/abundance of *Firmicutes* and *Bacillus* were found in the upper, middle, and down streams [42]. Besides, the results of redundancy analysis revealed that COD and phosphates were the primary environmental parameters that significantly affected the bacterial community structure [42]. Furthermore, the general trend of bacterial richness decreased in the order: downstream > middle stream > upstream [42].

Moreover, mercury is a carcinogenic heavy metal in the environment. The organic form of mercury, methyl mercury (MeHg), is more hazardous to human health [23]. Microbial communities are involved in controlling in MeHg toxicity and movement in the environment [17]. Therefore, the identification of microbial diversity involved in MeHg degradation in the TGRA is important for the ecosystem and public health [23]. A study reported by Xiang et al. [19] indicated that MeHg contamination in the TGRA soils was relatively higher in both summer and winter areas with the impoundment of 175–155 m. It was found that *Deltaproteobacteria* and *Methanomicrobia* showed higher abundance as Hg methylators seasonally [19]. In short, seasonal variations may enhance microbial community abundance as well as MeHg methylation [19]. Moreover, more detailed seasonal level studies are recommended to clearly comprehend the pollution situation in the TGRA.

6 Conclusion

In summary, this chapter aims to identify the sources and the distribution of the pollutants ubiquitously present in the TGRA. Various studies from 2008 to 2016 revealed elevated HM levels. Furthermore, PAHs fingerprints in the TGRA showed pyrogenic and petrogenic combustion activities. DEHP levels were found to be exceeded the WHO and US EPA guidelines, indicating high ecological risks. PPCPs, such as sulfonamides, are mainly derived from industrial/pharmaceutical and hospital waste. Moreover, microplastic pollution was also high mainly in the upper stream of the TGRA, and runoff from the river transport might be considered as the potential source of microplastic pollution. Microbe and DOM that indirectly aggravated pollution were also affected by the seasonal impoundment rate. In conclusion, more detailed temporal studies are recommended to depict a clear picture of the pollution load in the reservoir.

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Conflicts of Interest The authors declare no conflict of interest.

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Chapter 4
Migration and Transformation of Pollutants in the Reservoir

Naima Hamid and De-Sheng Pei

Abstract This chapter particularly aims to elucidate the factors involved in the migration and transformation of pollutants in the Three Gorges Reservoir Area (TGRA). However, limited seasonal studies available make it difficult to comprehend the real situation of different pollutants. More literature related to heavy metals (HMs) are available, which showed that, from 2003 to 2010, the HMs levels were found within the limits of safety guidelines as prescribed by the US EPA. Moreover, the hydro-fluctuation belt (HFB) greatly affected the migration of HMs from the inner layers of soil to the upper layers. In the upper reaches of the TGRA (2004–2015), the ecological health was found to be lower in summer, autumn, and spring, compared to winter. From 2003 to 2014, the chemical oxygen demand (COD) in the TGRA has increased, out of which domestic wastewater contributed about 68.2%. Overall, the ecological health of the TGRA for the past 12 years was relatively stable, except in winters when the pollution load was relatively high due to the varied impoundment levels.

Keywords Pollutants · Seasonal trend · Migration · Ecological risks · Reservoir

1 Introduction

In recent years, the migration of pollutants and their transformation into the freshwater ecosystem has become a global problem [1, 2]. Large-scale reservoirs have many diversified positive and negative impacts. Economic benefits, such as flood control, power generation, and water storage are some positive aspects [3, 4]. However, intensified anthropogenic activities and unsustainable practices become some negative aspects that amplified the pollutant levels in the reservoir [5, 6]. Generally, toxic pollutants are classified into two major categories, organic and inorganic pollutants [7]. Mostly, they are persistent in nature as well as possess long-distance transmission properties. The majority of the pollutants have high bioaccumulation and biomagnification properties and are difficult to degrade [8]. Besides, the biological effect of organic pollutants is more complex, because they can cause serious health problems, such as endocrine disruption abilities [9].

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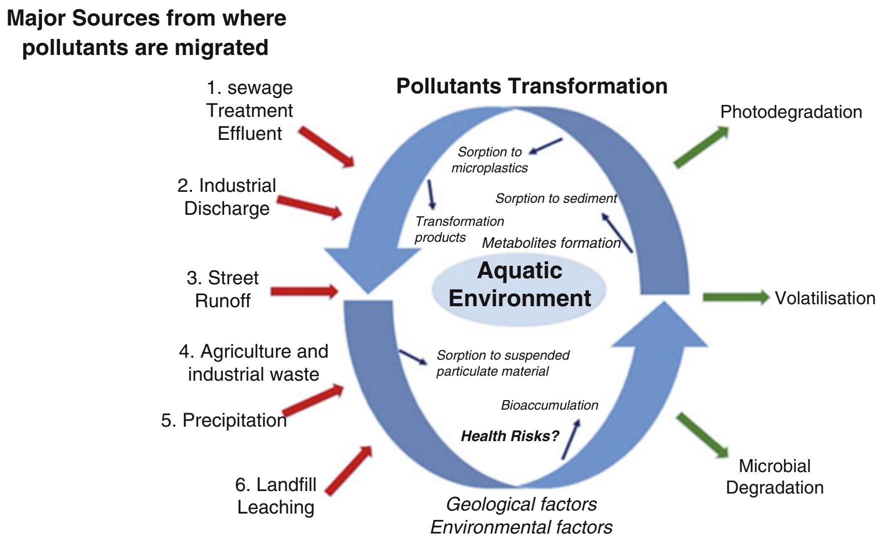

Fig. 4.1 Major factors that are involved in the migration and transformation of pollutants in the reservoir

Previously, it was reported that the majority of the persistent organic pollutants (POPs) are hydrophobic and tend to accumulate in aquatic species, whereas some are hydrophilic, which can easily migrate in water [10]. Polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), microplastics (MPs), microbial and algal pollutants, organic matter (OM), and pharmaceutical and personal care products (PPCPs) are the common pollutants ubiquitously found in freshwater, sediments, and fish bodies [11].

Bioavailability and transformation of the pollutants in the reservoir also depend on environmental/abiotic factors [12, 13], such as temperature, dissolved organic carbon, chemical oxygen demand, pH, precipitation, and humidity (Fig. 4.1). Moreover, biotic factors include living communities' feeding habits, exchange surfaces, and metabolic activity. Reservoir impoundment also has a significant impact on the migration and transformation of pollutants [14]. Sang et al. [10] reported that with the increasing water impoundment level and seasonal variations, pollutant load has also been enhanced [10].

2 Fluctuating Impoundment Level Effects

Water fluctuation level further regulates the pollutants transfer and migration along with the biogeochemistry of the sediments and the response of the microbial community, which helps in the sorption and desorption of the pollutants mainly heavy metals. The Three Gorges Reservoir Area (TGRA) is considered the largest

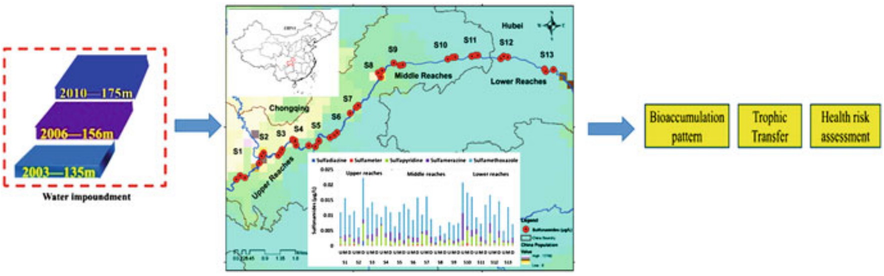

Fig. 4.2 Water fluctuation level affects the pollution load and elevates the health risk among aquatic species

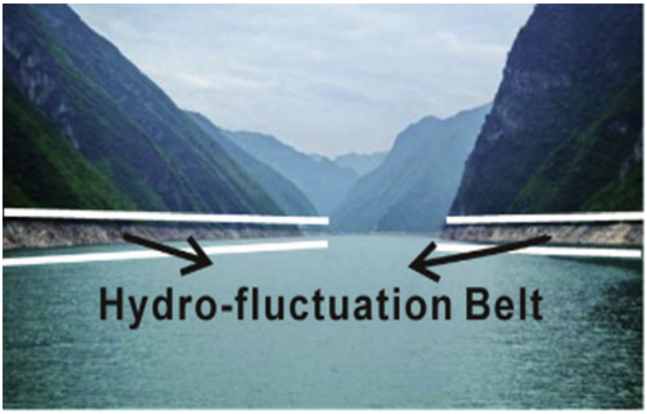

Fig. 4.3 Hydro-fluctuation belt observed across the TGRA

hydropower plant in the world, also attracts attention due to its variation in the water impoundment level for many years. Furthermore, the continuous contamination of persistent toxic pollutants in the TGRA poses high ecological risks to the aquatic species (Fig. 4.2).

From the previous studies, it was found that the water impoundment level may fluctuate from 145 to 175 m, which also varied from the hydrodynamic conditions [15, 16]. In the TGRA, a special zone has been created due to the seasonal impoundment, which is called the hydro-fluctuation belt (HFB) (Fig. 4.3) [17]. This belt comprises a vertical height of 30 m with a length of 662 km^2 and covered a total area of about 349 km^2 along the whole Yangtze River [18]. The HFB zone has gained much attraction from all over the world due to its unique characteristics [19]. Moreover, during wet and dry seasons, the rise and fall of the water levels in the TGRA is different from the natural water flow, resulting in higher chances of pollutants may enter in the reservoir, especially at lower water levels in summers with high precipitation [16, 18].

3 Status and Migration of Pollutants in the TGRA

A study reported by Zhang et al. [16] investigated the microplastic sources and sinks along with the influence of HFB in the TGRA. From the sediment samples, it was found that more microplastics were accumulated in the HFB area due to the influence of runoff or water impoundment, implying that the HFB acts as both the source and sink for the microplastics. However, additional studies are required to determine the real-time status and the transport and fate of the microplastics. Similarly, the heavy metals (HMs) quantified in the upper reaches of the TGRA were higher in concentration compared to the lower reaches, indicating that these chemicals were migrated from the domestic sewage and industrial waste [20]. On contrary, the HMs levels were decreased in sediments during the impoundment period, due to faster flow velocity, resulting in the low deposition of particles and high release of HMs from sediments [21].

In 2015–2016, heavy metals (As, Cr, Cd, Cu, Pb, and Zn) levels were quantified in 46 river sections of the mainstream and tributaries of the TGRA under high and lower impoundment levels and evaluated their health risks [22]. It was found that the levels of HMs were observed ranking as Zn $>$ As $>$ Cu $>$ Cr $>$ Pb $>$ Cd, which were lower than the permissible limit prescribed by the Chinese standard except for Zn [22]. Moreover, the results showed that As, Cd, Cu, and Cr exhibited a significant decrease in the levels from 2015 to 2016. Health risk assessment revealed that heavy metals caused higher risks in the mainstream, compared to the tributaries [22].

Similarly, the migration of HMs pollutants and their trophic transfer are found in aquatic species of the TGRA [10]. Results showed that, during the impoundment years (2003–2010), HMs levels were found within the limits of safety guidelines as prescribed by the US EPA [10]. Moreover, Cu, Zn, Hg, and Fe levels in fish were increased, compared to the concentrations reported before impoundment [10]. A study reported by Huang et al. [20] investigated the migration of HMs (Cd, Cr, and Cu) within the adjacent soil of the TGRA and tracked their adsorption and desorption in the reservoir. Results indicated that water fluctuation/impoundment level greatly affected the migration of HMs from the inner layers of soil to the upper layers. Moreover, hydraulic power further increased the release of Cd and Cu from the lower layer [20], while Cr was mostly released in the environment with continuous recession and inundation [21].

4 Temporal and Seasonal Variations of Pollutants

The published literature regarding the seasonal trend of pollutants is limited. Therefore, it is difficult to elucidate the seasonal status of pollutants [23]. Moreover, the water fluctuation level affected the water biochemistry of the aquatic ecosystem, which might increase the release of inorganic and organic pollutants

[18]. Consequently, more detailed seasonal studies and strict implementation of the industrial discharge rules should protect the ecological integrity of the TGRA.

Long-term temporal studies from 1992 to 2016 reported the levels of chemical oxygen demand (COD) through the potassium permanganate index [22]. The result demonstrated that total phosphorous levels decreased from 40.9% ± 9.9% in 2003 and from 22.2% ± 9.7% in 2016. However, the total nitrogen (1.3 ± 2.4%) and ammonia (8.2% ± 2.6%) levels were increased, respectively [24]. The significant reductions might be due to the decrease in the water flow, which leads to an increase in the sediment settlement/sink. Similarly, another study reported by the bulletin on the ecological and environmental monitoring (SEPA) from the years 2003 to 2014 found that COD in the TGRA has reached up to 1.98 million tons, out of which domestic wastewater contributed around 68.2% of the total COD [25] (Fig. 4.4).

Moreover, anti-seasonal operation levels with the water volume further elevated the pollutant dilution capacity [20]. Besides, the algal blooms and the growth rate of phytoplankton have been widely increased with a factor of 2.7% in the mainstream since 2004, which may deteriorate the water quality and intensify the pollution in the TGRA [22]. Moreover, phytoplankton growth in the TGRA is mainly supported by phosphorus during all seasons. Therefore, by limiting the growth level of phosphorous, short- or long-term eutrophication might be avoided.

5 Ecological Health of the TGRA

A study reported by Zhao et al. [22] found that ecological health was lower in summer, autumn, and spring, compared to winter. The ecological health status of the TGRA from the year 2004 to 2015 was determined using the chemical index method. It was found that the overall ecological health of the TGRA for the past 12 years was relatively stable. However, in winter, the pollution load was relatively high due to the varied impoundment levels (Fig. 4.5).

Various parameters play an important role in determining the ecological health of the TGRA, such as intensified anthropogenic activities (domestic, industrial, and agricultural waste) [26, 27] and natural processes (eutrophication, volatilization, photodegradation, and microbial degradation) [28, 29]. However, we cannot completely control natural activities, but it is in our hands to limit man-made pollution by strictly following the guidelines and complying with our safety limits [30], which ensures ecological integrity.

6 Conclusion

In summary, this chapter summarizes the factors involved in the migration and transformation of pollutants in the TGRA. However, it was found that HMs are available, which showed that from 2003 to 2010. HMs levels were found within the

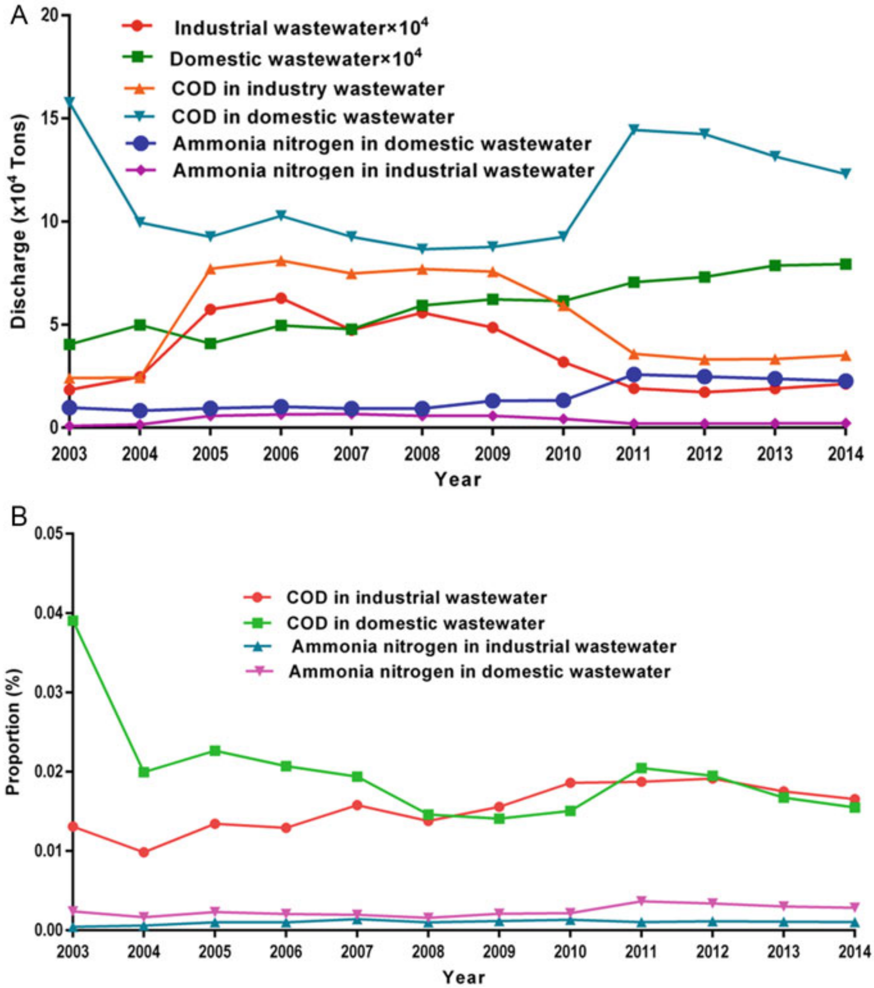

Fig. 4.4 Wastewater discharge and its contribution of COD and ammonia in the TGRA. (**a**) Annual temporal comparison of the domestic and industrial wastewater discharge. (**b**) its percentage contribution of COD and ammonia from 2003 to 2014 observed in the TGRA. (Source: SEPA, 2004–2015)

limits of safety guidelines as prescribed by the US EPA. Furthermore, the HFB greatly influences the migration of HMs from inner the layers of soil to the upper layers. The COD in the TGRA has increased up to 1.98 million tons from 2003 to 2014 and domestic wastewater contributed the maximum with 68.2%. In short, the overall ecological health of the TGRA in the upper reaches is relatively stable but it may vary due to fluctuation in the impoundment levels.

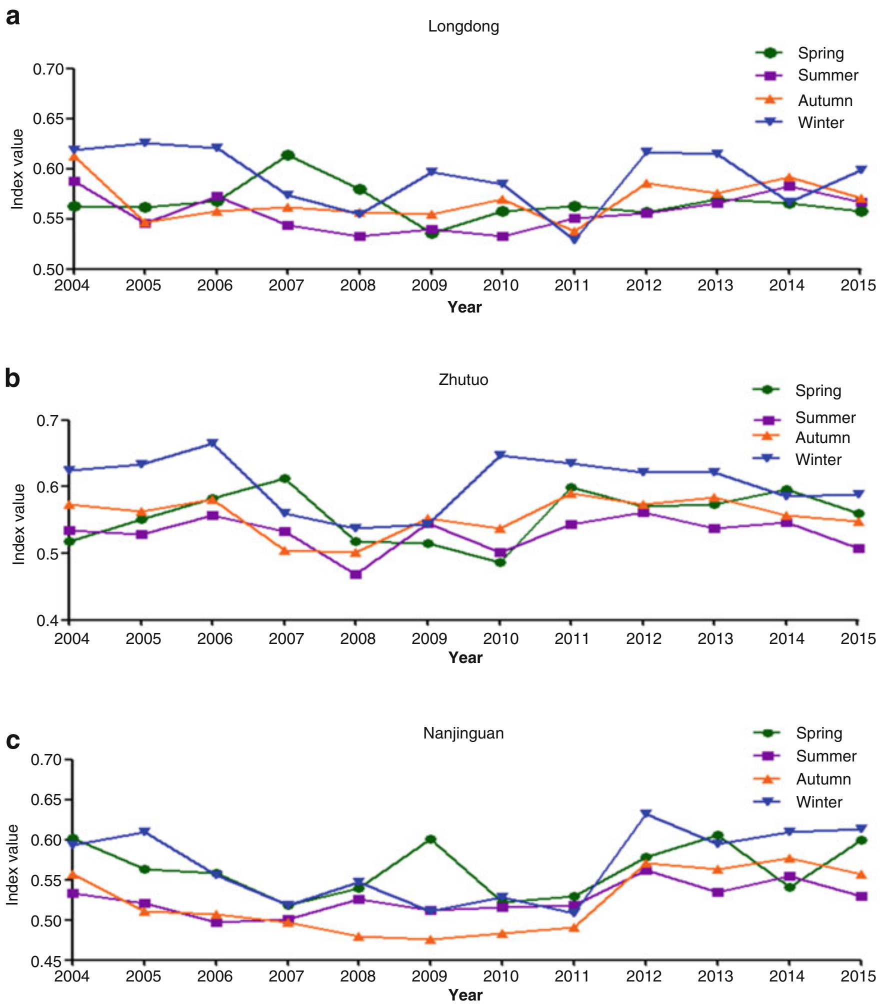

Fig. 4.5 Annual seasonal ecological risk index in the upper reach of the TGRA for the years 2004–2015 (Source: SEPA, 2004–2015)

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Conflicts of Interest The authors declare no conflict of interest.

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Chapter 5
Harmful Algal Bloom in the Reservoir

Suman Thodhal Yoganandham and De-Sheng Pei

Abstract The formation of harmful algal blooms is governed by a complex interaction of chemical, biological, physical, and geological processes along with reservoir-associated factors, such as water discharge, sediment deposition, and turbulence intensity. The abundant release of phosphates and nitrates in reservoirs from anthropogenic and natural sources leads to eutrophication, which accelerates the excessive growth of algae and induces algal blooms. Globally, algal blooms occurred in many reservoirs and become a core reservoir environmental problem. This chapter summarizes the types of algal blooms, elucidates their formation, and puts forward the methods for controlling harmful algal blooms, which may improve the management efficiency of reservoirs and lay the groundwork for future studies. Although there are various methods for treating harmful algal blooms in the reservoir, we can not only rely on any specific method to solve all problems. Therefore, an approach involving the combined methods is encouraged to apply to different types of algal blooms. Further, in-depth studies are needed to monitor and block invasive algal flora.

Keywords Harmful algal bloom · Reservoir · Formation of algal bloom · Toxin

1 Introduction

In the environments worldwide, the harmful algal bloom is a significant problem [1]. Algal blooms are not new and naturally occur in fertile regions for at least two centuries in history [2]. Nevertheless, with the growing environmental pollution over the last century, the frequency and geographical range of algal blooms have significantly increased in small and large lakes, rivers, reservoirs, wetlands, and other surface waters. As a result, a severe and persistent toxic algal bloom is currently spreading to large water bodies in the world, such as the Erie Lakes, Ontario, Okeechobee, Winnipeg (North America, Fig. 5.1), Kasumigaura (Japan), Taihu (China), the Caspian Sea (Europe), the Kinneret Lake (Israel), and Victoria (Africa) [3–5]. Some of the blooms are due to planktonic algae floating in the water, but often, the term may also apply to the aggregation of microscopic benthic algae or macro alga attached to the surfaces. *Planktothrix* spp., *Microcystis* spp.,

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Fig. 5.1 Toxic blue-green algal bloom in the Copco Reservoir of California, USA (*Photo from David McLain/Alamy*)

Cylindrospermopsis spp., *Anabaena* spp., *Oscillatoria* spp., and *Aphanizomenon* spp. produce cyanobacterial metabolites and toxins in freshwater, which may damage the human health and ruin the environment [6, 7].

Blooms are dense accumulations in marine, brackish, and freshwater bodies of microscopic algae or cyanobacterial cells, frequently contributing to noticeable discoloration of the water [8]. The effects of algal toxins on humans after acute or chronic exposure may cause kidney and liver toxicity, and the causal relations must still be identified in the long run [2, 9]. Besides, many algal blooms are often associated with the deaths of animals, pets, and birds because of environmental pollution [8]. Generally, toxins compounds produced by cyanobacteria are becoming increasingly problematic in drinking water reservoirs, and off-flavor blends are essential in aquaculture operations [10]. Cyanotoxins are the toxins produced by cyanobacteria, which are photosynthetic prokaryotes and are known to produce a wide range of secondary metabolites [11]. Depending on their toxic activity, cyanotoxins are divided into hepatotoxins (microcystins, nodularin), cytotoxins (cylindrospermopsin), neurotoxins (anatoxin-a, saxitoxin), and dermatotoxins (lyngbyatoxin A, aplysiatoxins, and endotoxin-LPS) [12]. Most reported incidents of poisoning by cyanotoxins were associated with hepatotoxic and neurotoxic blooms of cyanobacteria. However, exposure to cyanotoxins, such as microcystins (MCs) and anatoxin-a (ATX-a), leads to the death of terrestrial and aquatic animals, as well as humans [13].

Harmful algal blooms indicate environmental instability and are often triggered by multiple ecological changes. These changes fall into three general categories: (a) watershed development; (b) climate-related changes; and (c) biological changes affecting the consumption, integrity, and viability of cyanobacterial cells. In eutrophic and meso-eutrophic waters, harmful algal flora is generally more frequent and more extreme. It occasionally appears in underproductive systems, especially with human impacts, such as acidification and organic loading [14].

2 The Formation of Algal Bloom in the Water Bodies

While several kinds of algae can trigger harmful algal blooms in freshwater species, cyanobacteria usually contribute to the most frequent and severe blooms [15]. Blooms are created when the algae are high within a given region, originally due to sustained growth of algal populations, typically accompanied by the physical mechanism that further concentrates cells [16]. A common characteristic of several harmful algal species is their way of generating cysts or stages of rest [17]. The majority of bloom-forming algal are photoautotrophic, and light availability is essential. However, besides carbon, nutrients including nitrogen and phosphorus are often required to create cellular material. Silicon or iron may sometimes be restricted by algal growth. The availability of light and nutrients and cell distribution within the water body is influenced by physical factors. Similar principles can be used to control the production of toxic algal blooms in freshwater and marine environments. Marine algal-blooming species use proliferation strategies to accomplish high densities [16]. For example, large harmful algal bloom species form large, gelatinous colonies, extremely dense blooms, or allelopathic toxins that are toxic to naturally grazing organisms like zooplankton and fish [18].

Nutrients such as nitrogen and phosphorous are a natural part of aquatic ecosystems and essential for plant growth (Fig. 5.2). Yet, when they spill out of urban and rural surfaces and flow into a river, lake, pond, or reservoir, they serve as fertilizer and encourage algae and bacteria growth [18], whereas N_2 could be directly fixed

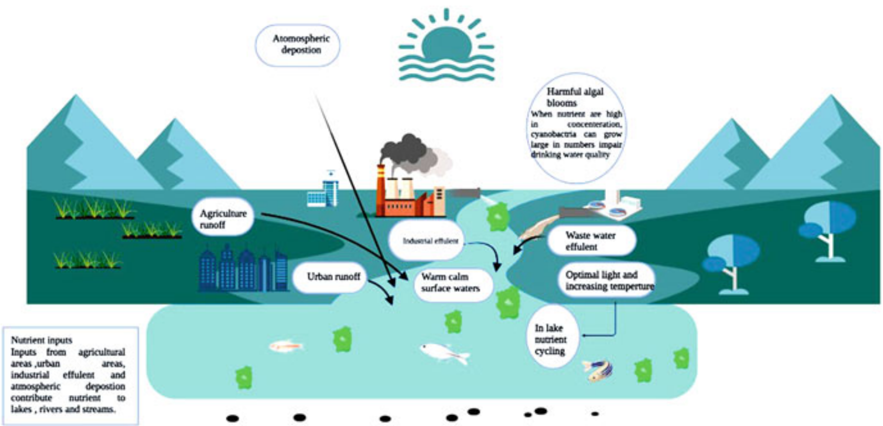

Fig. 5.2 Illustration of nutrient sources of harmful algal blooms

from the atmosphere for cell growth [19]. Warm water provides a competitive advantage for cyanobacteria. These bacteria grow more quickly at higher temperatures than benign algae, which can trigger a feedback loop after a bloom. The algae sludge absorbs more sunlight as the blooms grow thicker, resulting in algal growth. Eutrophic cyanobacteria (rich in nutrients) often substitute for other forms of algal biomass [7].

Increased anthropogenic fertilizer emissions affect lakes and reservoirs' ecology, chemistry, and physical properties. The cyano-harmful algal bloom often increases as an enrichment response [20]. Cyanobacteria survive well in the reservoir in high retention periods, because they have slow growth rates, compared to other algal types [21]. Thus, while most harmful algal blooms occur in standing waters, they can grow in very slow-flowing rivers. In 1991, after dry weather in Australia, the largest recorded cyanobacterial bloom of *Anabaena circinalis* formed 1000 km from the Barwon Darling River [22]. The stability of the water column is critical because cyanobacteria can control their growth by generating gas vacuoles to maintain an optimum position for light harvesting [23]. This mechanism is ineffective in gusty and turbulent situations, as cells are distributed over all mixed layers, but thick cyanobacterial scums can accrue on the water surface and concentrate more. When the wind blown onto the lake shores of the lake, they significantly increase the risk of toxic exposure [24].

3 Conditions that Stimulate Harmful Algal Blooms in the Reservoir

The dynamics of harmful algal blooms depend upon the synergistic stimulus of different environmental aspects, including nutrients inflow, temperature, light, pH, N_2 fixation, and hydrodynamics of the reservoir [21, 25, 26]. The construction of large-scale reservoirs is an efficient way of using water supplies, generating electricity from hydropower, and reducing flood risk. The hydrodynamic state with less water velocity and a longer residence duration causes water quality problems [27]. In man-made reservoirs, eutrophication has gained significant attention because of its harmful impact on the aquatic ecosystem and animal health [28]. There is a greater probability that algal blooms will occur, mainly while these blooms are related to (toxic) cyanobacteria. Under natural conditions, phytoplankton and cyanobacteria usually occur in aquatic environments. Unique features may allow cyanobacteria to prevail. These features include cellular physiology (for example, gas vesicles in cells allow for buoyancy regulation), physiological response (use of nutrients and light), general morphology, cell structure, and cell size. Under the condition of particular water temperature and optimum light intensity, cyanobacteria predominate over other species [29].

The available light, weather conditions, appropriate water flow, and optimized temperature are physical factors leading to the proliferation of all phytoplankton

Fig. 5.3 Harmful algal bloom at Starvation Reservoir (USA) has been spotted, and the public is forbidden from swimming in this area (*Photo from Duchesne County Sheriff's Office*)

species (including cyanobacteria). Chemical factors include pH changes, the loading of nutrients (mostly in various nitrogen and phosphorus forms), and trace metals. An algal bloom has traditionally been closely associated with high nutrient levels in water bodies with low turbidity, permitting light transmission across the water bodies. However, the conditional factors include reservoir morphology, water circulation, viruses, grazing pressure from plant-eating fish, and microbial mechanisms [30].

More reports have been published on producing harmful species emerging in the areas not previously experiencing problems, such as the cyanobacteria in eutrophic lakes in Florida (Fig. 5.3) [31].

4 Reservoir Algal Bloom in Different Countries

In China, the algal bloom in the Three Gorges Reservoir is watched closely by the Chinese government and researchers. The algal blooms were observed in the Three Gorges Reservoir tributaries, such as Xiangxi, Shennong, and Daning, instead of the mainstream of the Yangtze River [32]. These algal blooms were formed by the proliferation of harmful algae, such as dinoflagellate *Prorocentrum donghaiense*, due to the enhanced phosphorus limitation and Biogenic silica (BSi) sedimentation, which has limited the primary production of diatoms [33]. A significant occurrence of harmful algal bloom in a reservoir in Inner Mongolia, China was also reported, which prevailed for 2 months with *Dinobryon* sp. (*chrysophyte*) species being the predominant algal species (abundance of 88,520 cells/mL) [34].

In the USA, Kislik et al. reported the seasonal and interannual heterogeneity of algal blooms in Copco and Iron Gate Reservoirs using the Sentinel-2 satellite imagery, and found algae appeared to be the highest in the spring and summer with the highest peaks observed in 2019 [35]. Two reservoirs in the Highland Lakes of central Texas were observed for the duration of an extended drought period from 2010 to 2015 [36], and the result showed that an increase in inorganic and organic nitrogen following a period of drought amplified the potential for harmful blooms of *Aphanizomenon*. Tábora-Sarmiento et al. analyzed the association of air, land, and water variables with the current distribution of toxic *Prymnesium parvum* bloom in reservoirs of the Brazos River and Colorado River, Texas, USA, and found that higher salinity and wetland deficiency facilitated the establishment of harmful algal bloom [37].

In other countries, cyanobacterial occurring in the Spanish reservoirs indicate that they are toxic and dominate a significant proportion (35–48%) during the study period [38]. The toxic cyanobacterial communities in Amphur Muang (Khon Kaen Province, Thailand) were investigated in four recreational reservoirs (Bueng Kaen Nakhon, Bueng Thung Sang, Bueng Nong Khot, and Bueng). *Microcystis* sp. and *Cylindrospermopsis* sp. were the dominant species to generate algal bloom in the water samples of Bueng Nong Khot and Bueng See Than, Thailand [39]. *Saxitoxins* were found in two samples (7%) obtained from two different Czech water reservoirs at concentrations from 0.03 to 0.04 μg/L [40]. Microcystis colonies in the Isahaya reservoir (Japan) were estimated to be 34.5 kg in the water and 8.4 kg in the surface sediment of the reservoir [41].

5 Methods Used to Control Harmful Algal Bloom in the Reservoirs

Sustainable management of algae aims at limiting nutrient inflow into the water body. Effective monitoring of critical parameters for water quality and algae measurements contributes to mitigating and lowering the risk of algae growth. Phytoplankton dynamics, such as chlorophyll A, phycocyanin, temperature, DO, pH, and turbidity, can be used to forecast harmful algae blooms. Bloom's evaluation and assessment help to select appropriate safety strategies. Algae control methods include aeration, chemical & biological additives, or ultrasound technology. The control of cyanobacteria's spread has become a major global challenge. All existing approaches have major advantages and disadvantages. Many control methods, for example, algaecides, are not environmentally safe. Moreover, some approaches, such as aeration, are very expensive.

5.1 Chemicals

Algae treated with a biocide is used to control the growth of algae. Chemical lanthanum, copper sulfate, hydrogen peroxide, copper chelate, and endothall are algaecides. Copper sulfate ($CuSO_4$) accumulates in reservoir sediments that may harm fish. Hydrogen peroxide can easily be applied; however, there are uncertain long-term effects on biota. In a laboratory study, chlorine effectively removed harmful algal blooms [42].

Advantages and Disadvantages of Chemical Control First, chemical control is efficient when handling the entire surface, and it can significantly reduce algae populations, potentially eliminate toxins, remove nutrients from the water column, and control the growth of harmful algal blooms. Algaecides are costly and have to be dosed regularly. They should be carefully used as they can break the algal cell and release toxins into the water. Blooms with high algal toxins may be harmful to fish and plants. Algaecides may have significant long-term effects on the environmental quality of the lake. Moreover, they are not suited for large water surfaces [42].

5.2 Aeration

Dissolved oxygen quality in the reservoir is essential. Oxygen tends to break down nutrients and rot plants in the water. Besides, microorganisms break up the silt at the bottom. Both aerobic and anaerobic bacteria contribute to decay [43]. Aerobic degradation needs a complete oxygen supply. When dissolved oxygen levels are at their maximum, aerobic bacteria break down, and anaerobic decomposition takes longer to complete. The final products are organic compounds that smell bad, such as alcohol and organic acids [43].

Air pumping or aeration is typically used to oxygenate the smaller reservoir areas. These may be involved in airlifts, hypolimnetic diffusers, downward-flow oxygen contractors, and onsite oxygen generators. The most efficient aeration is to oxygenate the area between the top and the bottom layers. If the region between the upper and lower layers remains oxygenated, enough iron will react to phosphorus available, and oxidized iron may form. However, the residue is not toxic to organisms. Aeration is an eco-friendly way to increase the amount of oxygen in the small reservoir. Aeration systems can help prevent the use of chemicals and develop a safe environment. However, aeration is better suited for smaller, concentrated treatment areas that achieve positive results [43]. Moreover, construction costs may be if underwater dam structures are needed to maintain cool water zones. However, underwater dam structures can incur annual maintenance and operating costs.

5.3 Mixing

Mixing circulating water in reservoirs can cause desertification. The method consists of mixing water to remove layers of draping. Epilimnion and metalimnion are typically used for algae control. It is intended to clean the surface water from iron, manganese, and anoxic odors, usually in the hypolimnion layer. This reduces conditions for the growth of algae in specific layers [44]. Mixing is one way to reduce epilimnion temperature (e.g., mix cool lower layer with warmer upper layer) and create fewer favorable conditions for the evolution and continuity of a harmful algal bloom. Hypolimnetic aeration increases water quality. Discharge of nutrient-rich water prevents the formation of harmful algal blooms. Mixing can increase turbidity and may influence beneficial phytoplankton to foster invertebrate and fish species [44].

Advantages and Disadvantages of Mixing Artificial mixing does not harm the atmosphere as organic ingredients. In deep reservoirs, it is usually more successful (mean depth >15 m). However, water circulation requires high wear and tear system maintenance. There is debate about the impact on overall cyanobacterium levels. Only surface layers are frequently affected by blending in the reservoir. Mixing sediments will potentially increase the nutrients that are available in large systems. This leads to further growth of algae in the short term. Nevertheless, the decrease in algal bloom can be accomplished over a long time [44].

5.4 Ultrasound

The destruction of many cyanobacteria and algae is proven by cavitation and ultrasonic disruption methods. Cavitation processes are combined with secondary treatment methods for toxins, such as superoxide radicals or ozone [45].

Advantages and Disadvantages of Ultrasonic Algae Control Ultrasound treatment of algae is a well-known technique that has been used for many years. It has been demonstrated to be beneficial for blue-green and green algae. Ultrasound is eco-friendly and safe for plants or fish. This can be used on small reservoirs. Ultrasound incorporated into real-time monitoring enables algal bloom prediction and algal bloom prevention. The whole surface of the reservoir may be covered. To achieve maximum efficiency, each space spot must be held for a minimum time [45].

6 Conclusion

The prominent literature findings have clearly shown that the ecosystem is resilient to harmful algal bloom with increasing anthropogenic activities and the continuously changing environment. There are many approaches to dealing with toxic blooms of algal in reservoirs. In larger reservoirs, the effectiveness of these methods decreases. Neither approach solves all problems individually. A combination of methods is probably needed in larger reservoirs. Comprehensive and focused work is required to track and manage harmful algal flora in the area of reservoir water.

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Chapter 6
Microplastics Pollution in the Reservoir: Occurrence, Extraction, and Characterization

Marriya Sultan, Suman Thodhal Yoganandham, and De-Sheng Pei

Abstract Modern life's dependence on plastic materials has led to increasing microplastics (MPs) contamination in the reservoir. The increasing abundance of MPs (usually of a size less than 5 mm) has instigated the scientific and management communities regarding the monitoring of MPs in freshwater bodies. Worldwide natural freshwater bodies, such as lakes, rivers, and reservoirs, are widely documented with MPs, which interact with the organism and affect their natural habitat. This chapter highlights the relevant aspects of MPs in the reservoir system, such as their sources and abundance in major reservoirs across the world. Besides, different methodologies for MPs sampling, pretreatment, and characterization are also discussed.

Keywords Microplastics · Reservoir · Sampling · Extraction · Characterization

1 Introduction

Plastic is regarded as the most useful synthetic material developed by humans with wide application and versatile properties, such as electrical insulation, ultra-lightweight, tensile strength, durability, and anti-corrosive nature [1]. As a result, plastic production has steadily increased since 1950, with global production of about 368 million tons in 2019 [2]. Its improper use or disposal has caused severe environmental problems [3], instigating significant environmental management challenges. Annually, more than 8 million tons of plastic waste goes into the fresh and marine waters around the world [4].

Microplastics (MPs) are usually defined as tiny plastic particles with a size <5 mm, either manufactured intentionally for industrial or domestic use, such as exfoliating facial scrubs, toothpaste, and resin used in the plastic industry (primary MPs) that enters the environment directly or can be formed by fragmentation of larger plastic products by weathering or mechanical abrasion (secondary MPs) [5–8]. MPs consist of synthetic polymers, manufactured by polymerization of different monomers, which include a variety of materials like polyvinyl alcohol (PA), polyamide (PA), polyethylene (PE), nylons, polyethylene terephthalate (PET), polypropylene (PP), polystyrene (PS), and polyvinyl chloride (PVC) [9, 10] with different

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ratios of additives forming a variety of combinations. Due to their large surface area and hydrophobic nature, MPs adsorb persistent organic pollutants (such as OCPs, PAHs, PCBs, and PBDEs) and heavy metals, and carry them to other terrestrial and aquatic ecosystems [11]. Besides, because of their persistent nature and non-degradability, they pose threats to different aquatic and terrestrial organisms. Non-degradability of plastic granules affects various biological responses like cell apoptosis, necrosis, and genotoxicity. Further, it can also lead to several sub-lethal effects, such as growth inhibition, oxidative damage, and behavioral defects [12–17].

Recently, worldwide cognizance about MPs pollution is attaining momentum with important declarations from prominent world economies, such as the approval of a bill banning plastic microbeads from the varied range of consumer products in the USA and the implementation of a ban on MPs in cosmetic items in France and the United Kingdom. Similarly, some other countries are currently preparing decisions for the same cause soon [18].

Reservoirs are regarded as significant resources of freshwater as they are the main source of drinking, food production (via irrigation of agricultural areas, aquaculture, and fisheries), energy provision (hydropower dams), and the regulation of drought and flood [19]. Regardless of all the services reservoirs provide, they have not acquired the appropriate attention for monitoring MPs in reservoirs. While many research studies have focused on the evaluation of MPs in freshwater systems, such as streams, lakes, ponds, and rivers. Studies on the occurrence and distribution of MPs in reservoirs have emerged recently during the last 6 years [20]. Therefore, more research about the fate and distribution of MPs in reservoirs is required in the coming years to understand the potential risks of MPs contamination.

2 Sources of MPs in Reservoirs

The sources of MPs are diverse and show discrepancies geographically, depending upon the land-use practices. MPs in freshwater bodies, such as rivers, lakes, and reservoirs, are released via runoffs from landfills, agricultural waste discharge, and wastewater solids or effluents [21, 22]. The plastic waste from industrial sources usually ends up being dumped in landfill or open waste dumping sites in solid form or as released in surface water as industrial effluent. In developed countries, plastic waste is mostly disposed of in landfills [23]. In Europe, about 1000–4000 MPs/kg dry mass of sludge was observed in landfill and agricultural areas [24]. In agricultural zones, plastic mulch films, plastic-coated fertilizers, municipal waste, biosolids, atmospheric deposition, and irrigation runoff are the main sources of MPs in freshwater bodies, such as reservoirs [25].

Urbanization also intensifies the spatiotemporal distribution of MPs in reservoirs [26], as it is observed that reservoirs located in urban areas contained a higher abundance of MPs, compared to the others. Consequently, in highly populated regions, the contamination of MPs is more prominent, compared to other regions of the world [20]. However, in remote regions, the primary source of MPs is rainfall and atmospheric deposition. Besides, aquaculture and tourism are also regarded as prominent sources of MPs in rural and urban reservoirs [4].

3 Occurrence and Distribution of MPs in Reservoirs

MPs occurrence is distributed across wide geographic coverage with detections in different reservoirs from Asia, North America, and Europe [20]. However, the abundance of MPs in different reservoir varies widely across different regions due to differences in population statistics, economic development, and social status [27]. Table 6.1 presents the abundance of MPs in different reservoirs around the world. Ndlovu [28] reported elevated levels of MPs in Swedish Reservoirs Vombsjön and Bolmen with a mean abundance of 155.56 ± 91.6 particles/L and 80.56 ± 59.66 particles/L, respectively. Among the reservoirs in the USA, the abundance levels of MPs in Brownlee Reservoir, one of the biggest reservoirs in the western USA, were reported as 13.7 particles/m^3 [29], whereas in Lake Mead Reservoir (USA), comparatively low levels of MPs were observed with concentration ranged between 0.44 and 9.70 particles/m^3 [30]. Recent studies focusing on the spatial distribution of MPs in some main reservoirs of Asia also reported higher abundances of MPs in surface waters. For example, Turhan [31] reported the MPs abundance levels in the range between 106.6 and 200 particles/m^3 in Sürgü Dam, Turkey. Similarly, in the Aras Dam Reservoir (Iran), the MPs levels in surface water from 19 locations ranged from 1 to 43 items/m^3 (mean 12.8) with MPs of size 0.1–5 mm being more dominant [32]. Moreover, the MPs abundance in the Jatiluhur Reservoir (Indonesia) ranged between 0.71×10^4 and 4.59×10^5 particles/km^2 with Polyethylene (PE) being the common type of polymer found in surface water samples. Recently, Nousheen et al. [33] also assessed MPs occurrence in the Rawal Dam Reservoir (Pakistan) and observed the mean levels of 2.8 ± 1.44 particles/L (using the sieve method) and 0.025 ± 0.024 particles/L (using trawl method).

In China, the Danjiangkou Reservoir and Three Gorges Reservoir are the main reservoirs that reported the MPs distribution. MPs characteristics in these two reservoirs are comparable to the other freshwater bodies with fragments and fiber (size of most <1 mm) being more dominant, compared to the others [4]. Zhang et al. [34] assessed the MPs occurrence in the TGR region where the abundance was reported between 34.1×10^5 and 136.1×10^5 particles/km^2 (Yangtze River) and 0.55×10^5–342×10^5 particles km^{-2} (Xiangxi Bay, the TGR), respectively. Two years later, Zhang et al. [35] also studied the Xiangxi River in the TGR region and reported the abundance of MPs in a range between 550 and 14,580 particles m^{-2}.

Di and Wang [36] assessed the occurrence and distribution of MPs alongside the Yangtze River of TGR and reported the overall abundance of MPs as 1597–12, 611 n/m^3 in surface water with size 0.5–5 mm being more dominant. Tan et al. [39] reported the MPs in surface water of Feilaixia Reservoir with mean abundance levels

Table 6.1 Abundance levels of MPs in different reservoirs around the world

Reservoir	Abundance/levels in water	Size and polymer types	References
China			
Three Gorges Reservoir (TGR), Yangtze River	34.1×10^5 to 136.1×10^5 particles/km^2		[34]
Yangtze River (TGR)	1597–12,611 particles m^{-3}		[36]
Xiangxi Bay, the TGR	0.55×10^5–342×10^5 particles km^{-2}		[35]
Xiangxi River (the TGR)			[37]
Yangtze Estuary	500 n/m^3 to 10,200 n/m^3		[38]
Feilaixia Reservoir	0.56 ± 0.45 items/m^3	PP (52.31%) PE (27.39%)	[39]
Danjiangkou Reservoir	530–24,798 items/m^3 (7205 items/m^3)	PA(24.8%) PE (24%) PP (17.1%)	[40]
Jiayan Reservoir	1.10×10^4 to 6.17×10^4 items/m^3	<300 μm, PE	[41]
Other regions of the world			
Vombsjön Reservoir, Sweden	155.56 ± 91.6 particles/L		[28]
Bolmen Reservoir, Sweden	80.56 ± 59.66 particles/L		[28]
Brownlee Reservoir, USA	13.7 particles/m^3		[29]
Lake Mead Reservoir, US	0.44–9.70 particles/m^3	355–1000 μm (73.1%) 1000–5600 μm (26.5%)	[30]
Jatiluhur Reservoir, Indonesia	0.7×10^4–4.5×10^5 particles/km^2	PE	[42]
Sürgü Dam, Turkey	106.6–200 particles/m^3	Size:1–2 mm PP and PE	[31]
Rawal Dam, Pakistan	2.8 ± 1.44 particles/L (sieve method) 0.02 ± 0.024 particles/L (trawl method)	Size: 0.1–0.9 mm PP (40–74%)	[33]
Navua Irrigation Dam, Fiji	2.9 ± 0.4 particles/L	Size: 0.5–0.9 mm and 1.0–1.4 mm	[43]
Aras River, Iran	1–43 items/m^3 (mean: 12.8 items/m^3)	Size: 0.1 to 5 mm PE (36.6%)	[32]

of 0.56 ± 0.45 items/m^3. Recently, Lin et al. [40] assessed the MPs occurrence and distribution in the Danjiangkou Reservoir and reported very high levels with an abundance range of 530–24,798 items/m^3 (7205 items/m^3). Besides, Niu et al. [41] evaluated the MPs abundance in the Jiayan Reservoir and reported the levels within the range of 1.10×104 to 6.17×104 items/m^3 with MPs particles of size<300 μm as more dominant among others.

4 MPs Sampling and Analysis Techniques

The collection of MPs from water or sediment and their extraction from organic and mineral material is quite challenging. The choice of sampling and preservation techniques generally relies on the research aim, economic affordability of the methods, sample characteristics, and the study matrix [44]. The quantification and identification techniques for MPs are not comprehensively standardized, which could result in a significant difference in output results from different studies carried out in the same place and time [45]. The detection of MPs in the reservoir includes three basic steps, such as sample collection, sample pretreatment, and characterization of MPs [46].

4.1 Sample Collection

Based on the type of compartment that is required to be examined (water, sediment, or biota), different sampling strategies could be adopted. Reduced volume and bulk sampling are the two main methods for collecting surface water [47]. Bulk sampling does not result in a reduction of water volume unlike the reduced volume sampling technique, in which on-site filtration by nets or sieving is carried out [48]. For a volume-reduced sampling of surface water, nets are mainly used that directly filter the debris from the large volume of samples [49]. Manta trawl and neuston plankton net are usually used for collecting samples from surface water. The size and type of MPs that are collected while sampling largely depends upon the mesh size of the collecting tool. Such smaller-sized mesh tools for the collection of MPs could result in the collection of large amounts of fibers, compared to other types of MPs, and could also result in over or underestimation of the abundance of MPs [46].

An alternative method of collecting the sample is through a pump or grab sampler. A pump sampler consists of a motor containing an inline filter, and water is pumped manually into the sampler. In grab sampling, a bucket is used to collect and sieve the water in the field [46, 50]. The manta trawling and pump sampling methods give different results in the abundance, shape, and size of MPs as observed in a study, where both of these methods were employed for the MPs sampling of Lake Tollense, Germany. It was recommended to use pump sampling instead of manta trawl, which does not retain the fibers and small-sized MPs sufficiently. However, pump samplers could filter large volumes of water generating reliable results [51]. Barrows et al. [52] observed that grab samplers collected MPs three times more in magnitude than the conventional neuston net sampling technique. However, for effective monitoring, a combination of both volume-reduced (net-based) and bulk sampling (pump and grab-based) approaches is reliable depending upon the objective of the study.

The sampling tools for the sediment matrices are selected for collecting places and objectives. For sediment matrices, manual grab samplers, such as stainless steel spoons and hand spades, are used for sampling from littoral zones of reservoirs. However, for deep sediments, different types of grab and core samplers, such as box corer [53] or Van Veen grabber [54] are used.

4.2 Sample Pre-treatment

After the collection of MPs samples from water or sediments, extraction and purification is carried out. MPs particles are separated from the matrix as sometimes the plastic is confused with biological material (algae fragments). Therefore, a simple digestion method is required to reduce the amount of organic matter without affecting the chemical and chemical integrity of the MPs [46]. Hydrogen peroxide is a vigorous oxidizing agent mainly used to digest organic matter without affecting the integrity of MPs [36]. Samples are generally preserved with methyl aldehyde (5%) [34], fixed in formalin (2.5%) [38], or could be submerged in 40% ethanol (EtOH) and kept at 4 °C before analysis [55].

4.3 Characterization of MPs

MPs characterization is of two types including physical characterization and chemical characterization. Physical characterization involves the classification of MPs according to their sizes, shapes, and color; whereas chemical characterization refers to the composition of MPs [56]. For the physical characterization, a stereomicroscope is mostly used to gauge the size and count the abundance of MPs. However, the visual identification of MPs relies on the operator as the stereomicroscope has low magnification, and it is difficult to distinguish different fibers. Therefore, a higher magnification stereomicroscope should be used to confirm the nature of MPs [57].

For chemical characterization of MPs, different technologies have been used, such as gas chromatography coupled to mass spectrometry (GC-MS) [58], liquid chromatography (LC) [59], Raman spectroscopy [60], energy-dispersive X-ray spectroscopy (EDS), scanning electron microscope (SEM) [61], attenuated total reflection-Fourier transform infrared (ATR-FTIR) spectroscopy [62]. Moreover, in many research studies, two to three multiple technologies have been used to identify MPs, such as the stereomicroscope, ATR-FTIR, SEM, μ-FTIR, and EDS. However, the most commonly used technologies for MPs analysis are μ-FTIR spectrophotometer, μ-Raman, and SEM [61].

4.3.1 FTIR Spectroscopy

FTIR spectroscopy provides spectral ranges for identifying plastics from other organic and inorganic materials. The MPs particle is exposed to infrared radiation, which forms a spectrum with distinguishing peaks corresponding to definite chemical bonds between atoms. To ascertain the sample composition, FTIR spectroscopy obtained polymer spectrum is identified by the well-established polymer spectrum library [63]. FTIR spectroscopy enables spectra to be collected in attenuated total reflectance (ATR) or transmission modes. ATR-FTIR and micro-FTIR are the common FTIR techniques used to characterize MPs. ATR is a unique sampling technique with infrared spectroscopy, which directly enhances the study of the samples in liquid and solid states without further preparation [56]. Micro-FTIR has become increasingly popular, because it characterizes the sample size up to 20 μm. Micro-FT-IR analysis of plastics can be conducted in either transmission or reflectance mode. To obtain high-quality spectral data, infrared-transparent substrates are required in transmission mode [64].

4.3.2 Raman Spectroscopy

Raman spectroscopy is a vibrational spectroscopic technique, which produces a vibrational spectrum by the principle of inelastic stretching formed by the molecular vibrations of the MPs [37]. The Raman spectral data resembles a chemical structure with a fingerprint that allows the identification of components in the sample. The micro Raman combined with Raman spectroscopy is used to detect spatial resolution below 1 μm. In this technique, the studied samples are irradiated with laser light between the frequency of visible, infrared, or ultraviolet ranges [36]. It displays enhanced spatial resolutions (up to 1 μm) as compared to FTIR [65]. However, Raman spectroscopy is susceptible to fluorescence intrusion, caused by organic, inorganic, or microbiological entities in samples. Therefore, samples must be carefully purified before the analysis [59].

4.3.3 Scanning Electron Microscopy (SEM)

The SEM yields MPs images by scanning their surface with a concentrated beam of electrons, which characterizes the surface morphology of MPs [66]. Additionally, SEM-energy-dispersive X-ray spectroscopy (SEM-EDS) and environmental scanning electron microscopy-EDS (ESEM-EDS) are also used for identification of the elemental composition based on reflection and diffraction of radiations emanated from the surface of MPs surfaces alongside the characterization of surface morphology of MPs [22, 56].

5 Conclusion

With the increasing plastic utility, the discharge, distributions, and abundance of MPs in freshwater systems have increased, thereby increasing the health risks for the associated fauna. Owing to their health risks, MPs are widely documented in freshwater rivers and lakes but fewer studies have examined the MPs pollution in the reservoir system globally. Reservoirs, being an important source of water for various human activities, play a crucial role in maintaining the socio-economic well-being of the nations. Therefore, it is vital to assess the contamination of reservoirs in different countries. Moreover, the sampling and analytical techniques employed for MPs detection in reservoirs need to be standardized, because they vary dramatically among the published studies. Further, extensive research is required to develop an understanding of using appropriate sampling and analytical techniques for MPs detection in reservoirs.

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Conflicts of Interest The authors declare no conflict of interest.

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Chapter 7
Distribution of Microorganisms in the Reservoir

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Abstract This chapter aims to determine the microorganism distribution in the Three Gorges Reservoir Area (TGRA). However, limited seasonal studies make it difficult to comprehend its real situation. Previous literature found that, before the dam construction, *alpha and gamma proteobacteria* were more prevalent in all surface water samples. While in 2009, *betaproteobacteria*, *gammaproteobacteria*, and *deltaproteobacteria* were ubiquitously found in the TGRA. In 2015, the general bacterial richness trend was found in the order of downstream > middle stream > upstream of the TGRA. Seasonally, in summer, most bacterial and archaeal communities were found due to the increased nutrient levels and higher temperatures. Ecological risk via the bacteria-based index of biotic integrity (Ba-IBI) showed that 25% were Excellent, but 50% and 25% were Good and Fair among all sampling sites. In conclusion, the risk score for overall bacterial ecology was appropriate. However, more studies should be conducted timely to evaluate ecological health and make early warnings when abnormal microorganisms appear in the reservoir.

Keywords Microorganisms · Water quality · Ecological risks · Bacteria · Early warnings

1 Introduction

Microorganisms, known as microbes, are microscopic unicellular or cell-cluster organisms and infectious agents that include bacteria, viruses, protozoa, and microscopic fungi & algae. Among all microorganisms, bacteria are considered the most abundant microorganisms in the aquatic ecosystem [1]. Microbes have a great influence on determining the health and stability of the ecosystem [2]. They serve as decomposers and play an important role in the cycle of carbon, phosphorous, and nitrogen [3]. Microorganisms may influence the function, structure, and services of aquatic environments [4]. Microbial activity initiates the decomposition of organic matter (OM), maintains the whole-reservoir respiration and carbon flow to the higher trophic levels, and thus upholds the entire food web structure of the aquatic ecosystem [5]. In the aquatic environment, bacterial composition and diversity vary with

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the water quality. Many studies have been published previously focusing on the Three Gorges Reservoir Area (TGRA) regarding bacterial composition and diversity that shifts seasonally and annually with the pollution load. Similarly, nutrient pollution mainly from total nitrogen (N) aggravates the eutrophication process, which may change the microbial diversity in the TGRA [1].

Despite nutrient pollution, microorganisms play a mediated role in transforming mercury (Hg) to methyl mercury (MeHg) in sediments or water [6]. Recently, two genes *hcgA* and *hcgB* were discovered to play an important role in the methylation of mercury [7]. Moreover, *Deltaproteobacteria*, *Chloroflexi*, *Firmicutes*, and *Euryarchaeota* are involved in the methylation of mercury [6].

2 Factors Affecting the Microbial Diversity in the Aquatic Environment

Environmental factors, such as temperature, humidity, and precipitation, influence the microbial community pattern [1]. Similarly, seasonal variations and geographical distance alter the microbial distribution in the aquatic environment [8] (Fig. 7.1). The hydrological condition of water, such as flow velocity, water depth, water

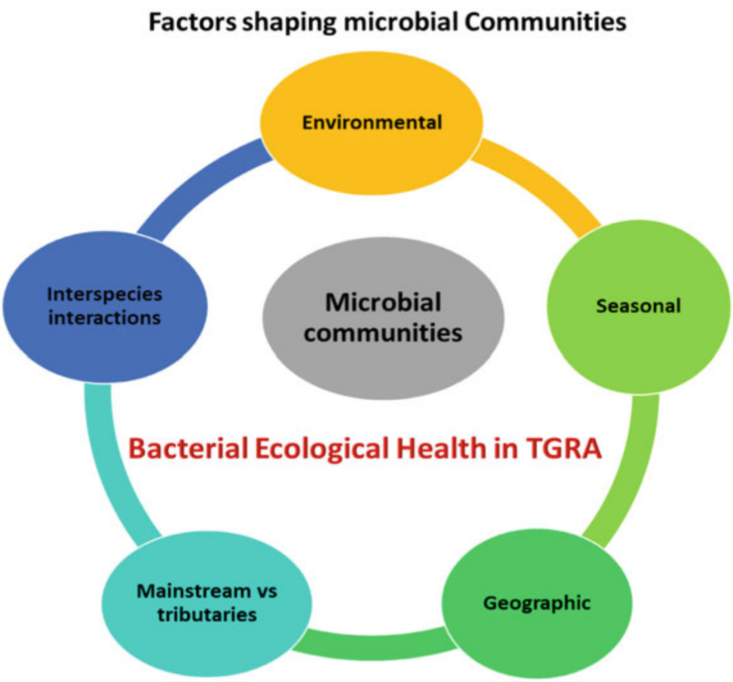

Fig. 7.1 Factors affecting the microbial diversity in the TGRA

temperature, pH, dissolved oxygen (DO), and nutrient concentrations may cause a spatiotemporal change in the microbial communities [9]. According to most of the previous studies, environmental factors were observed as more crucial to influence the aquatic microbial diversity, compared to spatial factors [1]. Notably, few other studies revealed that spatial factors significantly influence the distribution of microbes in water bodies [10–13].

Among hydrological parameters, pH plays a decisive role in nutrient metabolism. DO influences the efficacy of microbial degradation of substances and microbial growth [14]. Moreover, DO also regulates the redox reaction and affects loads of functional genes, involved in nitrogen and phosphorus cycling [15]. Different microorganisms have their physiological limitations for growth and reproduction within specific levels of DO, narrow ranges of pH, and nutrient availability, which influence the community structures. Moreover, the activities of microorganisms could alter the environmental properties, such as the concentrations of enzymes and nutrients, the form and amount of DO & carbon, and pH [16]. Seasonal and spatial variations also bring changes in concentrations of nutrients, which in return alter the microbial community composition [17]. Biotic processes regulating the seasonal variations of nutrients through the assimilation and mineralization processes are closely related to temperature variation, thus disturbing the propagation of bacterial communities in water bodies [18]. The spatial diversity of incoming water in the reservoir and climatic conditions cause variations in water temperature stratification patterns [19]. In the lower layer of the reservoir, the temperature is almost constant, compared to the upper surface layer, which also affects microbial diversity [14, 20].

3 Microbial Diversity and Phylogenetic Distribution Pattern in the TGRA

As the Three Gorges Reservoir (TGR) was established on the Yangtze River, it became a discontinuity for the riverine system due to the counter-season impoundment of the Three Gorges Dam (TGD). Thus, they affected river-borne nutrients transported by the river [21]. The upstream and downstream areas of the reservoir vary prominently in their physicochemical properties [22], which change the microbial communities. Sedimentation due to damming also shifts the proportion of free-living and particle-attached bacteria. The previous studies described the longitudinal shifts in bacterioplankton community structure that are recognized to be caused by prolonged water residence time [23]. Since the construction of the TGR, bacterial diversity had changed gradually from the upstream to the downstream [24]. After the construction of the reservoir in June 2003, changes in bacterial community structure in the Yangtze River-East China Sea estuary were also observed. According to the report from Jiao et al. [25], the overall bacterial diversity was decreased and a lower abundance of *Betaproteobacteria* was found. However, the diversity of

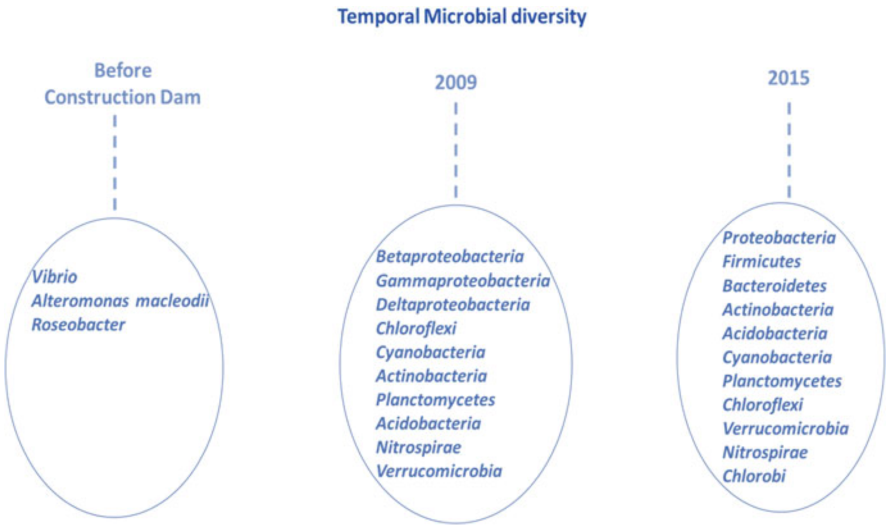

Fig. 7.2 The temporal microbial diversity observed in the TGRA

Alphaproteobacteria and *Cyanobacteria* was improved because of the abrupt decline of river runoff and subsequent intrusion of ocean currents [26].

Recently, from environmental samples, the bacterial distribution can be determined by sequencing 16S RNA [27]. Before the construction of the TGRA, it was found that *alpha and gamma proteobacteria* were ubiquitously presented in all samples [6] (Fig. 7.2). Based on genotype and phenotype analysis, *Alteromonas macleodii* and *Roseobacter* spp. were dominant in most surface water samples. Contrarily, in 2009, Wang et al. [28] found that *betaproteobacteria, gammaproteobacterial*, and *deltaproteobacteria* were dominantly presented in the surface water samples of the TGRA. In 2015, bacterial abundance and water quality were closely observed along the upper, middle, and lower reaches of the TGRA by Niu et al. [29]. Results indicated that at the highest chemical oxygen demand (COD) level, the highest number/abundance of *Firmicutes* and *Bacillus* were found in the upper, middle, and down reaches [29]. Besides, the results of redundancy analysis revealed that COD and phosphates were the primary environmental parameters that significantly affected the bacterial community structure [29]. Furthermore, the general bacterial richness trend decreased in the order of downstream > middle stream > upstream [29]. Although Proteobacteria dominated the bacterial communities, Ochrophyta, Chlorophyta, and Ciliophora became the majority during specific stages in the TGR (Table 7.1) [30–32].

Table 7.1 The microbial community observed in the TGRA

Microbial diversity of the TGR	Reference
Alpha-, Beta-, Gamma proteobacteria, Verrucomicrobia, and Planctomycetes Spirochaetes, Nitrospirae, Chloroflexi, and Acidobacteria	[28]
Acidobacteria, Spirochaetes, Chloroflexi, and Nitrospirae	[26]
Firmicutes, Proteobacteria, and Actinobacteria	[4]
Ochrophyta (Protist)	[30]
Ciliophora and Chlorophyta	[32]
Chironomidae, Heptageniidae, and Baetidae	[33]
Diatoma vulgare Melosira varians, Cocconeis placentula, Gyrosigma scalproides, and Oscillatoria tenuis M. varians, Cymbella affinis, D. vulgare, Eucapsis alpina, and M. granulata, M. varians, C. affinis, and C. placentula,	[34].

4 Effect of Environmental Variations on Microbial Diversity in the TGR

Environmental factors play an important role in shaping the microbial community structure in the aquatic environment [35, 36]. Canonical correspondence analysis (CCA) exhibited relatively high changes in bacterial community composition, which are primarily correlated with the surface water quality parameters, such as temperature, COD, phosphates, nitrates, carbonates, and DO [37]. For the year 2009, the water quality parameters were found to be relatively stable [37], whereas in 2015, the water quality parameters particularly, COD and phosphate were relatively high [29]. From the water samples in 2017, redundancy analysis (RDA) revealed that the microbial population showed a significant correlation/association with environmental factors, such as NO_3, NH_4, total organic carbon (TOC), and total phosphates [8]. Due to the TGRA being geographically divided into three longitudinal zones, including riverine, transitional, and lacustrine, different bacterial diversities were found in each zone [8, 38, 39].

Chen et al. [37] evaluated the seasonal comparison of the bacterial diversity at different impoundment levels in the TGRA for the year 2009. It was found that, in the summer, more bacterial and archaeal communities were commonly present in the surface water of the TGRA, due to high nutrient levels and temperature. In terms of the topological distribution, more diverse bacterial populations have been found downstream than that in the middle and upper stream of the TGRA [37]. Moreover, the bacterial diversity via Simpson's index and Shannon index revealed that bacterial diversity dramatically changed between dry and wet seasons. Phylogenetic analysis showed that *Betaproteobacteria* and *Actinobacterium* were found to be the most dominant bacterial populations observed in wet and dry seasons [37]. Further, with the high or low water impoundment level, a variety of bacterial niches were found in the TGRA, which needs to be studied in detail. Also, interspecies interactions of the communities should be explored.

Water level fluctuations in the TGRA may affect the soil microbial compositions. It is worth mentioning that microorganisms contained a variety of aerobic, anaerobic, and facultative populations. However, in soil, anaerobes are barely found, which cannot be ubiquitously distributed in the surface water of the TGRA. It was found that, at low water impounding levels, the mercury methylation activity by microbes was also low [6]. The 16S RNA sequence implied that bacterial richness in soil was relatively higher in the dry season, compared to the wet season, and the bacterial abundance fluctuates seasonally [6]. At the phylogenetic level, *Deltaproteobacteria* and *Methanomicrobia* were found to be higher, which were involved in Hg methylation, and showed increased bacterial activity in the dry season [6]. Moreover, soil nutrients, such as organic matter, nitrates, and phosphates, are found to be higher in content in the dry season than wet season, which aggravate the soil microbe's growth [40].

5 The TGRA Ecological Health via Bacteria-Based Index

In the past years, there was a dire need to quantitatively evaluate the ecological health of the TGRA using the bacteria-based index of biotic integrity (Ba-IBI). Li et al. [3] found the bacterial ecological status in surface water and sediments samples at different water impoundment levels, such as a low water level (September 2016), a high impounding period (December 2016), and a sluicing period (April 2017) [3]. Moreover, certain ecological reference conditions including land use, urbanization, pollution, river connectivity, soil erosion, sediment load, riparian zone, and biodiversity determine the anthropogenic disturbances and the hydromorphology of the TGRA (Fig. 7.3). From the environmental matrices, *Acidobacteria*, *Geobacter*, and *Gemmatimonadetes* were found to be dominant and were selected to indicate the bacterial ecological risk in the TGRA. Among 12 sampling sites, 25% were reported to be Excellent. However, 50% and 25% were Good and Fair according to bacterial

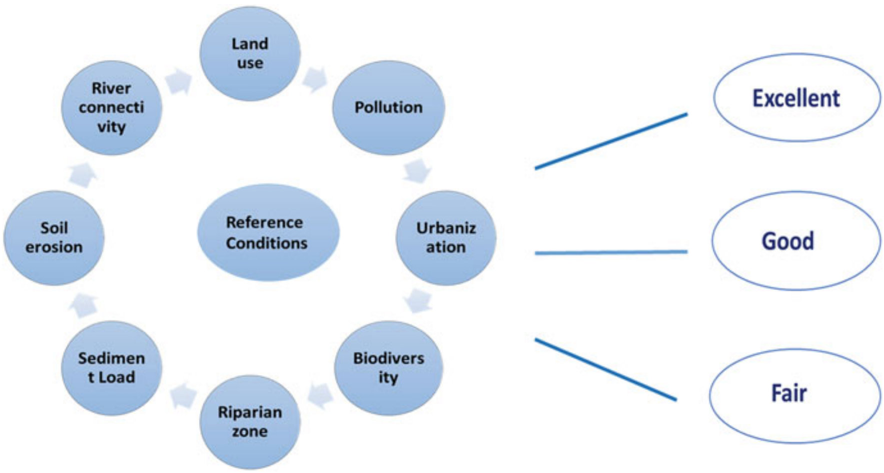

Fig. 7.3 Reference conditions for determining the bacteria-based index of biotic integrity

ecological health [3]. The biotic integrity was greatly influenced by ecological and environmental changes along with nutrient fluctuations [3, 41, 42]. Besides, the ecological health in the low impoundment level and the sluicing period was good compared to the high impoundment level, implying the hydrodynamic changes due to the seasonal increase or decrease of the water level.

Similarly, another recent study highlighted that relative microbial population distributions are affected by ecological variables [8]. Their results showed that homogeneous selection, dispersal limitation, ecological drift, and variable selection accounted for 51.3, 25.5, 21.2, and 2.0% of the microbial metacommunity distributed in the TGRA [8]. Moreover, it was found that variable selection processes were relatively higher in the summer and also reached up to 100%, compared to the winter [8].

6 Conclusion

In summary, this chapter summarizes the distribution of microorganisms in the TGRA at different impoundment levels. It was found that before the construction of the dam, *alpha and gamma proteobacteria* were dominant in all surface water samples. In 2009, *betaproteobacteria, gammaproteobacteria*, and *deltaproteobacteria* were ubiquitously found in the TGRA. Notably, the bacterial richness trend decreased in the order of downstream > middle stream > upstream in 2015. The season-wise comparison revealed that more bacterial and archaeal communities were commonly found in the summer due to high nutrient levels and temperature. Ecological risk via the bacteria-based index of biotic integrity (Ba-IBI) can indicate water quality. In short, the overall bacterial ecological health of the TGRA was found to be stable. However, it may vary seasonally, therefore, more temporal and seasonal studies are required to determine the in-depth mechanistic details according to the bacterial diversity.

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Conflicts of Interest The authors declare no conflict of interest.

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Part III
Study Methods of Ecotoxicology in the Reservoir

Chapter 8
Ecotoxicity Test Methods of Primary Producers in the Reservoir

Marriya Sultan and De-Sheng Pei

Abstract Primary producers occupy a bottom position in the food chain. For that reason, they play a vital role in the transfer of pollutants and ecotoxicity evaluations across the food chains. Ecotoxicity of freshwater bodies, such as reservoirs, is mainly evaluated using species of the primary producer, including *Vibrio fischeri* (bacterium), *Raphidocelis subcapitata* (microalgae), *Spirodela polyrhiza* (floating macrophytes), and *Myriophyllum* (submerged macrophytes). This chapter provides an overview of the use of primary producers in ecotoxicity assessment, standard ecotoxicity methods using primary producers, and the general procedure of some standard ecotoxicity tests given by international organizations. Besides, some recent literature regarding the use of ecotoxicity tests with primary producers in water quality assessment of reservoirs is reviewed and issues related to primary producers-based ecotoxicity tests are also highlighted.

Keywords Primary producers · Microalgae · *Vibrio fischeri* · Reservoirs · Ecotoxicity

1 Introduction

Freshwater reservoirs are considered important water reserves for human and animal consumption, irrigation, and recreational activities. Over the past few decades, a growing concern is witnessed with the decrease in water quality due to enhanced domestic, agricultural, and industrial outputs [1]. To improve the water quality, several legislations have been implemented across the world, which advises the assessment of important freshwater bodies for pollutant concentrations and collection of acute and chronic toxicity data on aquatic organisms (primary producers, invertebrates, and vertebrates) for setting the water quality standards [2]. In response to these legislations (such as Directive 2000/60/E), various organizations have developed guidelines for testing water quality. Among them, the most widely accepted one is the OECD guideline, which includes the collection of internationally accepted ecotoxicity testing methods that can be used by independent laboratories, industries, and governments to determine the safety levels of different pollutants in various ecosystems (terrestrial & aquatic) [3].

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Generally, chemical analysis is mainly carried out to monitor the pollutants, which does not give an accurate prediction of effects on organisms because it does not take into consideration of the direct interaction between organisms and the chemicals, such as dynamics of environmental heterogeneity, the bioavailability of the chemical, and organism life cycle. Conversely, bioassays provide more precise evidence of the effects produced after exposure to pollutants [2, 4]. In this perspective, ecotoxicological tests based on primary producers undertake an evident role in evaluating the quality status of freshwater bodies [5]. Being present at the bottom of the food chain, primary producers bioaccumulate the pollutants in water and transfer the pollutants to the herbivores and carnivores. Finally, humans will be contaminated along the food chain, resulting in the biomagnification process. Hence, ecotoxicological studies based on primary producers have the potential to identify the possible risks across the food chains [6]. Also, being the first interface amid abiotic and biotic constituents, plants react to water pollution earlier, compared to other organisms [7]. However, the use of primary producers, such as plants, in ecotoxicological tests is still limited [8, 9]. The most commonly used primary producers are bacterias (such as *Pseudomonas putida*) and unicellular algae [10]. Besides, aquatic micro- and macroinvertebrates (including *Daphnia magna* and *Gammarus duebeni*) and zebrafish (*Danio rerio*) are also frequently used organisms for ecotoxicity tests [7, 11].

2 Standardized Ecotoxicity Tests Using Primary Producers

Primary producer-based tests gained momentum during the last two decades [9, 12]. Microalgae were more commonly utilized as reference species, compared to other plant groups. Ecotoxicity tests using microalgae with various effluents and toxicants were the first to be designed and standardized since the 1960s by regulatory agencies, such as the ISO and the OECD [13]. The most common microalgal species used in ecotoxicology studies for a wide range of pollutants include *Pseudokirchneriella subcapitata* (*Selenastrum subcapitata*) and *Chlorella vulgaris* (Table 8.1) [7]. Afterward, since the 1970s, some aquatic pteridophytes (such as *Salvina molesta and Azolla pinnata*) and bryophytes (*Fontinalis antipyretica*) were also used for ecotoxicological tests with much lesser frequency, compared to other species. However, freshwater species, such as *Lemma* (floating macrophytes) and *Myriophyllum* (rooted flowering plants), have been used frequently for ecotoxicological studies since the 1950s.

Several standardized procedures for ecotoxicity testing have been proposed by globally recognized organizations, such as the US EPA (the United States Environmental Protection Agency), ISO (International Organization for Standardization), OECD (Organization for Economic Cooperation and Development), and ASTM (American Society for Testing and Materials) [28]. These organizations have given 13 standard tests using freshwater primary producers. Of which, three tests are selected for bacterial species, two tests for primary algal species (diatoms, green

Table 8.1 Standardized ecotoxicity test using primary producers

Aquatic primary producer	Species	Compartment	Organization	Test no.	References
Bacteria	*Pseudomonas putida*	Water	ISO	10712	[14]
	Vibrio fischeri	Water	ISO	11348-1	[15]
	Salmonella typhimurium	Water	ISO	11350	[16]
Microalgae	*Raphidocelis subcapitata*	Water	OECD	201	[17]
	Pseudokirchneriella subcapitata	Water	ASTM	D3978-04	[18]
	Anabaena flos-aquae	Water	USEPA	850.4550	[19]
Floating macrophytes	*Lemna minor*	Water	ISO	20079	[20]
	Spirodela polyrhiza	Water	ISO	20227	[21]
	Lemna gibba	Water	ASTM	E1415-91	[22]
	Lemna sp.	Water	OECD	221	[23]
	Lemna spp.	Water	USEPA	850.4400	[24]
Submerged macrophytes	*Myriophyllum aquaticum*	Sediment	ISO	16191	[25]
	Myriophyllum spicatum	Water	OECD	238	[26]
	Myriophyllum spp./ *Glyceria maxima*	Water & Sediment	OECD	239	[27]

algae, and cyanobacteria), five tests for floating macrophytes consisting of duckweed species, and three tests for submerged macrophytes (Table 8.1). The detailed procedure of some of the common standardized ecotoxicity tests using primary producers, such as bacteria *(Vibrio fischeri)*, microalgae (*Raphidocelis subcapitata),* and floating macrophyte (*Spirodela polyrhiza)* is given as follows.

2.1 *Luminescence Inhibition Test of* Vibrio fischeri

This test is based on the hypothesis that the luminescence of bacteria is reduced when exposed to pollutants and the toxicity is expressed as the concentration of the toxicants (pollutants) [2]. Water samples are placed in glass cuvettes and *Vibrio fischeri* bacterium is transferred to cuvettes. After 15 min, the toxicity of water samples is evaluated by computing a 50% reduction in luminescence using a temperate luminometer. And results are reported as percentage inhibition [2, 15].

In the control group, the change in luminescence containing the bacteria suspension is determined during the incubation time t. From this data, a correction factor f is calculated as follows:

$$f = L_t / L_0$$

Where L_0 is the initial luminescence and L_t is the luminescence over time. The corrected initial luminescence (L_{0C}) is calculated as

$$L_{0C} = L_0 \times 100$$

In the treatment group, the change in luminescence after the required incubation time (L_t) is determined as ΔL ($\Delta L = L_{0C} - L_t$).

Whereas the percentage inhibition of the test chemical is calculated as:

$$\mathrm{INH\%} = \frac{\Delta L}{L_{0C}} \times 100$$

2.2 *Growth Inhibition Test of* Raphidocelis subcapitata

This test is carried out in 24-well plates and a Woods Hole Marine Biological Laboratory (MBL) culture medium is used as a negative control. The natural water samples are assessed using three treatments and a blank (3 replicates each). In each treatment, the initial concentration of 5×10^4 cells/mL of microalgae (*Raphidocelis subcapitata)* is added. The microplates are then placed in a climatic chamber (Incubator) at 24 °C with a light intensity of about 7000 lux. The algal cultures are re-suspended every day to reduce sedimentation. After 72 h culture, the absorbance is measured in each well at $\lambda = 440$ nm using a UV-Spectrophotometer. In each treatment, the absorbance of the corresponding blank is removed to nullify the growth and existence of potential constituents that could affect the final results. Finally, the concentration of cells in each well is calculated using the following equation.

$$C = -17{,}107.5 + \mathrm{ABS} \times 7{,}925{,}350$$

Where C is the concentration of algae in cells/mL and ABS is the absorbance dimension ($\lambda = 440$ nm). The final results are quantified as yield, which is the difference between initial and final biomass (cell densities- cells/mL) [17, 29].

2.3 *Growth Inhibition Test of* Spirodela polyrhiza

Spirodela polyrhiza is carried out in glass vials and the Steinberg medium is used as a negative control for growth inhibition assay. The natural water samples are assessed under three treatments with 3 replicates for each treatment with a final volume of 100 mL. In each treatment and corresponding replicate, 9 fronds are added at the start of the test. The glass vials covered with perforated parafilm (for gaseous exchange) are retained in a climatic chamber (incubator water testing) for a week at 24 °C with a permanent light intensity of about 7000 lux. After 7 days culture, the number of fronds is counted and the total yield is calculated, which is the difference between the number of fronds at the end and the beginning of the test [21].

2.4 *Vegetative Shoot Inhibition Test of* **Myriophyllum spicatum**

Myriophyllum spicatum ecotoxicity test is to assess the pollutant-related effects on the vegetative growth of plants growing in freshwater water and sediment. The test is conducted by potting the shoot apices of healthy, non-flowering *Myriophyllum spicatum* plants in artificial sediment, supplemented with nutrients for the sufficient growth of plants. The potted plants are then retained in Smart and Barko medium. After an adequate period of root formation, plants are exposed to different test concentrations via water column or the sediment by spiking the sediment with the different concentrations of the test chemical. Later, plants are transplanted into the spiked sediment. After 14 days of culture, the effects on growth are assessed by numerically calculating the shoot length, fresh weight, and dry weight and by qualitative observations of growth deformities, or symptoms, such as chlorosis or necrosis [30].

For quantification of chemical-related effects, the growth of the plant in test solutions is compared with the control plants, and the concentration that causes growth inhibition is determined and expressed as the Effective Concentration (EC). Based on regulatory requirements, values of EC_{10}, EC_{20}, or EC_{50} are calculated.

The response variables, such as average specific growth rate and yield, are calculated. The average vegetative shoot growth rate is based on changes in the total shoot length, such as the total fresh weight of the shoot and the total dry weight of shoot. The mean weight and length of the three plants per test and the growth rate of each replicate are calculated using the following formulate:

$$\mu i - j = (\ln(Nj) - \ln(Ni))/t$$

Where μi-j is the mean specific growth rate from time i to j, Ni is the measurement variable in the vessel at time i, Nj is the measurement variable in the vessel at time j, and t is the time from i to j.

Percent inhibition of growth rate (Ir) is calculated for each test concentration according to the following formula:

$$\%Ir = \mu c - \frac{\mu t}{\mu c} \times 100$$

Where *% Ir* is the average growth inhibition in percentage, μc is the mean value for μ in the control, and μt denotes the mean value for μ in the treatment group.

Finally, the yield is calculated, based on variation in total shoot length, including total fresh weight and total dry weight of the shoot over time in both groups (controls and treatment). The mean percent inhibition in yield (*% Iy*) is calculated as:

$$\%Iy = bc - \frac{bt}{bc} \times 100$$

Where the *% Iy* is the percent reduction in yield, *bc* is the difference between final biomass from the initial biomass for the control group, and *bt* is the difference between final biomass from the initial biomass in the treatment group [26].

3 Ecotoxicity Assessment of Freshwater Reservoirs Using Primary Producers

The encouragement of using primary producers (mainly plants and algae) for ecotoxicity testing was given by the Toxic Substances Control Act (TSCA), which highlighted the sensitivity of freshwater algae to various pollutants [13]. Since then, various studies have used ecotoxicological tests with primary producers to determine the toxic effects of different pollutants, such as pesticides, herbicides, hydrocarbons, and trace and heavy metals. However, the studies regarding the use of primary producers for ecotoxicity testing of reservoirs are still scarce with few studies carried out during the last decades, featuring the freshwater reservoirs of Europe [1, 2, 31–33] and South America [34, 35] as shown in Table 8.2.

Pérez et al. [2] performed an ecotoxicity test using microalgae species, *Pseudokirchneriella subcapitata*, for evaluating the impacts of pesticides and heavy metals on primary producers of Alqueva Reservoir, Portugal. The results demonstrated that *P. subcapitata* was sensitive even toward the low concentrations of insecticides in the water. Baran et al. [32] evaluated the ecotoxicity of nutrients and trace elements in the sediments of Rożnów reservoir, Poland using *Vibrio fischeri* luminescence inhibition test and *S. alba* root and seed inhibition test, where the *S. alba* germination inhibition ranged between 22 and 53%, while root growth inhibition ranged within 23 to 63% with Germination Index (GI) values ranged between 30 and 152%. However, the *V. fischeri* luminescence inhibition was observed within the range of 28 to 57%. In another study, Szara et al. [33] verified the ecotoxicity level of sediment samples obtained from Rożnów reservoir, Southern Poland using *L. sativum S. saccharatum* and *S. alba*, and they found that the inhibition of root growth of these plants ranged between −70 to 37% for *S. saccharatum,* −47 to 45% for *S. alba* and −69 to 61% for *L. sativum*. L.

Table 8.2 Recent studies on the ecotoxicity of freshwater reservoirs using primary producers

Primary producer	Test species	Matrix	Stressors	Endpoint measured	Location	References
Algae	*Pseudokirchneriella subcapitata*	Water	Pesticides & heavy metals	Growth inhibition	Alqueva reservoir Portugal	[2]
	Raphidocelis subcapitata	Water	Pesticides & heavy metals	Growth inhibition	Aguieira reservoir, Germany	[1]
Floating macrophyte	*Spirodela polyrhiza*	Water	Pesticides & heavy metals	Growth inhibition	Aguieira reservoir, Germany	[1]
Bacteria	*Vibrio fischeri*	Water	Pesticides & heavy metals	Luminescence inhibition	Alqueva reservoir Portugal	[2]
	Vibrio fischeri.	Sediment	Trace metals	Luminescence inhibition	Rybnik reservoir, Poland	[31]
	Vibrio fischeri.	Sediment	Nutrients & trace elements	Luminescence inhibition	Rożnów reservoir, southern Poland	[32]
Plants	*Sinapis alba*	sediment	Nutrients & trace elements	Inhibition of seed germination & root growth	Rożnów reservoir, southern Poland	[32]
	Sinapis alba	Sediment	Heavy metals	Inhibition of seed germination and root growth	Ružín reservoir, Slovak Republic	[36]
	Sorghum saccharatum, Lepidium sativum & sinapis alba	Sediments	Trace elements	Inhibition of seed germination and root growth	Rożnów reservoir, southern Poland	[33]
	Lepidium sativum & Sinapis alba	Sediments	Nutrients and trace elements	Germination index	Rio Grande reservoir, Brazil	[34]
	Sinapis alba	Water	Nutrients & estrogens	Germination index	Billings reservoir, Brazil	[35]

Coelho et al. [35] evaluated nutrients and estrogens related to phytotoxicity using *Sinapis alba* in the Billings Reservoir (Brazil), and observed the GI values as 82.74% and 83.16% with no phytotoxic effect in site 1 and site 2 for wet and dry periods, respectively, whereas site 3 was classified as moderately phytotoxic with the GI values ranged between 60 and 80%. In another study, the growth inhibition ecotoxicity test with primary producers, such as *R. subcapitata* and *Spirodela polyrhiza* showed lower growth rates in the reservoir water during autumn and higher growth rates during the spring season. The growth inhibition of these two species during autumn was associated with the occurrence of herbicides, such as metolachlor, atrazine, and terbuthylazine, which are used mainly during summer in nearby agricultural areas of Aguieira reservoir, Germany [1].

4 Concerns in Standardized Primary Producers-Based Ecotoxicity Tests

In the previous study, several concerns have been reported regarding the use of these ecotoxicity tests, when these tests sometimes do not reciprocate the required results. The protocols of these ecotoxicity tests refer to their execution in controlled conditions of the laboratory that confine the ability of tests to deduce the real status of a natural system (such as other interactions and limiting factors that are ignored in lab-based experiments) [7]. Therefore, the obtained results do not fully reflect the real effects of tested pollutants on plants. For example, Ding et al. [37] studied the effects of silver nanoparticles (AgNPs) on *Lemna minor*, which are generally diminished in the humic acids (HA) as it reduces the absorption ability of AgNPs in *Lemna.* Besides, the exposure time given in standardized protocols does not always produce the anticipated response. For example, exposure extension (from 7 days to 14 days) could increase the intensity of the response providing an additional understanding of the pollutant toxicity [5]. Moreover, the use of standard growth undiluted growth medium (proposed by standard protocols) could also interfere with the tested chemical and could lead to various complexities in results interpretation. Gubbins et al. [5] found that silver nanoparticles (AgNPs) show different responses in the *Lemna* plant depending on the growth medium (either diluted or concentrated). As the interaction of AgNPs with the concentrated medium showed aggregation and sedimentation, a 100-fold diluted growth medium was used to reduce AgNP-medium aggregation. This assumes that the related problems might also occur with other standardized tests, therefore, preliminary tests are recommended to verify any interactions among pollutants and medium.

5 Conclusions

Because primary producers occupy the bottom place of the food chain, it is evident that they play role in pollutant transfer across the food chain and assist in ascertaining the possible toxic risks across the food chains. In this viewpoint, ecotoxicity tests based on primary producers are considered vital in evaluating the quality status of freshwater bodies. The guidelines for standardized ecotoxicity tests using primary producers are given by various organizations, such as the OECD, ISO, and ASTM, which have used sensitive species from different groups of primary producers for ecotoxicity tests. However, among the reported literature, the species of microalgae are more commonly utilized in ecotoxicity tests, compared to other plant groups. However, some of the common standardized ecotoxicity tests using primary producers include luminescence inhibition test with *Vibrio fischeri*, growth inhibition test with *Raphidocelis subcapitata, Spirodela polyrhiza*, and *Myriophyllum spicatum*. However, the standardized ecotoxicity test does not include the representative species from all major groups of primary producers and standard guidelines for ecotoxicological tests with some major groups, such as the missing mosses and lichens. Therefore, to increase ecological relevance, more test species representing all major groups are required to be incorporated in ecotoxicity testing, for which standard protocols are needed to be devised by authoritative organisms, such as the EPA, ISO, OECD, and ASTM. Besides, various concerns regarding ecosystem complexities, such as lack of understanding of synergistic effects, time scale, exposure time, and growth medium are required to be considered. Further, such tests should be designed to suitably represent the ecosystem complexity and achieve more consistent responses about processes occurring in the natural environment.

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Chapter 9
Ecotoxicology Methods of Reservoir Water Using Invertebrates

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Abstract This chapter explains different ecotoxicological methods utilizing invertebrates for the assessment of water quality of reservoirs and provides information on species of invertebrates that are involved mostly in ecotoxicological assessment assays. Invertebrates occupy a key position in the aquatic ecosystem and serve as intermediate consumers in the food chain, therefore, they are useful indicators of water toxicity. Various standard ecotoxicity tests approved by international organizations are being used in toxicity testing of water bodies, which includes acute and chronic toxicity tests using invertebrate species. The most acceptable organisms in ecotoxicity testing utilizing invertebrates are Daphids (*Daphnia magna*, *Daphina pulex*, and *Ceriodaphnia dubia*), which are also recognized as standard species. Previous studies also reported the use of crustaceans, such as amphipods, branchiopods, insect species, and rotifers.

Keywords Invertebrates · Ecotoxicology · Aquatic toxicology tests · *Daphnia magna*

1 Introduction

Inputs of various hazardous chemicals from agricultural activities, leaching of groundwater, sewage discharges, and runoff are affecting the overall quality of the freshwater reservoirs. Therefore, to reduce the risk instigated by hazardous compounds to the aquatic ecosystem, the assessment of water quality is essential for the execution of the monitoring programs. An integrated evaluation of harmful impacts of chemicals on aquatic ecosystems and populations is necessary, which must include characterization of chemicals complemented with bioassays as it allows the evaluation of effects from all components of water including effects from unknown substances [1]. For this purpose, ecotoxicological assessments are conducted to support the research on the harmful effects of pollutants on different organisms at various trophic levels [2]. Biological organisms mainly include vertebrates (such as fish and amphibians) and freshwater and marine invertebrate species. Different species are used in different toxicity tests. The difference is their proneness to chemicals due to their genetic factors and physiological factors, such as metabolic

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rate, excretion rate, dietary factors, and health of the organism. The most commonly used standard aquatic test species include zebrafish, fathead minnow, sheep head minnow, rainbow trout, grass shrimps, midges, daphnids, oysters, mysids, scuds, and mussels [3, 4].

2 Invertebrates for Ecotoxicological Testing

Invertebrates have been used for ecotoxicological assessments for decades. They are considered useful to detect pollutants mainly due to their bioaccumulation and biological characteristics. Moreover, they could also be used as early warning indicators to predict toxicological effects at the population and community levels [5]. Invertebrates like crustaceans and mollusks are integral to ecotoxicological studies and are extensively used as test species in a laboratory for ecotoxicological assessments. Daphnia and intertidal copepods like *Tigriopus japonicus* are classic invertebrate examples [6]. Besides, various other freshwater invertebrate models like arthropods, annelids, and bivalves are also used for ecotoxicology evaluation [7]. Their small life period supports them to be utilized to study the multigenerational and reproductive effects of harmful chemicals with limited resources [5]. European methods of inter-calibration practices have shown that approaches using benthic macroinvertebrates were the most effective among other methods [8]. Invertebrates are considered major targets in ecotoxicology because of their vital roles in ecosystem function, wide biodiversity, their suitability for experimental methodologies in the field and laboratory, and the available genomic data of ecotoxicology model species [9].

3 Invertebrate Test Species

It is observed that organisms inhabiting different habitats are suitable for holistic study of aquatic pollutants, because their health assessment after exposure to these pollutants at trophic levels is desirable. Therefore, they are also ideal invertebrates for reservoir ecotoxicity assessment [7]. Among crustaceans, intertidal copepods are considered suitable test organisms. The *Tigriopus japonicus* is considered susceptible to stressors like copper, fluorene, phenanthrene, UV, and gamma radiation [10–12]. Recently, *Paracyclopina nana* is considered for its efficacy as a test organism in ecotoxicological studies [7]. They are often used in ecotoxicological studies of moderately soluble chemicals like drugs and pharmaceuticals [13–17]. For toxicological studies, planarians are commonly utilized [18, 19] and considered suitable for examining developmental toxicity, potential genotoxicity, teratogenicity, and tumorigenicity of chemicals [20]. Acute toxicity test using planarian species has been conducted in many studies [21, 22]. In a previous study [23], the acute toxicity of various pesticides and metals using the freshwater planarians model has been reported, which demonstrates their susceptibility to cadmium, copper, mercury, tributyltin, and insecticides. The toxicities of ammonia and nitrite have also been evaluated using freshwater planarians [20].

Freshwater invertebrates, such as Daphnids (*Ceriodaphnia dubia*, *Daphnia pulex*, and *Daphnia magna*) were the recommended species for acute toxicity tests [24], because they could be cultured in the laboratory with ease and are sensitive to various classes of pollutants. Daphnids used for acute toxicity tests are usually less than 24 hours old. Other freshwater invertebrate species like mayflies, stoneflies, and amphipods should be in an early instar, whereas midges are in the second or third instar [25]. Besides, *Daphnia magna* is utilized in the majority of aquatic toxicity tests and cultured easily in the laboratory because of its small size, parthenogenetic reproduction, small reproductive cycle, and high fecundity [2]. The American Society for Testing and Materials (ASTM), American Public Health Association (APHA), Food and Agriculture Organization of the United Nations (FAO), and US Environmental Protection Agency (US EPA) are the known accepted regulatory units and give the list of some major species used as test organisms [3, 24, 25] (Table 9.1).

In benthic detrital and grazing food chains, the class of crustaceans and rotifers play a major role for primary consumers. Therefore, test methods with rotifers and crustaceans (copepods, amphipods, cladocerans, and insect larvae) have been developed, because they are considered representatives of major freshwater aquatic communities [3].

4 Ecotoxicity Tests

The toxicity tests provide qualitative and quantitative data for an ecological risk assessment, which aids in the evaluation of the adverse effects of environmental pollution on the survival of ecological receptors exposed to toxic compounds [2]. Aquatic ecotoxicology tests could be performed both in the field and laboratory. In the field experiment, multiple species are exposed to multiple toxicants, whereas in laboratory experiments exposure to single species is considered. To quantify the effects, a dose-response relationship is applied at certain criteria for adverse effects or selected end-points (lethal or sublethal effects) [4]. Toxicological assessments determine the potential bioavailability or biological damage caused by toxicants to tissues or organs. They are useful to describe the nature of a noxious effect [2]. Some generally include early life-stage tests for fish and invertebrates, whereas others are full life-cycle tests with invertebrates, such as shrimps and mysids [26]. The methods applied in reservoir water toxicity assessment could be classified as either instream or in situ observations on communities, instream bioassays, and laboratory tests (to assess acute or chronic toxicity to single species) [8] according to exposure time, test situation, and the effects to be measured [27].

A series of standard tests for ecotoxicity assessment have been published in scientific literature by widely accepted regulatory agencies, such as the APHA, US

Table 9.1 A list of major freshwater invertebrate species used for toxicity assessments

Phylum	Order/Species	Listed by organization
Protozoans	Ciliates	
	Tetrahymena pyriformis	APHA
Vermes	Annelids	
	Branchiura sowerbyi	APHA, FAO
	Limnodrilus hoffmeisteri	APHA, FAO
	Stylodrilus heringianus	APHA
	Tubifex tubifex	APHA, FAO
	Platyhelminthes	
	Dugesia tigrina	ASTM
Molluscs	Gastropods	
	Amnicola limosa	ASTM
	Physa integra	ASTM
	Physa heterostropha	ASTM
Crustaceans	Amphipods	
	Gamrnarus lacustris	ASTM, USEPA, APHA, FAO
	Garnrrwrus pseudoIimnaells	ASTM, APHA, US EPA
	Gammarus fasciatus	ASTM, APHA, US EPA, FAO
	Hyalella azteca	APHA, FAO
	Pontoporeia affinis	APHA
	Hyalella spp.	US EPA
	Branchiopods	
	Daphnia magna	APHA, FAO, ASTM, US EPA, OECD
	Daphma pulex	ASTM, US EPA, OECD
	Daphnia pulicana	ASTM
	Daphnia spp.	OECD
	Ceriodaplmia spp.	US EPA
	Decapods	
	Cambarus spp.	APHA, US EPA, FAO, ASTM
	Orconectes rusticus	APHA
	Orconectes spp.	ASTM, US EPA
	Procambarus spp.	ASTM
	Pacifastacus leniscuIus	ASTM, US EPA
	Palaemonetes cummingi	APHA
	Palaemonetes	APHA
	Mysids	
	Mysis relicta	APHA, FAO
lnsect larvae	Plecopterans	
	Pteronarcy dorsata	APHA
	Pteronarcy califonica	APHA
	Pteronarcy spp.	ASTM, FAO
	Hesperoperla lycorias	APHA

EPA, International Organization for Standardization (ISO), Environment and Climate Change Canada (ECCC), and Organization for Economic Co-operation and Development (OECD) [4]. The nature of the test design is subjected to available resources, time, laboratories, type of chemical pollutants, and physical and chemical properties of environmental matrices under investigation [4]. These tests are useful to characterize the spatial distribution of toxicity and temporal trends by monitoring at different times in different locations [2] (Table 9.2).

Organisms are exposed to the selected chemical pollutant based on four systems, including static assemblies (organisms are exposed to toxicants in standing water and the test solution is not altered [27]), the renewal test (a static test but the test solution is renewed after intervals), the recirculating system (organisms are retained in a chamber and the test solution is pumped inside through a filter maintaining oxygen and nutrient levels, which is usually used for chronic exposures), and a flow-through test (the test organisms are exposed to a test solution with flow through the compartments in which the test organisms are placed) [4].

4.1 Acute Toxicity Tests

Acute toxicity tests are short exposure period tests that generally range from a few hours to several days, and are used to assess the growth, immobility, and mortality of an organism [27]. In short-term exposure, organisms are exposed to a high dose of chemicals either in single or multiple events and produce instantaneous effects. Such tests are carried out using test species during a specific life cycle of the organism, usually called a partial life-cycle test. These tests are not considered valid if mortality is greater than 10% in the control [56].

Presently, *Daphnia* is the most commonly used for acute toxicity tests, which is recognized as the standard aquatic toxicity assay. These tests are comparatively easily performed when a good culture is established and are cost-effective as they are of shorter duration (48 vs. 96 hrs). Moreover, the maintenance of their culture needs less effort, equipment, and space [3]. Ecotoxicology protocols recommend *Daphnia magna* or *Daphnia pulex* as a test invertebrate species. *Daphnia magna* less than 24 hours of age are commonly used for this test [2]. Mortality and immobility are noted at 24 and 48 hours and matched with the values of control. The concentration of the test substance, dissolved oxygen, and pH are examined at the beginning and the completion of the test. The LC_{50} or EC_{50} is a major parameter to measure acute toxicity [2, 3].

Embryos of rotifer *(Brachionus calyciflorus*) used in this toxicity test are hatched from cysts by incubation in dilute water for 24 h at 25 °C in darkness. Commercially cultured strains of cysts are recommended because of their well-characterized sensitivity. In each culture plate, 10 newly hatched neonates are placed with 1 ml of test solution. At the end of the test, living and dead rotifers are counted and percent survival is noted with a dissecting microscope in each test chamber [57].

Table 9.2 Studies reporting the assessment of various toxicity endpoints using invertebrates species

Species	Stressors tested	Endpoint measured	Type of test	Reference
P. nana	UV	Mortality, reproductive parameters	life cycle	[28]
P. nana	Food (5 microalgae)	Fecundity, mortality, growth	diet	[29]
P. nana	Temperature, salinity,	Gene expression	mRNA expression	[30]
P. nana	Gamma radioisotope	Growth, fecundity	radiation	[28]
P. nana	Heavy metals, EDCs	Molecular, Vg expression	EDCs, HM	[31]
P. nana	UV	Clutch number, growth pattern, assimilation of diet, DNA repair	UV exposition	[32, 33]
P. nana	Light intensity	Survival, growth, productivity	light exposure	[34]
P. nana	Density, antioxidants	Naupliar production, gene expression	culture density	[35]
Acartia tonsa	Diflubenzuron	Reproductive rate, development Morphological abnormalities,	Life-cycle (part.)	[36]
Acartia tonsa	TBT	Development, acute toxicity, and mortality	Life-cycle (part.)	[37]
	Methyltestosterone, fenarimol		Exposition	
Acartia tonsa	Nickel	Mortality	Acute	[38]
Acartia tonsa	Zinc	Mortality	Acute	[38]
Acartia tonsa	Synthetic musks	Acute toxicity development, mortality	Partial life-cycle (larval development)	[39]
Acartia tonsa	Polybrominated diphenyl ether hydroxyecdysone, tetrabromobisphenol A	Acute toxicity development, mortality	Partial life-cycle(larval development)	[40]
Amphiascus tenuiremis	Atrazine	Reproduction, population growth rate, malformation,	Acute	[41]
Amphiascus tenuiremis	Fipronil	Mortality, reproduction	Population growth rate, acute	[42]
Amphiascus tenuiremis	Fipronil	Reproductive rate	Full life-cycle	[43]
Amphiascus tenuiremis	Fipronil, vitellin (VTN)		Full life-cycle	[44]
Amphiascus tenuiremis	Single-walled carbon nanotubes		Full life-cycle	[45]

(continued)

Table 9.2 (continued)

Species	Stressors tested	Endpoint measured	Type of test	Reference
Eurytemora affinis	Cadmium, copper	Mortality	Acute	[46]
Daphnia magna	nonylphenol	Mortality, Reproduction	Acute	[47]
Daphnia magna	Juvenile hormone	Mortality, Immobility		[48]
Ceriodaphnia dubia	Styrene	Reproduction rate	Chronic	[49]
Daphina magna	Bisphenol A	Reproduction rate	chronic	[50]
Daphnia magna	Cadmium	Survival rate	Acute	[51]
Ceriodaphnia dubia and Daphnia carinata	Zinc, Copper, Lead	Reproduction and Mortality	Acute	[52]
Daphnia pulex	Organic selenium	Mortality	Acute	[53]
Daphnia magna	Fenvalerate (insecticide)	Mortality, survival	Acute	[54]
Daphnia magna	Propiconazole (pesticide)	Reproduction rate, offspring growth	Life cycle	[55]

4.2 Chronic Toxicity Tests

Long-term exposure tests that last from weeks to months or years relative to the life span (usually >10%) are known as chronic tests. The test organisms have to come in contact with low and frequent doses of chemical pollutants. These toxicity tests allow the long-term evaluation of concentrations of chemicals and produce sublethal effects [27]. Chronic toxicity tests are considered full life cycle assessments as they cover the full reproductive life cycle and are not regarded as valid if the percentage of mortality is more than 20% in the control sample. The results are stated in EC50s, the lowest observed effects level (LOECs), and no observed effects level (NOEL). There are sub-chronic exposures that are applied in early life stages and include the sensitive life stage of the organism (also called the critical life stage). Endpoints include reproduction survival and developmental changes.

4.2.1 The Survival and Reproduction Test of Ceriodaphnia dubia

The *Ceriodaphnia dubia* reproduction and survival test is a seven days chronic toxicity assay, which was proposed by Mount and Norberg [58]. Later, it was adopted by the US EPA and ASTM as a standard toxicity assay for chronic toxicity tests. *Ceriodaphnia dubia* is preferred because of its short exposure time, compared to *Daphina magna* [58]. In this test, juvenile *Ceriodaphnia* is treated with different concentrations of pollutants in a static renewal system for 7 days. Each neonate is placed in 15 mL of test solution. During this period, the control organisms produce three broods. Each day organisms are fed and numbers of young produced and survived are noted. Results are centered on the reproduction capability and survival ratio. Developmental parameters, LOEC, and NOEC are determined [3]. Results are considered valid when 60% of survived females must produce three broods with each female producing 15 offspring and at least 80% of overall control organisms will survive [59].

4.2.2 The Reproduction Test of Daphina magna

In this chronic bioassay, the effect of a contaminant on the reproduction of *Daphnia magna* during a 21-day exposure is assessed. The test is set up by placing young female neonates ($n = 60$) in a test solution of five different concentrations (each neonate in 50–80 mL solution) in a static renewal system for 3 weeks. The numbers of surviving and produced organisms are determined after every two days. The experiment results are based upon the comparison of the offspring number per surviving female in the toxicant treatments with the reproductive output, compared to controls. The test is ended on the 21st day, and the LOEC and NOEC are calculated [3].

5 Bioaccumulation

Bioaccumulation tests are commonly utilized for chemicals that are hydrophobic as these chemicals could be stored in the fatty tissues of aquatic species. Due to they have low water solubilities, the species can accumulate such toxicants at a high level [60]. Their storage within the organism's body may cause cumulative toxicity. To predict the concentration of chemicals within the body of organisms, bioconcentration factors (BCF) are used. It is the ratio of average chemical concentration in the tissues of the organism, compared to the mean concentration in the water. There are different standard methods for reservoir bioaccumulation tests. Bioaccumulation tests could be performed in many ways like active bioaccumulation, passive biomonitoring laboratory setup, and simulating methods [4]. Four types of bioaccumulation monitoring are usually followed, such as stream active bioaccumulation monitoring, stream passive bioaccumulation monitoring, and laboratory simulation approaches. For carrying out active biomonitoring, invertebrate species are collected from unpolluted sites and then exposed to polluted sites in the field for a definite duration. The most commonly used organisms are freshwater mussels like *Anodonta anatina* and *Dreissena polymorpha* because of their extensive existence and potential of resisting the high concentration of toxic substances [61].

For regulatory purposes, bioconcentration factors are determined according to the OECD documents using fish flow-through tests, which are expensive, time-consuming, and many organisms during the experiments. To overcome these issues, the freshwater amphipod species, *Hyalella Azteca*, is used as a substitute test species for such studies because of its high reproduction rate and fast growth, which meets the requirement of a large number of organisms for study [62]. Previous numerous laboratory tests have been conducted to assess the bioconcentrations of metals, organo-metals, insecticide DDT, and polycyclic aromatic hydrocarbons in *Hyalella azteca* [63–70]. Amphipods are raised in the laboratory, and 50 test organisms are in a 2 L flask. After 8-week development, they become mature enough to be used for bioconcentration studies. A 20 L of test solution is stocked with about 1200 test organisms and kept in 8/16 h dark. The uptake phase lasts from 2–12 days and organisms are exposed to a constant concentration of test chemicals [71]. For each chemical, the exposure period is adjusted based on the previous studies to make sure whether the equilibrium state has been achieved or not. After the completion of uptake, test organisms are moved into a new aquarium. Samples of test organisms are analyzed according to the standard procedure. BCF factors are calculated according to the quantity of the test chemical in the tissue represented as (Ch) and their comparable levels in water (Cw), which is given by the below equation [62].

$$\mathbf{BCFss = Ch/Cw}$$

Besides, amphipods, calanoid copepods, *Pseudodiaptomus annandalei*, and *Eurytemora affinis* have also been used to study the bioaccumulation of heavy metals from surface water [72]. *Gammarus pulex* and *Notonecta glauca* were reported to study the uptake and bioaccumulation of pharmaceuticals [73].

6 Conclusion

Formerly, various chronic and acute toxicity tests were developed for reservoir ecotoxicity analysis, but only a few tests have been accepted as standard toxicity tests for routine toxicity assessments. The best recognized are chronic and acute toxicity tests with *Daphnids*. Invertebrates are ecologically diverse and there is great phylogenetic complexity among them. Consequently, they are sensitive to many ecotoxicological responses and exhibit variability after exposure to different stressors, which are considered suitable for ecotoxicity testing. Research efforts for developing new standard toxicity procedures using invertebrates are required.

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Chapter 10
Ecotoxicology Methods of Reservoir Water Using Fish

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Abstract This chapter aims at elucidating the use of model fish species in ecotoxicological studies and the methodology of ecotoxicity testing based on fish. Chemicals are released into the reservoir and pose a harmful impact on the biota. Water quality and fish health are associated with each other. Therefore, fish is considered a suitable pollution indicator for monitoring reservoir pollution. To assess the effects of these chemicals, different standardized toxicity methods using fish have been developed by international organizations, which include acute and chronic toxicity tests. Species including zebrafish, rainbow trout, Japanese medaka, and fathead minnow are the most recommended fishes and are selected for a toxicity test according to the contaminant's type and the endpoints to be measured.

Keywords Fish · Aquatic toxicology test · Zebrafish · Acute toxicity · Chronic toxicity

1 Introduction

For decades, the assessment of reservoir ecotoxicological risks and the elucidation of molecular mechanisms of contaminant-induced dysfunctions have remained unambiguous [1]. The formal beginning of ecotoxicology as a separate science was procured in the late 1960s, and reservoir ecotoxicology is proposed in this book. Recently, many studies based on the risks of reservoir ecotoxicology are being conducted including the contamination characteristics, source apportionment, climate change, and potential ecotoxicological effects [2–4]. Environmental toxins profoundly affect the fish population and the health of wildlife and humans. Despite the presence of various species in the aquatic environment, fish is considered a more suitable indicator of water pollution. Fish health is associated with water quality when water contamination affects fish populations by disrupting lifespan, embryonic development, and reproductive health [5]. Toxicological testing using fish has been a common practice in the past, and fish death has been recognized as a general water pollution indicator. Current fish toxicology has moved towards mechanistic and multidisciplinary approaches [5].

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2 Ecotoxicological Importance of Fish and Its Use in Ecotoxicity Testing

Fish is being utilized as sentinel organisms in ecotoxicological studies because of their capability of inhabiting all zones of the aquatic habitat where prevails suitable conditions for their survival, feeding habits, physiological diversity, reproductive strategies, and economic significance [6]. For anthropogenic pollutants, reservoir aquatic environments are acknowledged as the absolute sink, and fish death could be observed as a consequence of the toxic action of the pollutants. Thus, fish is commonly recognized as the substitute for evaluating the deleterious effects of contaminants on reservoir ecosystem health. The first ecosystem disturbances studied using fish were the impacts of mine-tailing effluents as reported by Carpenter [7]. In the middle of the twentieth century, techniques were standardized for acute fish toxicity testing. Toxicological tests were developed and validated to the internationally agreed testing procedures used by laboratories, government, and industry to illustrate prospective hazards of novel and existing contaminants. About 20% of the tests recognized by the OECD (Organization for Economic Co-operation and Development) for evaluating health effects on living systems are conducted using the fish model [8]. In the 1960s, concerns about long-term exposure of organisms to contaminants were raised and flow-through techniques were developed. Various biomarkers and endpoints were developed by studying the effects on early-life stages, reproductive cycles, and complete life cycles. New molecular techniques were developed and research was more concentrated on the detection and understanding of the toxicity mechanism of chemical substances [5]. The practice of using wild fish population indices for the assessment of ecological status in water bodies has also grown since the 2000s [9, 10]. Recently, the assessment of emerging contaminants, such as microplastics, pharmaceuticals, and pesticides, has been performed based on fish acute and chronic toxicity, and lethal and sublethal effects are evaluated using novel approaches of modern molecular biology. The acute and chronic toxicity of emerging chemicals using different fish species are presented in Table 10.1.

3 Fish Models

For ecotoxicity testing, fish species are selected based on different factors, such as size, ease of laboratory maintenance, suitability for testing, known sensitivity, available data, and the availability of test procedures and protocols that could be followed [8]. Zebrafish, rainbow trout (*Oncorhynchus mykiss*), Japanese medaka (*Oryzias latipes*), and fathead minnow (*Pimephales promelas*) are the most common species used for ecotoxicological studies. Zebrafish is considered the most popular testing model, because it shares common anatomy and development features, metabolism, as well as physiological and chemical-induced organ responses with

Table 10.1 Acute and chronic fish toxicity studies

Contaminant	Test Organism	Exposure duration	Toxicity	References
Diazinon	*Anabas testudineus*	96 h	Acute (LC 50) 6.55 ppm	[11]
Diazinon	*Channa punctatus*	96 h	Acute (LC 50) 3.09 ppm	[11]
Cadmium, copper, zinc	*Rainbow trout (O. mykiss)*	–	Acute and chronic (LC50 116 (μg/L)	[12]
Permethrin	*Cyprinus carpio*	24 h	Acute (LC 50) 35 μg/L	[13]
Dichlorovinyl Dimethyl phosphate	Zebrafish	24 h	Acute LC 50 39.75 mg/L,	[14]
Methyl parathion	*Catla catla*	96 h	Acute (LC 50) 4.8 ppm	[15]
Cypermethrin	*Colisa fasciatus*	96 h	Acute (LC 50) 0.02 mg/L	[16]
Malathion	*Labeo rohita*	96 h	Acute (LC 50) 15 mg/L	[17]
Endosulfan	*Channa striatus*	96 h	Acute (LC 50) 0.0035 ppm	[18]
Cypermethrin	*Labeo rohita*	96 h	Acute (LC 50) 4.0 μ/L	[19]
Endosulfan	*Labeo rohita*	96 h	Acute (LC 50) 2.15 μg/L	[20]
Dimethoate	*Labeo rohita*	96 h	Acute (LC 50) 24.55 μg/L	[21]
Poly Ethylene	*Pomatoschistus microps*	96 h	Acute: Reduced AChE activity	[22]
Poly Ethylene	Japanese medaka	2 months	Chronic: Histopathological alterations	[23]
Poly Ethylene	Japanese medaka	2 months	Chronic: Altered expression of a gene mediated by the estrogen receptor in the liver	[24]
Poly styrene	Zebrafish	7 days	Uptake and bioconcentration Accumulated in fish gills, liver, and gut	[25]
Chromium	*Channa punctatus*	60 d, 120 d	Chronic: 2.6 mg L^{-1} LDH activity inhibited in liver and kidney.	[26]
3-benzylidene camphor	*Pimephales promelas*	14 d, 21 d	Chronic: VTG LOEC 435, 74 μg L^{-1}	[27]
Oxybenzone UV filter	*Oncorhynchus mykiss*	14 d	Chronic: VTG LOEC 749 μg L^{-1}	[28]
Triclosan	*Oncorhynchus mykiss*	96 d	Chronic: Hatching, Survival No Effect, LOEC 71.3 μg L^{-1}	[29]
Triclosan	*Oryzias latipes*	14 d	Chronic: Hatching LOEC 213 μg L^{-1}	[30]
Triclosan	*Oryzias latipes*	21 d	Chronic Growth, Fecundity, HSI and GSId, VTGe LOEC 200 μg L^{-1}, No Effect,	[30]
Fluoride	Salmon	–	Chronic: 0.5 mg/l Significant disruption of PGC migration	[31]

(continued)

Table 10.1 (continued)

Contaminant	Test Organism	Exposure duration	Toxicity	References
3-benzylidene camphor	*Pimephales promelas*	14 d	VTG, Reproduction, Gonad Histology LOEC 434.6, 74 μg L^{-1}	[32]
Benzylparaben	*Pimephales promelas*	48 h	Acute LC_{50} 3.3 mg/L	[33]
Isobutylparaben	*Pimephales promelas*	48 h	Acute LC_{50} 6.9 mg/L	[33]

humans. Moreover, other characteristics, such as small size, rapid development, the optical transparency of embryos, low cost, and easy maintenance, make it to be an ideal model species [34]. Zebrafish is also responsive to chemical and genetic screens and has a fully sequenced genome [35]. Further, zebrafish offers in vivo high-throughput assays, which are less costly than rodents. The huge population size of zebrafish provides a prompt assessment of multiple toxicity testing and facilitates the study of molecular mechanisms, and developmental and health effects related to exposure to contaminants across a population of organisms [36]. Thus, the OECD recommends the use of zebrafish as a model organism [34]. Japanese medaka has been used for toxicity testing for over 50 years, which is a small-sized (2–4 cm) freshwater fish and is well characterized as a model species because of being tolerant to wide salinity and temperature ranges. Japanese medaka is also being used as a model in the OECD test guidelines for developmental stages like early-life stages, juveniles, and adults [34].

The fathead minnow is also extensively used as a model species, especially in endocrine disruption studies [1, 37, 38]. It is native to North American and temperate waters and inhabits muddy pools of small rivers and streams. It is also among the three species validated by the OECD for ecotoxicity testing and has a huge toxicological database [39], which is mostly favored for embryotoxicity testing because of its well-known rapid development, transparent chorion, and sensitivity to toxic contaminants. The OECD has validated a test guideline for fathead minnow, zebrafish, and Japanese medaka [34]. Some of the fish species recommended by the European Center for Ecotoxicology and Toxicology of Chemicals (ECETOC) are given in Table 10.2 [40].

Fish species are selected and preferred according to the type of test and type of endpoint to measured, such as for toxicity test of early-life stage smaller species. For example, zebrafish, fathead minnow, and Japanese medaka are favored instead of rainbow trout because of their shorter test duration (30 days *versus* 90 days). Conversely, for longer exposure tests, rainbow trout are preferred to check endpoints [8].

Table 10.2 Recommended fish species for ecotoxicity testing

Recommended species	Recommended test temperature range (°C)	Recommended the total length of test fish (cm)
Zebrafish *Danio rerio* (*Cyprinidae*)	21–25	2.0 ± 1.0
Fathead minnow *Pimephales promelas*	21–25	2.0 ± 1.0
Cyprinus carpio (*Cyprinidae*)	20–24	3.0 ± 1.0
Rice fish *Oryzias latipes* (*Cyprinodontidae*)	21–25	2.0 ± 1.0
Guppy *Poecilia reticulata* (*Poeciliidae*)	21–25	2.0 ± 1.0
Blue gill *Lepomis macrochris* (*Centrarchidae*)	21–25	2.0 ± 1.0
Rainbow trout *Oncorhynchus mykiss* (*Salmonidae*)	13–17	5.0 ± 1.0

4 Fish Reservoir Ecotoxicity Tests

Reservoir toxicity tests aim at determining the level of biological response shown by adverse effects demonstrated by test species after being exposed to reservoir chemicals of concern. These tests are often conducted in controlled laboratory settings where the exposure concentration is of primary concern mainly regarding adverse biological effects associated with chemicals [41]. To enhance comparability, test methods are standardized. Toxicity tests are either conducted in situ or ex situ. Water samples may be collected from the contaminated area of the reservoir or prepared for simulated water after composition analysis [42]. Comprehensive assessments of the growth and reproduction of fish are more anticipated for population, multispecies, and community-level studies where reduction in the growth or the reproduction of particular species could be inferred regarding its ecological importance. For such kinds of reservoir toxicity assessments, standard toxicity tests including early-life stage fish tests are more suitable, compared to the other tests [43]. In reservoir environments, most of the exposures are chronic apart from those caused by accidental spill discharges. The results of acute and chronic tests are extrapolated to fluctuating aquatic environments and could be used to predict the effects of chemicals on the reservoir ecosystem [44].

Most of the time, the interaction between a fish and a contaminant under laboratory conditions is mostly more important. However, it is equally important to narrate the effect of chemicals on the fish population in reservoir ecosystems. The status of the fish population at the different concentration levels of contaminant is assessed in field surveys and results are then compared with data acquired from laboratory tests. Due to the fish being mostly exposed to multiple contaminants at a time, a combined toxicity assay in the laboratory is desirable. Besides, *in situ* experiments with caged fish in the reservoir are encouraged to conduct, which may provide more real data on reservoir toxicology [45].

Table 10.3 List of standard fish toxicity tests

Guideline designation	Organization	Title
203	OECD	Fish acute toxicity test
204	OECD	Fish prolonged toxicity test
210	OECD	Fish early-life stage toxicity test
212	OECD	Fish short-term toxicity test: embryo and sac-fry stage
215	OECD	Fish juvenile growth test
229	OECD	Fish, short-term reproduction assay
230	OECD	21-day fish assay
234	OECD	Fish, sexual development test
236	OECD	Fish, embryo acute toxicity test
850.1075	US EPA	Fish acute toxicity test, freshwater and marine
850.1085	US EPA	Fish acute toxicity mitigated by humic acid
850.14	US EPA	Fish early-stage toxicity test

Because of concerns about resource management and the release of chemicals from industries and factories into surface water bodies, aquatic toxicity tests were developed by world-renowned organizations on environmental protection like the US Environmental Protection Agency (US EPA) and OECD. Fish tests mostly relied on species, such as fathead minnow, zebrafish, and the cold-water rainbow trout [46]. A list of the standard fish toxicity test recommended by the OECD and US EPA [5, 46] is presented in Table 10.3.

4.1 Acute Toxicity Tests

When chemicals are released into the environment, they will find their way to enter lakes, rivers, and reservoirs. The EPA data require that fish acute toxicity tests should be typically conducted in three different fish species, including a cold-water freshwater species, a warmwater freshwater species, and a marine/estuarine species. Usually, an acute toxicity test is designed to check the safe concentration of pollutants, which gives a measure of acute lethality [45]. Despite being less ethically accepted as compared to tests with plants and invertebrates, fish acute toxicity commonly required ethics approval authorized by the official animal care and use committee [35, 47]. According to the EU Directive on animal protection utilized for scientific purposes, death as the endpoint should be avoided and substituted by some early endpoints [48]. The OECD guidelines for the testing of chemicals provide a helpful tool for assessing the potential effects of chemicals on human health and the environment. The OECD test guideline of fish acute toxicity tests (OECD TG 203) was published on July 17, 1992, in which fishes are exposed to the test substance for 96 hours under static or semi-static conditions [49]. Fish are exposed to five different concentrations of test chemicals for 96 h and deaths are documented at 24, 48, 72, and 96 h, respectively. Moreover, EC50 is determined to evaluate the concentration of the chemicals that gives a half-maximal response using a log-logistic model [48]. Recommended species are bluegill sunfish, common carp, zebrafish, fathead minnow, Japanese medaka, guppy, and rainbow trout.

4.1.1 Fish Larvae Test for Acute Toxicity

Rainbow trout acute toxicity test is 96 h static assays in which solutions are not renewed. Fish larvae are exposed to test solutions and are aerated at a rate of 6.5 ml/min at 15 °C and a photoperiod of 16 h light: 8 h dark is maintained. In each test tank, ten fish larvae are retained. Fish are not fed during the experiment and even not 16 h before the test. After exposure for 96 h, numbers of survived fish larvae from each test concentration are counted and mortality is determined [50].

Fathead minnow toxicity test is conducted using a semi-static assay during which solutions are renewed after exposure for the first 48 h. Three replicates are prepared for each test concentration. Ten 4–6 days-old larvae are introduced into each tank with different concentrations. After 24 h of exposure to the test solution, fish larvae are transferred to a clean water container. Then, for the 72 h test, containers are placed in a controlled incubator at 25 °C with a 16 h light: 8 h dark photoperiod. To remove metabolic waste containers are renewed with fresh water. Finally, after exposure for 96 h, survived larvae are recorded from each concentration, and mortalities are observed [50].

4.1.2 Fish Embryo Test (FET) for Acute Toxicity

The fish embryo test (FET) is a potential animal alternative for the acute fish toxicity (AFT) test. The OECD test guideline for fish embryo acute toxicity test was published on July 26, 2013 (OECD 2013). In the beginning, this test was intended to ascertain the acute toxicity of chemicals at fish embryonic stages and was designed to replace the fish acute toxicity test later [35]. Because the early-life stages like embryogenesis are the sensitive period of the life cycle, the study of adverse impacts of chemicals on developmental processes is more discrete. Moreover, embryos are not anticipated under animal welfare regulations and could be utilized as screening tools [34].

Taking zebrafish as an example, the fertilized zebrafish eggs are exposed to the test chemical for 96 h with five different concentrations. After every 24 h observations, the indicators of acute toxicity are measured, which include the thickness of fertilized eggs, the absence of somite formation, the lack of heartbeat, and the non-detachment of the tail bud from the yolk sac. After the completion of the test, acute toxicity is determined based on the presence of any four of these observations, and LC50 is calculated. The test report should contain important information on physicochemical properties, such as pH, temperature, water hardness, the concentrations of the chemical being tested, and the conductivity [35, 51].

4.2 Chronic Toxicity Tests

Commonly, chronic toxicity tests are conducted to evaluate the adverse effects of contaminated medium, such as water, soil, or sediment under long-term exposure [46]. In 1956, a chronic exposure test was conducted by Olson and Foster to assess the toxicity of sodium dichromate to successive life stages (eggs, fry, and early juvenile) stages of salmonids [43]. During the exposure process, at least 10% of the test species remain alive after a complete life span. In chronic tests, survival is monitored and sublethal effects, such as reproductive success and growth, are observed. Statistical endpoints that are taken into account include no-observable (NOEC) and the lowest observable effect concentration (LOEC), which shows the maximum concentration of test substance that does not show any effect on the responses of test species under observation, and the minimum concentration of test chemical that shows the substantial effect on the response parameter compared to the control, respectively [52]. During life cycle tests, fish's younger developmental stages have constantly been shown as more sensitive than others. Short-duration tests using early developmental periods could also predict chronic toxicity. Chronic toxicity tests are considered more sensitive, compared to acute tests, because toxicity actions emphasize no adverse effects levels [46]. The conditions for chronic tests are different for different species. The selection of test species depends upon the consideration of whether the desired endpoints could easily be measured using the selected species or not. When the critical endpoints are the secondary sexual characters, the species nominated for testing should be fathead minnow or Japanese medaka instead of zebrafish. However, while using the endpoints such as fecundity, egg hatchability, and body size, zebrafish are preferred [8].

4.2.1 Full-Life Cycle Tests

Full-life cycle tests using fish were first carried out by Mount and Stephen [53]. Toxic effects were assessed for at least one generation under continuous exposure to chemicals [43]. Life cycle tests are carried out for a year or more to reveal more information, compared to the other tests, because hidden effects of contaminants could be exposed. They can provide evidence of not only fecundity and progeny, but also growth rate and disease resistance. The downside of long-term life cycle tests is that only a limited number of species could be investigated, because these tests are time-taking and few contaminants could be assessed under limited exposure conditions [45]. They are carried out using rapidly growing and small-sized warmwater fish, such as zebrafish and fathead minnow [54]. For the life cycle toxicity test, freshwater fish like fathead minnow or zebrafish is cultured in the presence of the test chemicals from one stage of life to the other (whole life cycle) till the same stage of the next generation F_1. The concentration of the test substance in the water is administered periodically during the experiment. By the end of the experiment, the reproductive, behavioral, pathological, and physiological effects are assessed, and egg numbers, spawning ratio, fertility, and fecundity are recorded [55].

4.2.2 Partial-Life Cycle Tests

Before 1970, it was observed that when carrying out full-life cycle tests with numerous species after exposure to various chemicals, greater sensitivity has been shown by the early developmental stages, such as embryo, larvae, and juvenile stage, compared to the adult life stages [56, 57]. Consequently, in the mid-1970s, embryo-larval stages (30- to 60-day post-hatch) were proposed as a replacement for full-life cycle tests to lessen the time and cost [43]. embryo-larval stage tests intend to define the lethal and sublethal effects of a chemical on embryonic development, hatching, and larval growth are assessed [54]. According to the OECD guideline 210, these tests are recommended as a suitable and sensitive method for toxicity evaluation of chemicals [54]. In such tests, the embryo-larval stage of fish is exposed to three to five concentrations of the test chemicals under flow-through or semi-static conditions. Lethal and sublethal effects are evaluated and the lowest observed effect concentration (LOEC) is determined. The concentrations of the test chemicals are measured at regular intervals [58].

4.3 Bioconcentration and Bioaccumulation Tests

The uptake of pollutants from the external environment (usually water) is referred to as bioconcentration, and bioaccumulation is the absorption of a contaminant in biological tissues. In these tests, organisms are exposed to sublethal concentrations of the chemical, and their residues in the tissue of exposed organisms are evaluated until a steady state is achieved. Fish is usually used for such studies, because it is consumed by humans. Besides, soil invertebrates are also being assessed by fish for chemical uptake [52]. For fish, bioaccumulation and bioconcentration studies are carried out under flow-through and semi-static conditions. The test is divided into two phases: phase 1 is the uptake phase (exposure), which lasts normally 28 to a maximum of 60 days. During this phase, four fish of one species are exposed to at least two concentrations of the test chemical in separate groups. The second phase is the post-exposure or depuration phase, and fish are transferred into a medium devoid of the test chemical. Besides the two test concentrations, a control group without exposure to test chemicals is also performed in parallel. The concentration of the test chemical is monitored in fish in both phases of the test. Physicochemical parameters like pH, TOC, dissolved oxygen, salinity, total hardness, and temperature are also measured inside the test containers during the test. The lipid content is determined and the bioconcentration factor (BCF) at apparent steady state and the kinetic bioconcentration factor (BCFK) are calculated. Bioconcentration is expressed as a ratio of lipid content *versus* the whole bodyweight of fish [59].

5 Limitations of Fish Ecotoxicity Testing

Chronic fish toxicity tests are considered more sensitive than acute tests for the reason that the estimation of toxicity emphasizes endpoints other than survival, which can define better the no adverse effects levels. Moreover, chronic tests also provide a sound measure of responses for a population in the field. However, acute toxicity tests are regarded as fewer sensitive measures of toxic conditions, compared to chronic tests. Notably, chronic tests might not identify all sublethal effects [60]. Among chronic tests, the life cycle test is considered superior, but there is a limitation on time, space, and type of species that can be used. Other tests guarantee only a partial understanding of the impact of pollution on the fish's survival ability [8]. FET is considered a robust test and used as an alternative to the OECD 203 fish acute test [60].

6 Conclusions

Fish has been used as a sentinel organism for reservoir ecotoxicological testing. Various standardized tests have been designed according to contamination type and condition for evaluating the impacts of water-borne chemicals on fish. Standard toxicological tests are performed for acute lethality, fish embryo acute toxicity test, and chronic toxicity tests (full-life cycle toxicity tests). FET for acute toxicity. Fish bioaccumulation and bioconcentration tests are important because they reflect the reservoir ecotoxicology through the food chain. Reservoir toxicological studies also prefer to use small-size freshwater fish species like zebrafish, Japanese medaka, and fathead minnow. To enhance the predictive value and the extrapolation of acquired data at the ecosystem level, biochemical and molecular tools that can characterize the mode of action of chemicals should also be developed.

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Chapter 11
Ecotoxicology Methodology of Sediment Toxicity in the Reservoir

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Abstract The reservoir sediment can act as a drain and contaminant source. Once contaminants are released into the water column, they can be toxic for biota. This chapter details various ecotoxicological studies using sediment derived from reservoirs. Aquatic organisms are critical in the aquatic environment as intermittent consumers in the food chain and valuable indicators of sediment toxicity. International organizations have developed numerous standardized toxicity methods for aquatic species, including acute and chronic lethal sublethal toxicity measures, to determine the effects of this sediment toxicity. Aquatic species, such as algae, amphipods, and bivalves, are the best recommended for a specific test depending on the type of test, the final measurement, and the easy use of ecotoxicity.

Keywords Sediment · Toxicology · Aquatic organism · Acute toxicity · Chronic toxicity

1 Introduction

Human health and growth rely on the efficiency and abundance of freshwater, mainly through food and water supply [1, 2]. Intensive urbanization and industrialization risk the quality of the water. Anthropogenic effluents discharged into aquatic systems known as industrial, farming, and domestic activities contain mixtures of organic and inorganic pollutants, which have negative impacts on ecosystems and organisms [3, 4].

In water irrigation, power generation, and flood control, reservoirs play essential roles. Dam construction alters the hydrological system, and the sediment of reservoirs contains multiple heavy metals (HMs) [5–8]. To monitor aquatic ecosystems, the bottom sediment is significant [9] for the assessment of pollution levels [10, 11] and the evaluation of environmental risks [12]. As large and small dams are built globally, water reservoirs are increasing [13, 14]. According to the report from Zarfl et al. [13], the number of dams is anticipated to double [13].

The sediment acts as a sink for pollutants [15]. But inside the water column, metals may be resuspended and a diffuse source of contamination, depending on environmental conditions [16]. In addition, the sediment can be essential to the

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ecosystem's trophic dynamic by geochemically interacting among the sediment and water column, including nutrient release [17]. Thus, surface sediment studies and water from the bottom and column may be used to elucidate pollutants' temporal and spatial dynamics and test reservoir management effectiveness.

Sediment toxicity testing under controlled exposure suggests toxicity assessment of the organism in the site [18–22]. Toxicity rate can be derived from chemical analyses, and pollutants can be quantified and compared to the sediment quality criteria, in which sediments are considered low or highly sensitive to adverse effects. However, chemical analyses can not evaluate the possible impacts of non-media pollutants that might lead to outcomes. Although chemical analyses can give details on potential pollutant bioavailability, sediment toxicity testing responses reflect bioavailable contaminant fractions, cumulative effects of pollutant mixtures, and the interaction of non-contaminant stressors as they influence test organisms. The sediment toxicity tests generally include further factual proof of pollutant effects than the benthic ecological, since community endpoints are difficult to interpret [23–25].

2 Sediments Collection

Surface sediments are most frequently collected to determine risks associated with sediments. Generally, epifaunal and infaunal organisms occupied the top 10 cm of sediments. Epibenthic groups, including shrimps and amphipods, can only be subjected to surface sediment contaminants (0–1 cm). In contrast, others including bivalves and polychaetes may mainly be subjected to pollutants several centimeters deep. Epibenthic species can only contain surface sediment. Adequate information should be achieved to evaluate contaminant concentrations of sediments in both 0–2 cm and 2–10 cm depth for assessing significant pathways of exposure to contaminants in most species.

Many studies are available for different collection conditions [26]. Sediment processing systems need to limit the deterioration of sediment, otherwise, extensive erosion and other forms of sedimentary structural degradation may occur. The mixing can also demonstrate a possible disturbance with layers of varying particle dimensions and composition of preceding redox stratified chemical substrates that affect the bioavailability and potential for sediment toxicity [27].

The sediment quantities to be obtained are based on the tests and analyses to be carried out. Usually, 1 kg sediment should be adequate for most pollutants from each site in research. Both samples should be processed with the appropriate equipment and procedure for the study requested. Furthermore, bioaccumulation or toxicity tests require 2–3 kg, and these samples should be kept cold (not frozen).

Plastic devices and containers used for sample collection are soaked in 10% nitric acid and thoroughly washed [26]. Nitric acid is not ideal for the analysis of nitrogen forms. The sampling system must be cleanly rinsed with water from the sampling station before and after collecting samples. More cleaning of the sample may be appropriate for evaluations, including (I) water and soap washing, (ii) rinsing with distilled water, (iii) rinsing acetone or ethanol, and (iv) on-site rinsing water. In the case of heavily polluted sediments, reference sites should be first sampled to avoid cross-contamination [18].

Before taking sediment for the subsequent biological, physical, toxicity, or chemical analyses, it is necessary to verify the reliability of the sample collected. Grab samples are acceptable when the surface layer appears unchanged, and the volume of the sediments is sufficient. For the desired material quantity, several replicate samples are needed for both grab and core sampling.

3 Manipulations of Sediments Before Testing

Until chemical or toxicity testing, sediments are frequently processed in the laboratory or field. Manipulation may include sieving to extract large particles and waste and homogenizing large samples so that multiple biological and chemical tests can be carried out [28]. Most sediment manipulations can influence the sediment's properties and pollutant bioavailability, and these effects will have to be tested. Therefore, the methods designed to process sediment samples for tests and analyses should eliminate perturbations.

It is desirable to examine how sample manipulation can affect the concentration, toxicity, and bioavailability of the pollutants in the sediments. Later, bioavailability and toxicity analysis will help to interpret the data of preliminary redox potentials, total organic carbon, pH, AVS, distribution of the iron and weakly extractable pollutants, and pore water contaminants in newly collected sediment samples that have been minimally manipulated. However, pH might be influenced if sediment samples are subjected to storage above 4 weeks longer [26].

4 Pathways for Pollutant Exposure

Sediment-dwelling organisms are vulnerable to pollutants by inadvertent and direct sediment intake, pore water, and excess water intakes [28, 29]. Contaminants can be released from sediments to water columns through absorption, disruption, and the distribution of dissolved chemicals into the water column [30]. In surface layers, normal sediment resuspension often preserves pollutants in oxidized types. For many benthic species, sediment-water exposure is the most critical measurement since feeding is conducted on organically rich particles in this area. Field studies have shown that sediment resuspension for transport and internal recycling in aquatic ecosystems is significant [31, 32]. Laboratory studies demonstrated a substantial rise in hydrophobic contaminant concentration in surrounding water in connection with simulated resuspension events [33, 34].

The benthic species play a significant role in sediment bioturbation. The deposition feeders consume organic/inorganic particles from sediment or within their sedimentation surface. Polychaete and oligochaete consume sub-surface lower sediments and transport them as fecal pellets to the sediment. A second relevant factor of bioturbation is the capacity, through feeding and continuous burrowing of diverse sub-surface feeders to increase the sediment/water area and the toxicants and oxygen fluxes. Several authors explain substantial rises in the flow of sediment-related pollutants from overlying water and sediment to pore water [35–38]. Moreover, the bio-mediated resuspension of particles can be considered under certain conditions and even surpass that of physical disruptions.

5 Test Organisms for Sediment Toxicity

The sensitivities of benthic organisms to sediment pollutants vary widely because of different organism properties, such as burrowing, life cycles, routes of pollutant exposure (for feeders to filter and deposit), and the contaminant's characteristics (such as partitioning and bioavailability). Therefore, in sediment quality assessments, it is critical that toxic exposure may use various organisms with diverse feeding approaches and behaviors. Bivalve (clams, mussels, and oysters), insect larvae (chironomid midges and mayflies), algae, nematodes, copepods, worms, and snails, may be used to examine the toxicity of sediments (Table 11.1). Despite the organism's close connection with sediment, large crustaceans, such as crabs, are reported for the toxicity test of sediments [46].

Table 11.1 Freshwater whole sediment toxicity tests

Organism	Test species	Duration/End point	Acute/ Chronic	References
Algae	*Chlorella vulgaris*	72 h/growth	Acute	[39]
Amphipod	*Hyalella azteca*	10- and 28-d survival and growth, 42-d survival, growth and reproduction	Chronic	[40]
	Corophium volutator	Survival	Chronic	[41]
Bivalve	*Hyridella australis*	28 d, growth, antioxidant.	Chronic	[42]
Worm	*Limnodrilus hoffmeisteri*	mortality and autotomy rates	Chronic	[43]
	Chironomus larvas	mortality and growth inhibition rates	Chronic	[43]
	Caenorhabditis elegans	Growth reproduction	Acute	[44]
Crustacean	*Daphnia magna*	48 h survival	Acute exposures	[45]

5.1 Algae

Algae are the common food source for many invertebrates, so transmitting harmful sediment pollutants to higher trophic levels is possible [47]. In sedimentary stimulation, toxic effects have no masked effects of ammonia stimulation [48, 49]. Regarding sediment research, algal inhibition appeared to be more suitable than whole algal growth as an endpoint. Flow cytometry is a speedy measurement of algal cells inside a moving fluid [50].

5.2 Amphipods

Amphipods are an important component of aquatic ecosystems (rivers and reservoirs). They are the main prey of fish, birds, and large invertebrates. Thus, they become crucial for moving pollutants from sediments to higher trophic levels. Amphipods have an environmental significance and vulnerability to polluted sediments, due to their large numbers, extensive distribution, ease of handling, and their suitability for sediment toxicity research. Many amphipods eat sediments directly and are treated with sediment-based pollutants combined with pollutants in overlying waters. There are many standardized or peer-reviewed methods for measuring survival, reproduction, development, and sediment avoidance in various fields for whole sediment toxicity studies [21, 51–53]. *Gammarus pulex* for freshwater sediment tests is widely used. The life cycle of amphipods was limited to ten days. Thus, bioassays of those species were commonly used to test sublethal reproductive effects [21]. As we know, amphipod species are ideal models for in situ sediment toxicity studies [54].

5.3 Bivalves

Bivalve is a significant and essential part of benthic estuarine, marine communities, and freshwater ecosystems [55]. Bivalves include clams, mussels, and oysters. Bivalves sometimes bury themselves in the top two to twenty centimeters of silt or sandy sediments. The bivalve species feed on organic-rich particles and algae from the surface layer with their siphons or filtering vast water column quantities. The bivalves are adversely affected by aqueous pollutants, such as pore water, burrow water, and overlying water. Their food contains bacteria, algae, plants, and inorganic sedimentary products unintentionally ingested [56–58]. Bivalves are vital preies of many fishes and invertebrates. Therefore, they are likely to transfer the contaminants from sediments and water to predators at the top of the food chain.

Freshwater bivalve has an extraordinary life history and physiological and anatomical qualities that make them beneficial organisms for detecting pollutants from contemporary and historical sources and determining the ecological value of pollution in reservoir ecosystems [59]. Therefore, Australian freshwater bivalves, including *Velesunio ambiguous*, *Velesunio angasi*, and *Hyridella depressa* were employed to examine metal accumulation from the sediment [60].

6 Toxicity Endpoints

Test endpoints are generally chronic or acute but can also be sublethal or lethal. Acute toxicity is usually a negative impact resulting from a short exposure period to a chemical, compared to the organism's lifespan. On the other hand, chronic toxicity is generally related to the negative effect on the organism of the lifetime or adverse outcome on a sensitive early life stage due to chemical exposure caused by a substantial concentration. A significant proportion of an organism's life will be over 10% [61]. Generally, a minimum of 48 h for a short life cycle and a 4 to 10 d test for a long life cycle. Thus, chronic sediment toxicity tests should be >10 days. Thus, 28–42 or 60 days are used for evaluating the longer-term effects of amphipod, bivalve, and worm species for survival, growth, and reproduction [20, 53, 62–66].

The organism's survival is the most common endpoint for sediment quality testing and is a general acute endpoint. The juvenile stage is more critical than the adult stage. These sublethal responses usually act as chronic endpoints and provide more detail about possible long-term consequences at the level of individual populations [19, 21, 66].

To have effective decisions on the mitigation alternatives for contaminated sediments, one needs to know about test endpoints' biological and ecological value. There are increasingly biochemical and physiological responses, and biomarker approaches give higher sensitivity and less variability than well-defined sublethal endpoints in whole sediment tests [21]. Direct associations between impaired reproduction and lysosomal instability are more susceptible to specific species [67]. The main goal of eco-genomics is to detect triggered genes so that molecular fingerprints are unique to that bioavailable chemical fraction [68, 69].

7 Conclusion

This chapter covers the basics of ecotoxicology techniques, focusing on sediment toxicity in the reservoir. Possible evaluations of sediment quality include acute and chronic lethal and sublethal toxicity measurements. Based on the kind of test, final measurement, and ease of using ecotoxicity, aquatic species including algae, amphipods, and bivalves are appropriate models for the sediment toxicity of reservoir sediment. How to design toxicity tests, what to evaluate, and how to interpret the toxicity results deserve us to systemically consider before the beginning of sediment toxicity in the reservoir.

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Conflicts of Interest The authors declare no conflict of interest.

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Chapter 12
Mesocosm Study in the Reservoir Ecosystem

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Abstract This chapter discusses the significance of mesocosm systems in studying the reservoir ecosystem. The use of various types of mesocosm to study the effects of contaminants on freshwater bodies has been in practice for the past three decades. They have been used widely to assess the toxic effects of water-borne contaminants on various biological levels of organizations and their impact on the overall ecosystem. Various studies have been conducted to evaluate the impact of contaminants like pesticides, microplastics, and persistent organic pollutants on macroinvertebrates and fish species, and endpoints like species richness, species diversity, and morphological and physicochemical parameters have been observed. These systems allow the control of various environmental factors and imitate the natural environment to create real exposure scenarios according to the study objectives, available cost, and time. However, if their design does not take account of the physical dimensions and their suitability with the use of suitable test organisms, the replicability and reproducibility of these systems get affected.

Keywords Mesocosms · Reservoirs · Freshwater ecosystem · Ecological realism

1 Introduction

Studying the effects of already present issues like eutrophication, removal of biota, and the addition of toxicants to aquatic ecosystems poses an additional challenge in assessing the overall effects [1]. Controlled laboratory toxicity tests using single species do not account for the complex biological, chemical, and physical interactions that occur in the natural environment. Such tests can only evaluate the real effects of a particular chemical or toxicant for a particular test organism used in the experiment. Thus, this approach is not useful to make comparisons with real-world data. On the contrary, field studies require adequate time, money, and replication to carry out such experiments. Moreover, the conditions are not controlled and a range of natural and anthropogenic stressors can affect the results. Therefore, for examining the aquatic toxicity, an improved test, which enhances the accuracy of ecotoxicological assessments and incorporates environmental realism, is required. For this purpose, some improvements in the design of toxicity testing have been made by the

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development of aquatic mesocosms [2]. Aquatic mesocosms are the well-designed systems that assess the physical and functional parameters of aquatic ecosystems that could not be assessed in laboratory bioassays [3]. According to the description by Alexander et al. [4], mesocosm is a fusion of laboratory and field systems that permits the control of various factors of concern, such as species and habitats, and allows settings close to the natural conditions to create factual exposure setups than laboratory tests. Their originality is mainly based on ecological realism, which is achieved by incorporating basic constituents of natural ecosystems and maintaining biological, physicochemical, and toxicological parameters and controlled to some extent [5], among which biological characteristics include food chain and food web interactions and physical characteristics contain temperature-salinity flow, as well as chemical characteristics cover toxicant mixtures or fate and transport of chemicals [2]. They are designed in such a way, so that they may imitate the features and functions of shallow lake and reservoir ecosystems [6]. According to the International Union of Pure and Applied Chemistry (IUPAC), a mesocosm is an enclosed experimental system that mimics the natural setting and is used to evaluate the effects of contaminants on a greater scale, compared to a laboratory microcosm [7].

2 Use of Freshwater Mesocosms in Lakes and Reservoir Ecosystems

Mesocosms have been used by toxicologists, ecotoxicologists, and environmental scientists for about the past three decades to study aquatic and terrestrial ecosystems [2]. The term "mesocosm" was first used by Eugene P. Odum to describe moderate-sized, simulated test setups, in which populations and ecosystems could be examined simultaneously [8]. Mesocosms have been used in experimental ecology for studying freshwater ecosystems since the twentieth century. Different approaches like freshwater *in situ* enclosure systems, small-bottle incubations, and multitrophic *in situ* enclosures containing water columns from surface to sediments have been utilized to assess acidification and eutrophication since the early 1970s [9]. In the USA, in the 1980s, large-scale mesocosm systems were employed for new pesticide registration. However, in the early 1990s, these test systems were restricted because their cost-effectiveness was questionable. Currently, in the USA, the assessment of the pesticides' adverse ecological effects is gathered using a tiered approach, and mesocosm studies are employed at the final tier for ecological risk assessment [10]. Various studies have been conducted using mesocosm to assess the effects of contaminants on freshwater bodies such as streams, rivers, lakes, ponds, and reservoirs. Studies using mesocosms mostly focus on understanding the effects on biological components of ecosystems, such as effects caused by chemical nutritional dilutions on trophic-level species richness, diversity, zooplankton, and phytoplankton community and the effect of chemical contaminants on physicochemical endpoints like turbidity, total suspended solids, total nitrogen phosphorus, *etc*. In most freshwater studies, fish species are used as indicator organisms. Moreover, macroinvertebrates, amphibians, and plankton communities are also being used. In several previous studies [11–15], the effects of pesticides, insecticides, and herbicides on phytoplanktonic, amphibians, and macroinvertebrates communities have been investigated. While other studies have focused on the impact of nutrient enrichment on various physicochemical parameters of freshwater using indicator species, such as fish [16, 17]. Recent studies have concentrated on the effects of microplastic resins on the diversity of aquatic organisms and reproductive toxicity at various trophic levels [18]. Studies carried out using the freshwater lake and reservoir mesocosms during the last two decades are presented in Table 12.1.

3 Significance of Mesocosms in Ecotoxicity Testing

The mesocosm tests are vital for understanding the impacts of activities, such as habitat fragmentation, species incursion, and habitat fragmentation (Stewart et al. [25]). They are considered useful when they replicate cause–effect relationships. An ideal mesocosm scheme fulfills the criteria of good spatial and temporal replication of the natural ecosystem, which establishes a representative community and covers appropriate predator–prey relationships in natural settings [4].

Mesocosm testing eliminates the need to assess biological and physicochemical parameters separately and provides hazard and exposure assessment in a single experiment [26]. The main objectives of applying the mesocosm approach in ecotoxicological research are to determine the fate and behavior of contaminants, and to observe the effect of toxicants on individual species, populations, food webs, and communities residing in a particular ecosystem [7]. In experimental ecology, they offer an association between field observations and controlled laboratory experimentations. They are applied to appraise the response of individual species or communities in their changing environment, such as the increases or decreases in pH, temperature, and CO_2. In mesocosms, various perturbations are facilitated together with species diversity, environmental variation, and the replication of experiments [9]. Moreover, mesocosms are also employed to refine NOECs and PECs approximations by exposing multiple species belonging to various taxa *via* fate studies. They could also be used to assess the recuperation of contaminated systems [10]. Thus, mesocosms work as efficient assays to investigate assumptions and generate new hypotheses [26].

4 Size and Types of Mesocosms

The size of mesocosms ranges from constructed ponds, ditches, and fabricated tanks (synthetic enclosure systems) to littoral enclosures, which are the quarantined parts of the natural habitat [27]. The OECD recommends that a size of 1 to 20 m^3 is

Table 12.1 The use of lentic reservoir mesocosms to assess the effects of different environmental toxicants

Mesocosm	Toxicant	Duration	Organisms	Endpoint	Reference
Outdoor tanks, 5000 L	Atrazine, herbicide	25 d	Freshwater Phyto pK community	Chlorophyll fluorescence tolerance	[11]
Outdoor artificial ponds, 1480 L	Carbaryl, insecticide Atrazine, herbicide	56–88 d	Amphibian community	Taxonomic composition Survival, body size, development, and time to metamorphosis	[12]
Pond enclosures (0.06 ha)	Trophic level	3 m	Common carp (*Cyprinus carpio*) Catfish (*Ictalurus punctatus*)	Turbidity, suspended solids, total phosphorus	[19]
Six macrophyte-dominated lakes: 11 mesocosms	Nutrient enrichment	5–6 w	Fish	pH, alkalinity, TSS, total phosphorus, ammonium, phytoplankton, and zooplankton.	[16]
Shallow eutrophic reservoir: 2 enclosed systems with 360 L water	Oligo-meso and eutrophic treatments	31 d	Phytoplankton	Dissolved oxygen, species diversity, richness, and dominance, ammonium, free CO_2	[20]
Outdoor artificial ponds, 12,000 L	Ivermectin veterinary drug	265 d	Zoo Pk	Abundance Species richness	[21]
Outdoor tanks 11 m3	Chlorpyrifos OP insecticide	99 d	Zooplanktons	Population and community effects	[13]
Outdoor lentic microcosm (1.05 m; height: 0.9 m,780 L)	Gamma-cyhalothrin Pyrethroid	73 d	Lentic invertebrates	Population dynamics species composition Chlorophyll-*a* (phyto pK) macrophyte biomass leaf decomposition	[14]
Tropical reservoir in Brazil: outdoor 20 enclosures ($9.8 m^3$)	Nutrient enrichment	5 w	Fish (*Nile tilapia*)	Total phosphorus and nitrogen, phytoplankton, and zooplankton biomass	[17]
Subtropical mountain reservoir 0.30 m depth	Solar UV-B	4 w	Phytoplankton	Chlorophyll-a soluble carbohydrate	[22]
Outdoor ponds 9 m^3		10 m	Benthic macroinvertebrate communities	Drift, the abundance of different taxa decomposition of leaf litter	[15]

	Different pesticides used in crop protection programs				
Outdoor: 6 enclosures of 400 L	Cyanobacteria, Microcystin		Bivalve (*Limnoperna fortunei*)	Total phosphorus and nitrogen, phyto-plankton periphyton, chlorophyll-a	[23]
Lentic mesocosm, 20 m long × 1 m wide	Bisphenol A Plastics and resin constituent	165 d	Different trophic levels	Watercress volume macroinvertebrates community structure, abundance of fish and gonad morphology	[18]
50 L transparent polyethylene bags	Cyanotoxins	12 d	Zooplanktons and algal biomass	Increase and decrease in microcystin at different times	[24]

suitable for outdoor mesocosm studies. However, according to the literature, the average size of mesocosm in many studies was 1.7 m^3 with 49 days of exposure duration [4]. For studies of shorter duration (3–6 months) using smaller organisms like planktonic species, smaller mesocosm with 1–5 m^3 is more appropriate. However, for studies of longer periods (6 months or more), larger systems are recommended [28].

Mesocosms are either large enclosures placed in lakes and reservoirs or enclosed in artificial canals or ponds [29]. They can be small plastic enclosure systems, which are open-air [30], or large flexible/rigid polyethylene enclosures or ponds constructed by retaining water masses in dams or lagoons by placing bag systems, pond systems, or tanks. According to design and shape characteristics, various types of mesocosm systems are being used in different ecology studies [25].

The bag system comprises transparent, black-colored polyethylene or polyvinyl chloride bags, through which the separation of a considerable volume of water is attained. These bags are knotted to a floating wharf and have a narrowed bottom with a hose attached outward for water renewal. They are filled with filtered water and seeded with microalgae. Later, water is fertilized to promote the growth of algal blooms, and then planktons are added. After their development, the larvae of test organisms are released and exposed to environmentally relevant concentrations of contaminant [31]. Another variation of the system includes land-based dug-out ponds, which are easy and economical to construct and operate. After digging out the pond, it is sheltered with a plastic liner to avoid water leakage. Ponds are then exposed to sunlight for 3–4 days. Test organisms are cultured separately and then transferred to the ponds [32].

Cement tanks are also a common type of mesocosm, which are usually up to 50 m^3 in size [33]. They generally vary in volume from 2000 to 20,000 L and represent a simple food web with ambient environmental conditions like light and temperature [34]. Biological communities are treated easily due to the presence of water and sediment from the same source. Cement tank mesocosms have low cost and cause less contamination of the natural surroundings. However, small-sized tanks cannot retain large predators and can cause wall effects, albeit large tanks may also induce wall effects in long-term mesocosm tests (>50 days) [4, 35]. The most promising tanks for mesocosm testing are super intensive mesocosm tanks known as the maximum tank system, which is intensified and controlled by steady readjustment using a computer-based subjective decision manipulation program. This system can control pH, nutrients, temperature, light, and biotic components (plankton, predators, and bacterial production) [33]. Circular tanks are more promising, compared to square designed tanks. They ensure high carrying capacity and deliver good flows with the advantage of self-cleaning ability because of continuous fish swimming. However, this design does not work well for small-sized less energetic organisms that could not overcome a higher water flow. Rectangular design tanks, also known as raceways, do not imitate usual environmental situations. They do not have good water flow, so higher velocities are not attained to self-clean the tank system [4]. Another mesocosm system, known as the Swedish pond, is considered to be the best for testing organisms, because it has more surface area with square tanks and rounded edges. Moreover, it also offers a self-cleaning process like a circular tank [36].

5 General Design of Aquatic Mesocosms

5.1 Preliminary Aspects

Conventional ecology comprising soil, water, rocks, air, and living biota is the prerequisite of the mesocosm system. However, for designing mesocosm, ecological engineering is also needed, because the system is developed artificially. The enclosed structure is constructed using plastic, metal, or cement. Besides, automated and electrical components are also required. The design and maintenance of ecological and engineering features of mesocosms and their interaction with each other are very crucial for the function of the mesocosm system [37].

Before carrying out any mesocosm test, it is important to define the objectives to determine a suitable experimental design according to the relevant endpoints. Questions, such as probable entry route of contaminants in the water body, frequency of entry, and physicochemical properties of contaminant, must be ascertained before the beginning of the experiment to identify components that are at risk as well as the sampling strategy and frequency. Preliminary studies and lab testing must be conducted before undertaking the mesocosm study when information related to a particular test design is not available [28]. The most important objective of mesocosm studies is to sustain realism, which could be achieved by adding basic components of the natural ecosystem into the mesocosm system. Although the reconstructed system may not mimic the exact natural conditions, it could be simplified by adding key features to ensure ecological representativeness [5].

While designing mesocosms, various scale-related parameters are kept in mind, such as size (radius, depth, and volume), the overall duration of the experiment, and sampling frequency. Moreover, the life cycle of test organisms utilized in the experiment, proper light intensity for temperature balance, primary productivity, construction material and cleaning frequency, temperature control for biogeochemical activities, selection of test organisms or communities, and horizontal and vertical mixing and flow of nutrients should be well considered [38].

5.2 Mesocosm Assembly

Mesocosms can be assembled using inert material either stainless steel, glass, or sealed concrete. To prevent water, exchange systems should be lined using PVC. Water added to the system should be originated from where organisms and sediments are collected. Besides, the physicochemical properties (pH, alkalinity, hardness, dissolved oxygen, and turbidity), chemical contaminants, and nutrients should also be characterized. During the entire study, the water level should be retained at similar levels. Species communities should be developed by adding organisms from suitable external sources. Free-living species are not recommended, especially in cases where effects on plankton and macrovertebrates have to be studied [28].

For generating communities in mesocosm systems, organisms from laboratory cultures are used to standardize the system to assure the initial similarity of the replicates, although the communities will be unlike the natural communities of the surroundings. The other alternative is that the organisms are taken from natural surroundings and allowed to develop into communities for reaching equilibrium before the beginning of the experiment. Communities of fish, macroinvertebrates, and planktons are usually cultured in the systems [39]. Macrophytes can offer sanctuary for the mesocosm fauna and facilitate the diversity of invertebrates and algae [40]. Notably, they should be maintained according to the requirement of the study. For example, if the mesocosm system focuses on plankton, then macrophytes are recommended to limit their development to 20–30% of the bottom surface. However, if the study emphasizes macroinvertebrates, then, macrophytes should be enhanced to promote a large number of macroinvertebrates. According to study objectives, benthic and planktonic invertebrates are also required to be added to the mesocosms along with water and sediments. Invertebrates such as zooplanktons from phylum *Rotifera* (*Branchiopoda*) and benthic organisms from the phyla *Annelida* (*Hirudinea Oligochaeta*), *Arthropoda* (*Insecta*), *Crustacea* (*Amphipoda*), *Mollusca* (*Bivalvia*, *Gastropoda*), and *Platyhelminthes* (*Turbellaria*) are mostly investigated in the mesocosm studies. For mesocosm studies using fish, only endemic species are recommended to avoid the contamination of habitats of the native fish, and they should be added when the test system gets stabilized. Fish species used in most of the mesocosm studies include bluegill sunfish (*Lepomis macrochirus*), carp (*Cyprinus carpio*), fathead minnow (*Pimephales promelas*), golden orfe (*Leuciscus idus*), sticklebacks (*Gasterosteus aculeatus*), mosquito fish (*Gambusia affinis*), and rainbow trout (*Oncorhynchus mykiss*). Large mesocosms are recommended for fish studies and fish species are selected according to the size of the experimental system and the objective of the test. Before chemical dosing, mesocosms are adapted to ascertain the growth of a population community in terms of age and sex structure. The adaptation period varies with the origin of the water introduced in the system [28].

5.3 Protocol of Mesocosm Preparation

Mesocosm systems are first either dried for four days or treated with chemicals, such as HCl, to get rid of predators. Systems are then filled with nearby reservoir water, which is fertilized using poultry manure of about 40 g.m^{-3} along with chemical fertilizer (2.4 g superphosphate of lime, ammonium sulfate 1.6 g, and urea 1.08 g) for three days. Then, different planktonic community starts to develop in a process termed succession. The diatom may emerge first, followed by nano and dinoflagellates. However, the rotifers and ciliates develop at the end. When an adequate population is established, cultured larvae of test organisms are added to the mesocosm system. Various biotic and abiotic factors are maintained and synchronized during the whole experiment, such as the maintenance of plankton growth rate and related environmental conditions (nutrients, temperature, and light intensity). Moreover, water quality parameters and other biotic factors are also regularly monitored. During the rearing period of test organisms, water analysis is carried out at regular intervals and food consumption is also observed [30].

6 Limitations

Mesocosms experiment contributed to understanding ecology community and ecosystem [41]. However, these tests are criticized for being unrealistic and less related to natural ecosystems. Other general limitation includes oversimplification and worse repeatability. Furthermore, the findings of mesocosm tests are difficult to extrapolate to bigger and natural ecosystems [42]. Mesocosms are generally considered replicable to the natural ecosystem. However, there is more variability among the biological parameters assessed at the individual level than those estimated at the community level [43]. Notably, large mesocosms can be replicable, if they are permitted to grow naturally and placed adjacent to the source system [44]. Achievement of an appropriate level of ecological realism is dependent on the project level and available funds. At a lower level of biological organization, there is high reproducibility but low realism. Larger systems, despite being ecologically realistic, still cannot offer better replication, compared to small systems [4]. The realism and replication aspects of mesocosm in the time and space domain are explained in Fig. 12.1, implying that there is a trade-off between these aspects and that none of the approaches is perfect [25]. Moreover, in the mesocosm test, the environmentally relevant levels of chemicals show less control and precision over the test progress. Certainly, concentrations up to sub-ppm to ppb can be detected, considering the existing instrumentation availability. However, the approaches used to determine the species distribution need concentrations away from the upper limit of the expected levels. Therefore, the physicochemical and biological parameters that could be assessed using standardized tests cannot be determined using mesocosm testing. Nevertheless, this limitation could be overcome by improving the instrumentation [26].

7 Conclusion

Mesocosm test is the fusion of laboratory and field experiments and has been used efficiently for the past few decades to assess the ecotoxicological impacts of contaminants at various biological scales. However, due to complex biological

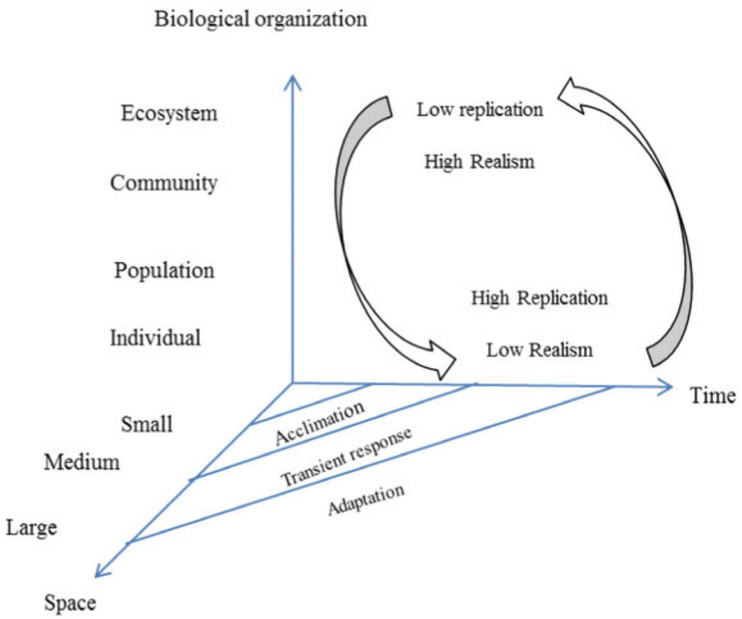

Fig. 12.1 Conceptual diagram representing space and time domain for mesocosms

interactions, it is difficult to achieve reproducibility and ecological realism. Thus, their development and use at higher organizational levels should understand the knowledge of the environmental processes, interrelation of species and their habitat, and the engineering design of the system. Thus, before carrying out such experiments on bigger scales, pilot studies must be conducted to avoid the wastage of time and money. To implement scientific research, the community is required for bringing forth novel ideas to modify such systems on an engineering and ecological basis and improve the realism and replicability of these systems.

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Part IV
Ecotoxicological Effects and Mechanism of Pollutants in the Reservoir

Chapter 13
Molecular Toxicity Mechanism of Heavy Metals in the Reservoir

Suman Thodhal Yoganandham and De-Sheng Pei

Abstract Protecting water quality related to metal exposure is a significant problem in a reservoir. Most heavy metals are carcinogenic substances. For example, arsenic, cadmium, chrome, and nickel are listed by the International Cancer Research Organization as carcinogens and are used extensively. Metal exposure derived from water, air, or food may result in acute or chronic toxicity. The bioaccumulation of heavy metals may cause toxic effects on different tissues and organs. Growth, proliferation, differentiation, and damage repair are all impeded after exposure to a high concentration of heavy metals. This chapter mainly discussed the molecular pathways of heavy metal toxicity in the reservoir, including reactive oxygen species (ROS) formation, antioxidant defense balance, enzyme inactivation, DNA damage, and cell death.

Keywords Heavy metals · Antioxidant · DNA damage · Cell death · Reservoir

1 Introduction

Heavy metal contamination is a major concern due to its serious health effects on the reservoir [1]. Because of the pervasiveness and persistence of chemicals, it is also regarded as a critical environmental problem [2]. Therefore, heavy metal pollution in the trophic chain must be quantified, characterized, and analyzed [3, 4]. Toxicity and bioaccumulation of heavy metals in the reservoir may have serious human health consequences, such as hepatitis, kidney damage, nervous system disorders, coronary artery disorders, hematological implications, reproductive outcomes, and cancer [4–9].

Environmental matrices in the reservoir, such as soil, air, and water also contain lead (Pb), cadmium (Cd), chromium (Cr), mercury (Hg), nickel (Ni), copper (Cu), zinc (Zn), iron (Fe), and manganese (Mg) [2]. However, heavy metals such as copper, iron, manganese, molybdenum, zinc, and nickel are essential micronutrients for the body's metabolism. Nevertheless, other heavy metals, such as mercury, cadmium, lead, and chromium, do not play a positive role even in minimal concentration and cause health risks [10–12].

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Metals can be found in dissolved, suspended, colloid ions, and solid sediments in the reservoir [13, 14]. The concentration of these metal ions is heavily influenced by redox potential, biological processes, pH, ion strength, and the behavior of organic and inorganic chelators [15]. Over the last few decades, the contamination of water supplies by the indiscriminate dumping of heavy metals has caused global concern. Rapid population growth have contributed to significant water contamination in many developing countries, such as rivers, lakes, and reservoirs [16]. However, heavy metals can be accumulated in the sediments of reservoirs [17, 18]. Artificial reservoirs provide a significant role in functioning as sediment accumulation, which has attracted more attention recently [19].

2 Heavy Metal Pollution in the Reservoir

As shown in Table 13.1, in China, from 2014 to 2016, six heavy metals (zinc, chromium, cadmium, nickel, copper, and lead) were investigated in the Three Gorges Reservoir (TGR) to assess ecological risks. In the mainstream of the TGR sediments, concentrations of heavy metals and pollution levels were not significantly different between 2014 and 2016. Cadmium was high, while other metals were extremely low [20]. In Nigeria, the sediment composition of the Asejire Reservoir was examined to determine the heavy metal content. Twenty stations were chosen, sampled, and measured using standard methods. The findings revealed that sediment in all stations was mildly acidic, with low conductivity and organic matter. In Ghana, Anash et al. [22] investigated the concentration and distribution of heavy metals in the Weija Reservoir. Heavy metals in water, suspended particles, and sediments

Table 13.1 Heavy metal pollution in the reservoirs

Sample collection site	Sampling time	Elements	Determination method	References
Three Gorges Reservoir, China	2016–2014	Cd, Cr, Cu, Ni, Pb, and Zn	ICP-MS	[20]
Asejire Reservoir, Nigeria		Fe, Pb, Cu, Zn, Mn, Al, Ba, Ni, and Cr		[21]
Weija Reservoir, Ghana		Cd, Cu, Ni, Pb, and Zn	AAS	[22]
Biliuhe, Tanghe, and Dahuofang Reservoirs	2015	(Fe, Mn, Cu, Cd, Pb, Zn, and Cr	AAS	[23]
Chah Nimeh water reservoir, Iran	2012	Cr, Cd, Cu, Mn, Fe, Pb, Zn, and Ni	AAS	[24]
Manwan Reservoir, China.	2011	As, Cd, Cr, Cu, Pb, and Zn	ICP-AES	[25]
Guanting Reservoir, China	2009	Cu, Zn, Cr, Ni, Cd, Pb, and As	ICP-MS	[26]
Hongfeng and Baihua Reservoirs, China	December 2010 and April 2012	Hg, Cd, Pb, Cr, Cu, and As	AAS and GFAAS	[27]

were measured using atomic absorption spectrophotometry. This study discovered that heavy metal accumulation concentrations in the sediments of Weija Reservoir are ranked in the following order: copper> manganese> iron> zinc> nickel> chromium> lead> arsenic> mercury> cadmium [22]. A high concentration of copper was found in the sediment [22]. Notably, a sampling campaign was carried out in February 2015 to investigate the heavy metal accumulation and potential toxicity of sediment cores from 5 reservoirs in the northeastern region of Liaoning and Jilin, China. The concentrations of most metals (manganese, iron, cadmium, copper, chromium, zinc, and lead) were detected. Interestingly, cadmium is accumulated significantly according to the findings [23]. In Iran, the concentrations of heavy metals (cadmium, chromium, manganese, copper, lead, iron, nickel, and zinc) were measured in the Chah Nimeh Reservoir of water and sediments [24]. Heavy metal concentrations in sediments were found to be higher in the reservoir water. The ranking concentrations of heavy metals in the sediments are: Iron > manganese > zinc > nickel > lead > chromium > cadmium > copper [24]. In 2011, sediment samples were collected from Manwan Reservoir (China) sites to detect the source of heavy metals (aluminum, arsenic, copper, iron, cadmium, chromium, manganese, lead, and zinc). The results showed that heavy metal sources were mainly categorized into natural and anthropogenic forms. Cadmium, arsenic, copper, chromium, zinc, and lead concentrations in some sediment areas exceed the standard's requirement [25]. In Guanting Reservoir (China), topsoil samples were assessed for the concentrations of zinc, nickel, chromium, lead, arsenic, and cadmium. Mean copper, zinc, chromium, nickel, cadmium, lead, and arsenic were quantified at 16.8, 59.4, 37.8, 18.3, 0.32, 20.1, and 8.67 mg/kg, respectively [26]. Further, mercury, cadmium, lead, chromium, and copper were detected in surface water and in the sediment of Hongfeng and Baihua Reservoirs (China) [27].

3 Bioaccumulation of Heavy Metals in the Reservoir's Organisms

Six commercial fish species were found in three key Cauvery Delta River Reservoirs, India, which were accumulated with heavy metals (iron, manganese, copper, chromium, lead, nickel, and zinc) [28]. The highest concentration of iron was contained in fish specimens, subsequently followed by lead, zinc, manganese, chromium, nickel, and copper (Table 13.2). The concentration of lead, chromium, and zinc in several samples was above the permitted limits of the Food and Agriculture Organization (FAO) of the United Nations [28]. In Rawal Lake Reservoir (Pakistan), four edible fish including *Cirrhinus mrigala*, *Tor putitora*, *Channa punctatus*, and *Labeo calbasu* were investigated. The results showed that the concentrations of Ni, Cr, and Pb in the muscle of four fish were higher than that requested by the World Health Organization (WHO) [29]. Heavy metal levels in the kidney and liver were relatively high. Metal concentrations were higher in

Table 13.2 Heavy metal accumulation in the reservoir's organisms

Sample collection site	Organism	Elements	Determination method	References
Three major reservoirs of the River Cauvery Delta Region, India	Fish	Fe, Mn, Cu, Cr, Pb, Zn, and Ni	AAS	29
Rawal Lake Reservoir, Pakistan	Fish	Zn, Cu, Cd, Pb, Co, Ni, Mn, and Cr	AAS	30
Keban Dam Reservoir, Turkey	1 mussel, 1 crayfish, 6 wild fish, and 1 farmed fish	As, Cd, Cu, Pb, and Zn	AAS and GFAAS	31
Shah Jamal Reservoir of India	Fish	Cr, Ni, and Pb	AAS	32
Wanzhou section, Three Gorges Reservoir, China	Fish	Pb, Cr, Cd, As, and Hg	HG-AAS	33

post-monsoon fish organs (skins, muscles, and gills) than in pre-monsoon fish organs. Heavy metals concentrations were decreased in the following order: zinc > lead > iron > nickel > chromium > nickel > cobalt> copper > cadmium in the pre-monsoon season, while the post-monsoon season was followed by iron > lead > chromium > nickel > zinc > copper > manganese > cadmium. Similarly, the Cu levels were similar to that of *Cirrhinus mrigala*, but lower in *Tor putitora* and *Channa punctatus* [29]. In 2017, Varol and Sünbül examined the concentration of five heavy metals in aquatic organisms from the Keban Dam Reservoir (Turkey) on the Euphrates River [30]. The highest concentrations of cadmium, lead, and arsenic were found in mussels, while the highest concentrations of copper and zinc were observed in crayfish. The effects of heavy metal pollution on *Heteropneustes fossilis* and *Channa striata* were also investigated in the Shahjamal Reservoir (India) [31]. A considerably higher concentration of heavy metals such as nickel, lead, and chromium was measured in many fish tissues (muscle, liver, gill, and kidney) in this reservoir. The genotoxicity of the heavy metal for fish was confirmed using a micronucleus erythrocyte test and comet assay. The concentrations of heavy metals (chromium, nickel, and lead) have highly increased compared to the recommended values of the Federal Environmental Protection Agency (FEPA) [31]. Similarly, the muscles of eleven fish collected from the Yangtze River in China were tested for arsenic, chromium, lead, mercury, and cadmium. Hg, Cr, Pb, As, and Cd levels were found to be below the recommended limits [32].

4 Molecular Toxicity Mechanism of Heavy Metals

The detailed toxicological mechanism of heavy metals is discussed in Fig. 13.1. Initially, the reactive oxygen species (ROS) level increases and reduces the antioxidant level. The cells are protected from free radicals by antioxidants, such as

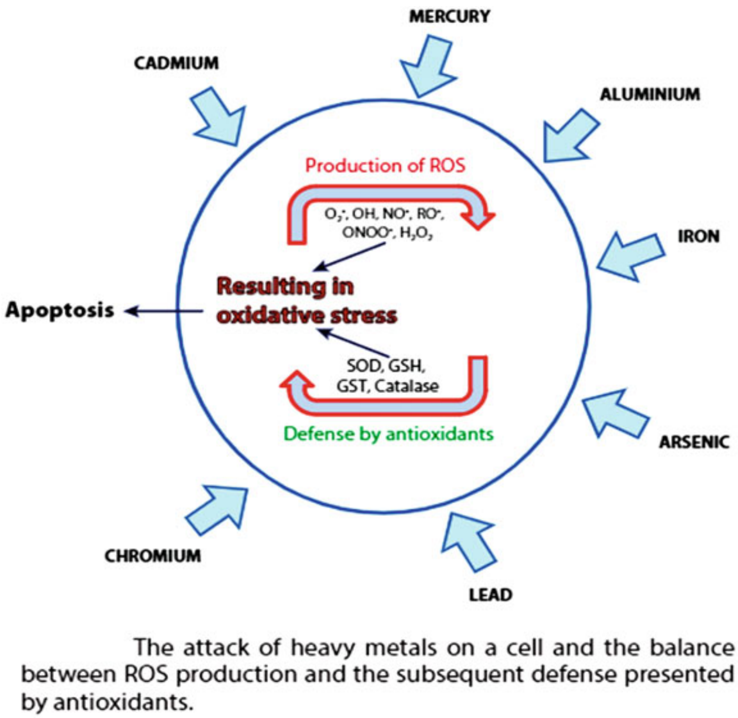

Fig. 13.1 The attack of heavy metals on cells and the balance between ROS production and the subsequent defense by antioxidants [33]

glutathione in its reduced form (GSH). The enzyme of glutathione peroxidase easily converts GSH into oxidized conditions states (GSSG). In normal conditions, GSH is 90% of the total glutathione amount, and the oxidized GSSG is 10%. However, the GSSG concentration under oxidative stress is higher than the GSH and catalyzed by a protein disulfide isomerase [34]. A further biomarker for oxidative stress is lipid molecules within the cell membrane, leading to lipid peroxidation [35, 36]. Moreover, ROS can cause significant damage to proteins, cells, nuclear acids, lipids, and membranes at high concentrations [37].

Toxic heavy metals, such as Cd and Hg, disrupt certain body functions in humans. Metallothionein (MT) can theoretically react with heavy metals and act as an antidote by removing heavy metals from the body [38]. SOD was determined to produce primarily H_2O_2 through superoxides, which can benefit species against free radicals. CAT can catalyze H_2O_2 into harmless H_2O and O_2. Xu et al. found that the MT in *Crassostrea hongkongensis* could potentially respond to the presence of Cd and they established a practical approach to specifically monitor ChMT from the oyster tissues [39]. Chen et al. [40] found that Cd exposure altered the levels of transferrin in the livers of *Pseudosciaena crocea*. Feed ions in the *Pseudosciaena* serum expanded rapidly following the treatment of heavy metal Cd ions

[40]. Another study indicated that Cd and Zn were significantly absorbed in *Salmo platycephalus* gills after 15 days of exposure to Cd and Zn [41]. Moreover, oxidative stress caused by ROS is a well-known mechanism of heavy metal-induced damage, and apoptosis, caspase activation, and ultrastructural changes were observed after exposure to heavy metals [42].

5 Mechanism of Heavy Metal's Detoxification

Metal ions may bind to specific ligand molecules of living organisms under a phenomenon known as chelation [43]. Phytochelatins (PCs) are plant-derived protein-ligand molecules that chelate metal ions when plants are exposed to heavy metals [44–46]. Several studies have shown that PCs are synthesized and formed by a glutathione (GSH) PC syntheses enzyme [47, 48]. Metal ion PCs are effectively delivered and isolated from cellular proteins into vacuoles, reducing damage caused by heavy metal ions. ROS formation prevents DNA repair, and DNA cross-connection with proteins is a crucial factor in heavy metal carcinogenesis [49]. A homeostasis imbalance between the antioxidants and the pro-oxidants induced by the ROS, which contains radical hydroxyls (HO), radical superoxides (O_2^-), and hydrogen peroxide (H_2O_2), which cause protein, DNA, and lipids to oxidative damage. Intracellular antioxidants inhibit this process by oxidation and reacting with free radicals [50, 51]. Intracellular antioxidants are different from complex systems, including heme oxygenase 1, GSH, NAD(P)H: quinone acceptor oxidoreductase 1 (NQO1), catalases, and superoxide dismutase (SOD) [51–54]. Moreover, nuclear factor (ERD 2)-like protein (NRF2) is a well-known antioxidant element regulator to respond to oxidative stress.

6 Conclusion

This chapter has addressed the critical sources of heavy metal exposure in a reservoir and its toxicological mechanistic pathways. Artificial reservoirs generally trap more sediments. Through the aquatic food chain, heavy metals in the water column eventually make their way into human bodies. Heavy metal exposure, whether direct or indirect, disrupts various intracellular processes. These mechanisms could be target markers of heavy metal-induced carcinogenesis. As, Cd, Cr, and Ni toxicity are frequently caused by oxidative pathways. Furthermore, research into heavy metal-induced cancers and diseases will comprehensively understand these complex mechanisms through pathway analysis.

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Chapter 14
Molecular Toxicity Mechanism of Persistent Organic Pollutants (POPs) in the Reservoir

Naima Hamid and De-Sheng Pei

Abstract Due to the ubiquitous environmental existence, persistent organic pollutants (POPs) and their harmful effects have drawn attention among scientific communities. This chapter particularly emphasizes the mechanistic toxicity pathways of POPs when interacting with aquatic species in the reservoir. Among previously published studies, these emerging chemicals mainly disrupt the aryl receptor (AhR) signaling pathway. Besides, some pesticides groups like polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and organochlorinated pesticides (PCBs) induced neurodevelopmental disorders (NDD) and caused endocrine disruption, when exposed to mice and zebrafish at low environment-relevant concentrations (ERC). Furthermore, the increased oxidative stress and genetic toxicity with increased apoptosis levels and DNA damage were also observed even at low exposure doses. In summary, POPs possess deleterious effects when interacting with any biological organism. Therefore, regulative authorities should properly implement the rules and control the release of POPs in the environment.

Keywords Persistent organic pollutant · AhR signaling pathway · DNA damage · Oxidative stress

1 Introduction

Over the past decades, persistent organic pollutants (POPs) are recognized as silent killers due to their bioaccumulative potential and their ubiquitous presence in the environment [1]. Generally, persistent organic chemicals belong to the industrial chemicals or the byproducts released from the combustion. They have been identified as significant components in the global ecosystem and cause health risks to human adipose tissue, blood, and fish [2, 3], which may be organochlorine pesticides, including DDT, lindane, chlordane, heptachlor, dieldrin, hexachlorobenzene, and halogenated hydrocarbons. The industrial compounds mainly comprise polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polychlorinated naphthalene (PCNs), polybrominated biphenyls (PBBs), dibenzo-p-dioxins (PCDDs), polybrominated diphenyl ethers (PBDEs), and polychlorinated dibenzofurans (PCDFs) [1, 4, 5]. These different classes of chemicals cause various

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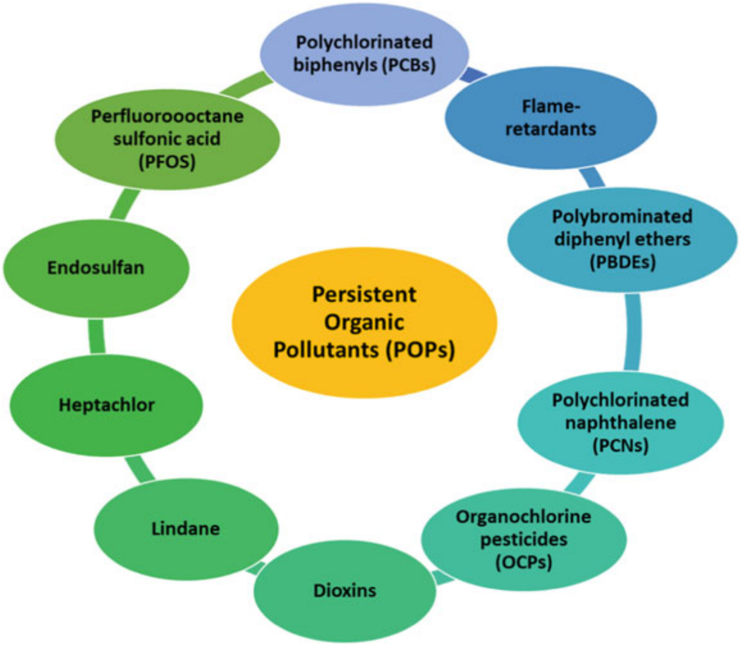

Fig. 14.1 General classification of the POPs

diseases through cytotoxicity, mutagenicity, neurotoxicity, genetic toxicity, and estrogenicity [6], when interacting with any biological organism [3] (Fig. 14.1).

The integrated biomarker approach has been used widely for vertebrates and invertebrates for environmental monitoring [7]. However, the xenotoxicity mechanism of POPs is still elusive. Therefore, more in-depth studies are required to evaluate the mechanistic toxicity of the POPs. Moreover, the toxicology of POPs is highly complicated, and different chemicals can elicit diverse toxic responses in target organs, tissues, and species in a sex-dependent manner [8, 9]. Most xenobiotic chemicals can be mediated *via* the AhR signaling pathway and target different organs, including endocrine, neurological, and cardiovascular, leading to several well-characterized diseases [10–12].

Previously, various model animals including mouse, *Daphnia magna*, *C. elegans*, zebrafish, and marine medaka have been used to investigate the toxic potential of POPs, such as PFOS, PAHs, PCBs, and PAEs [6, 13–15]. Besides, zebrafish is considered a perfect animal model due to its 85% similarity to the human genome, high growth rate, and reproduction [6]. However, in toxicological studies, various exposure concentrations, exposure duration including acute or chronic, and multigenerational exposures are required to determine the complete toxicity scenarios [16–20]. Similarly, different chemical exposure durations may result in different toxic responses [21].

2 POPs Mechanistic Toxicity in the Three Gorges Reservoir (TGR)

The Three Gorges Reservoir (TGR), one of the largest hydropower reservoirs, extends 670 km and covers an area of 1084 km^2 from Chongqing to the Three Gorge Dam (TGD) in China. In the TGR, POPs toxicity has gained much intention owing to their increasing ecological risks. However, detailed mechanistic toxicological studies in this area are still elusive. In a recent study, PAHs pollution in the TGR and its toxicological risks were evaluated using the zebrafish model by Tang et al. [22] at the maximum impoundment level (175 m) [22]. Results revealed that transgenic fish *Tg*(*cyp1a:gfp*) induced by PAHs showed the highest fluorescent levels, particularly in the middle and lower reaches of the TGR. Moreover, the increased levels of oxidative stress further confirm the findings. Interestingly, in 2011 and 2012, sediment and benthic fish species were sampled and elucidated the organic pollutants levels and their mutagenic and genotoxic potentials [1]. It was found that PAHs, their derivatives, and non-target compounds are considered the main causative agents for the genotoxicity of the TGR's fish [1].

3 AhR Metabolism Mechanism

It is believed that POPs induced transcriptional changes in the detoxification of AhR pathway genes, resulting in the disturbance in AhR levels and induction of CYP1A [23, 24]. Zhou et al. conducted a study to determine the phylogenetic analysis of the AhR mechanism after exposure to the POPs in aquatic animals, and each species behaved differentially when exposed to different classes of POPs [24]. Generally, in the regulation of the AhR pathway, cytochrome P4501 is the major target molecule. When a xenobiotic compound, such as POPs, contacts any biological organism, CYP1A1, a xenobiotic metabolic enzyme, is activated and forms Ahr–ligand complexes. Therefore, AhR-dependent CYP1A1 has a high binding affinity against POPs metabolism [24]. When the AhR–ligand complexes translocate from cytoplasm to the nucleus, it induces the formation of AHR-ARNT and binds a precise xenobiotic response element (XRE) in the promotor region, resulting in disturbing the expression levels of the downstream genes and elevating the oxidative stress levels (Fig. 14.2).

4 POPs Toxicogenetic Endpoints *In vitro* and *In vivo*

Different model species have been performed in the past to determine the mechanistic toxicity mechanisms of the POPs. Here, a summary of numerous *in vivo* and *in vitro* studies on POPs' toxicity is presented in Table 14.1. The majority of the

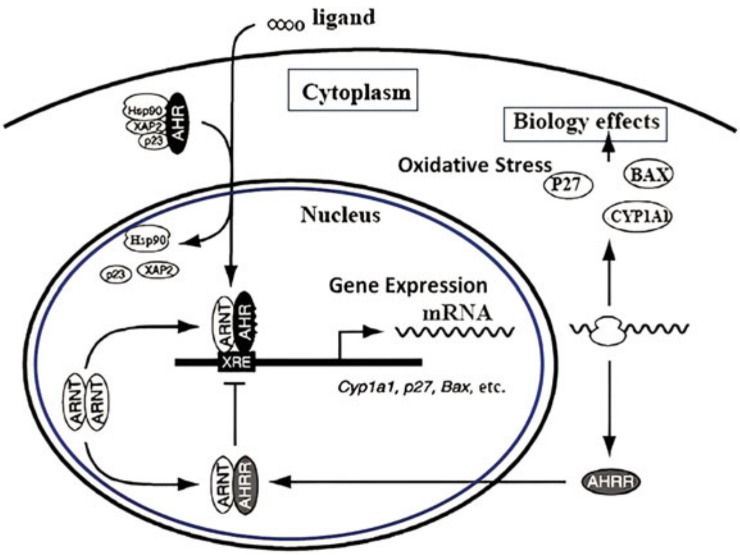

Fig. 14.2 A detailed regulation description of the AhR pathway for the POPs. The figure was adapted from a previously published research [25]

previously published studies have focused on determining developmental toxicity *in vivo*, such as mortality, deformity, and oxidative stress [33, 34]. Likewise, perfluorooctane sulfonate (PFOS) exposure to *C. elegans* elevates the reactive oxygen species (ROS) and increased apoptosis levels with the significantly upregulated *HUS-1* expression [24]. When *C. elegans* was exposed to endosulfan, the expression levels of the germ apoptosis-related genes were increased [27] (Table 14.1).

Even at lower exposure concentrations of Pesticides, polychlorinated biphenyls (PCBs), and organochlorine phosphate (OCPs) perturb the central glucose metabolism signaling pathway, and caused reproductive toxicity and severe liver damage [7, 24]. Previously, a study published by He et al. [32] indicated the reproductive toxicity of the PCBs exposed to adult mice at low exposure concentrations [32]. DNA methylation was observed with the significant upregulation of the associated genes (*Dnmt1, 3a, 3b, 3 l, Uhrf1, Tet2*, and *Tet3*) in mice F_1 generation [32]. Therefore, it can be concluded that the PCBs' transgenerational effects were more related to epigenetic regulation. Furthermore, Lindane exposed to *C. elegans* activates the insulin-growth factor (IGF) pathway by decreasing the transcriptional gene expression (such as *daf-2, sgk-1, akt-1*, and *daf-16*) [30].

Interestingly, some pesticides of POPs induce neurotoxicity with acute exposure to aquatic species. For example, a recent study highlighted that PCBs disturbed the neurological networks and caused neurodevelopmental disorders (NDD) in mice even at low exposure concentrations [31]. The author further demonstrated that chronic exposure to low doses of many pesticides caused serious neurodegenerative diseases, particularly Parkinson's disease, resulting in silent neurotoxicity that may

Table 14.1 Toxicogenetic endpoints of the POPs observed *in vitro* and *in vivo* assays at environmentally relevant concentrations (ERCs)

Chemical	Species	Observation	Toxic endpoints	Reference
Perfluorooctane sulfonate (PFOS)	*C. elegans*	Oxidative stress, cell metabolism	↑ROS; ↑Cell apoptosis; ↑Distinct foci of HUS-1:GFP	[26]
Endosulfan	*C. elegans*	Cell apoptosis	↑Germ cell apoptosis in mev-1(kn-1) mutant; ↓Apoptosis cep-1(w40), egl-1 (n487), and hus-1 (op241); ↑HUS-1: GFP foci	[27]
Perfluorooctane sulfonate (PFOS)	*C. elegans*	Insulin/IGF-1 signaling pathway	↓Average lifespan in daf-2(e1370) and daf-16b(KO) mutants	[28]
Polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDE), polychlorinated dibenzo-p-dioxins (PCDDs), and polychlorinated dibenzo-p-dioxins/ furans (PCDD/Fs)	Aquatic birds	Developmental toxicity	Non-dioxin-like PCBs were ten times higher toxic for birds eggs than other analyzed PCB congeners	[29]
Lindane	*C. elegans*	Insulin/IGF-1 signaling pathway	↓Level of insulin; ↓daf-2, sgk-1, akt-1, and daf-16 genes	[30]
DDT (1,1,1-trichloro-2,2-bis(*p*-chlorophenyl) ethane)	Mice	Liver damage, reproductive toxicity, glucose physiology, and central signaling pathway	200 hepatic genes affected by *p, p'*-DDE, perturb lipid metabolism, mitochondrial dysfunction, and alterations in glucose and central signaling pathways. Also impairs testosterone metabolism in the liver revealing endocrine disruption properties.	[7]
Polychlorinated biphenyls (PCBs)	Mice	Neurodevelopmental disorders	Neurodevelopmental disorders (NDD), including autism spectrum disorder (ASD), a high prevalence of gastrointestinal (GI) distress.	[31]

(continued)

Table 14.1 (continued)

Chemical	Species	Observation	Toxic endpoints	Reference
Polychlorinated biphenyls (PCBs)	Mice	DNA methylation, reproductive toxicity	DNA methylation patterns of the genes *H19, Snrpn, Peg3*, and *Igf2r* as well as the high expression levels of *Dnmt1, 3a, 3b, 3 l, Uhrf1, Tet2*, and *Tet3* in fully grown germinal vesicle oocytes were found in mice offspring after exposure to PCBs.	[32]
Tetrachlorodibenzo-p-dioxin (TCDD)	*D. rerio*	Aryl hydrocarbon receptor repressor (AHRR) xenobiotic metabolism pathway	Disturb cytochrome P450 1 (CYP1) metabolism detoxification pathway using environment-relevant concentrations (ERCs).	[24]

disturb the nervous system entirely in old age [31]. In summary, POPs can induce different toxicity mechanisms even at lower exposure concentrations and significantly affect the integrity of the whole ecosystem.

5 Conclusion

In summary, POPs are ubiquitously present in the environment and exhibit intricate exposure scenarios for aquatic species. Although published data regarding the mechanistic toxicity of POPs in the TGR is limited, it can be found that some POPs, when interacting with any biological organisms, may target the AhR signaling pathway. For example, mice and zebrafish after pesticides exposure may induce neurodevelopmental disorders (NDD) and caused endocrine disruption properties, such as testicular toxicity even at low exposure concentrations. Exposure to PFOS in *C. elegans* elevates oxidative stress and cell apoptosis. However, there are significant challenges in determining the interactive effects of POPs mixtures. Thus, regulative authorities should focus their attention and ensure that it is adequately banned both in developed and developing countries to combat its serious health effects.

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Conflicts of Interest The authors declare no conflict of interest.

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Chapter 15
Molecular Toxicity Mechanism of Microplastics in the Reservoir

De-Sheng Pei and Suman Thodhal Yoganandham

Abstract Recently, the toxicity of microplastics (MPs) has attracted global public health concerns. In this chapter, we reviewed the published studies on MPs toxicity in the reservoir to identify knowledge gaps about the harm caused by MPs. Most toxicology studies of MPs have focused on ecotoxicity using apical endpoints, with only a small number of studies addressing molecular toxicity mechanisms. Moreover, MPs in the reservoir are easy to adsorb environmental pollutants, including heavy metals and organic pollutants, implying combined toxicity effects on organisms. Adverse Outcome Pathways (AOPs) framework may integrate published data and identify data gaps in the toxicity mechanisms of MPs. Generally, these findings point to the generation of reactive oxygen species (ROS) as the molecular initiating event (MIE) and then cause developmental and reproductive failure. However, there is not enough data about the association between key events (KEs). Thus, further research is needed to fill the gaps in the toxicity pathways of MPs.

Keywords Microplastics · Reservoir · Oxidative stress · Adverse outcome pathways

1 Introduction

Plastic materials have excellent properties, such as lightweight, robustness, flexibility, and low production costs [1]. However, the wide use and environmental effects of plastic waste have raised worldwide concerns. In 2016, approximately 322 million plastic products were produced globally [2]. Around 10% of plastic waste enters the aquatic environment due to widespread utilization, increased plastic product development, and poor management [3]. Plastic waste can reduce the aesthetic value of the water ecosystem, cause biodiversity loss, and influence public health [4]. Small plastic debris (<5 mm), commonly called microplastics (MPs) may cause a greater and broader ecological risk [5], due to their small size, difficult degradation [6], and easy adsorption of toxic organic and inorganic contaminants [7]. Generally, the polymers of MPs contain polyethylene terephthalate (PET), polystyrene (PS), polypropylene (PP), etc. Notably, in 2021, MPs were first reported in the human placenta

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[8]. Although the health risks of MPs have aroused great attention, the molecular toxicity mechanism of MPs in the water environment, especially in the reservoir, is still elusive [9]. Therefore, this chapter will clarify the MPs distribution in the reservoir, elucidate toxicity mechanisms caused by MPs, and reveal their combined toxicity and adverse outcome pathways (AOPs) relevance to MPs.

2 MPs Distribution in the Reservoir

MPs contamination in the reservoir is recently raising increasing concerns worldwide. Their distribution in the reservoir was reported in different countries, but lacks a holistic understanding of their occurrence. Guo et al. [10] constructed a global MPs dataset containing 440 collected samples from 43 reservoirs worldwide, which aimed to provide a comprehensive understanding of the drivers and mechanisms of MPs pollution in reservoirs from geographical distribution, driving forces, and ecological risks aspects. They found that small-sized MPs (<1 mm) accounted for more than 60% of the total MPs found in reservoirs worldwide; seasonal variation, geographic location, and land-use type were the main factors affecting MPs abundance.

Liu et al. [11] investigated the horizontal and vertical distribution of MPs in the Guanyingyan Reservoir (China), a dam reservoir, and found that MPs abundance in the horizontal distribution of the reservoir decreased significantly, and the vertical distribution of fibers MPs had less variation in the surface, intermediate, and deep layers, compared to other types of MPs. Moreover, MPs with a size <0.5 mm occupied the majority portion. Interestingly, Lin et al. [12] measured the distribution and source of MPs in the Danjiangkou Reservoir, China's second-largest reservoir. They found that MPs accumulated in the middle layer of the reservoir, and the MPs appearance, such as size and color, varied from the surface to the bottom, implying that surface water sampling cannot determine the MPs contamination for deep-water reservoirs. Shen et al. [13] monitored MPs pollution in the vicinity of ten dams in the Shaying River, a typical multigate dam-type river, by collecting water, sediment, and biological tissues. They found that dam construction altered the suspension, transportation, and deposition of MPs at different dams. Gao et al. [14] investigated MPs pollution at a sandy beach near the outlet of a major reservoir in north Mississippi (USA), and they found that the major form of MPs was fibers (64%), followed by fragments (23%), beads (7%), and films (6%). Further, more in-depth studies should be conducted to provide a comprehensive understanding of the occurrence, drivers, and potential risks of MPs in the reservoirs.

3 Toxicity Mechanisms of MPs

MPs exposure has been shown to inhibit different enzymes and metabolic pathways in invertebrates and vertebrates [15]. Exposure to MPs may disrupt detoxification systems and induce a high expression of genes involved in fundamental physiological processes, such as the arrest of cell cycle growth, oxidative stress, and apoptosis [16].

In our lab, Suman et al. [17] highlighted the molecular toxicity mechanism of MPs exposure using transcriptome analysis. Acute and chronic toxicity in brine shrimp were determined after exposure to polystyrene MPs with various concentrations, and the generation of reactive oxygen species (ROS) was observed. The histopathology analysis revealed the deformation of epithelial cells in the midgut region of brine shrimp. Moreover, the transcriptome analysis was performed after chronic exposure to polystyrene MPs, and the differential expression gene was further confirmed using qRT-PCR. Venn diagram of the transcriptome indicated that 3770 and 5448 genes were differentially expressed in the MPs exposure groups and the controls; 14,930 unigenes were co-expressed both in the treatment and controls (Fig. 15.1a). Compared to the control, 292 and 429 differentially expressed genes (DEGs) were significantly expressed after exposure to polystyrene MPs, indicating their vital role after exposure to PS-MPs (Fig. 15.1b). Enrichment analyses of KEGG and GO revealed the functional clusters and biochemical pathways of those DEGs. According to the biological process, the significantly DEGs were closely involved in the energy derivation by oxidation of organic compounds, polysaccharide biosynthetic process, cellular nitrogen compound metabolic process, glucan biosynthetic process, cellular polysaccharide biosynthetic process, RNA capping, and 7-methylguanosine RNA capping (Fig. 15.2a). Interestingly, 155 pathways were achieved through KEGG enrichment, including viral myocarditis, phagosome, arrhythmogenic right ventricular cardiomyopathy, fluid shear stress, hypertrophic cardiomyopathy, and atherosclerosis. Further, the regulations of actin

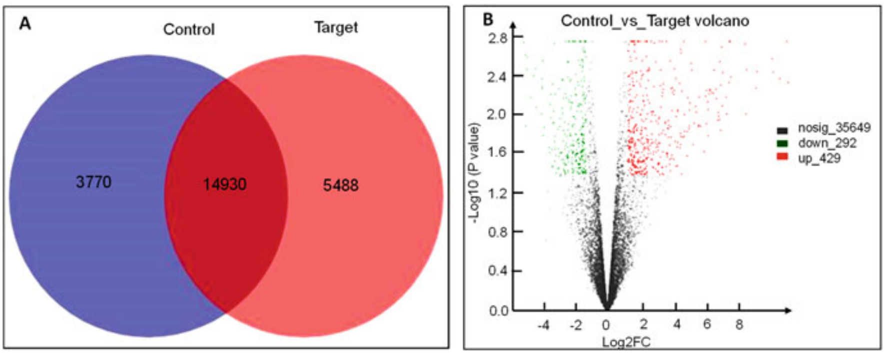

Fig. 15.1 The Venn diagram and volcano plot depicting the DEGs profile of brine shrimp treated with polystyrene MPs for 14 days. (**a**) Venn diagram shows the number of unigenes and changed expression after MPs exposure. (**b**) Volcano plot describes the DEGs profile after MPs exposure

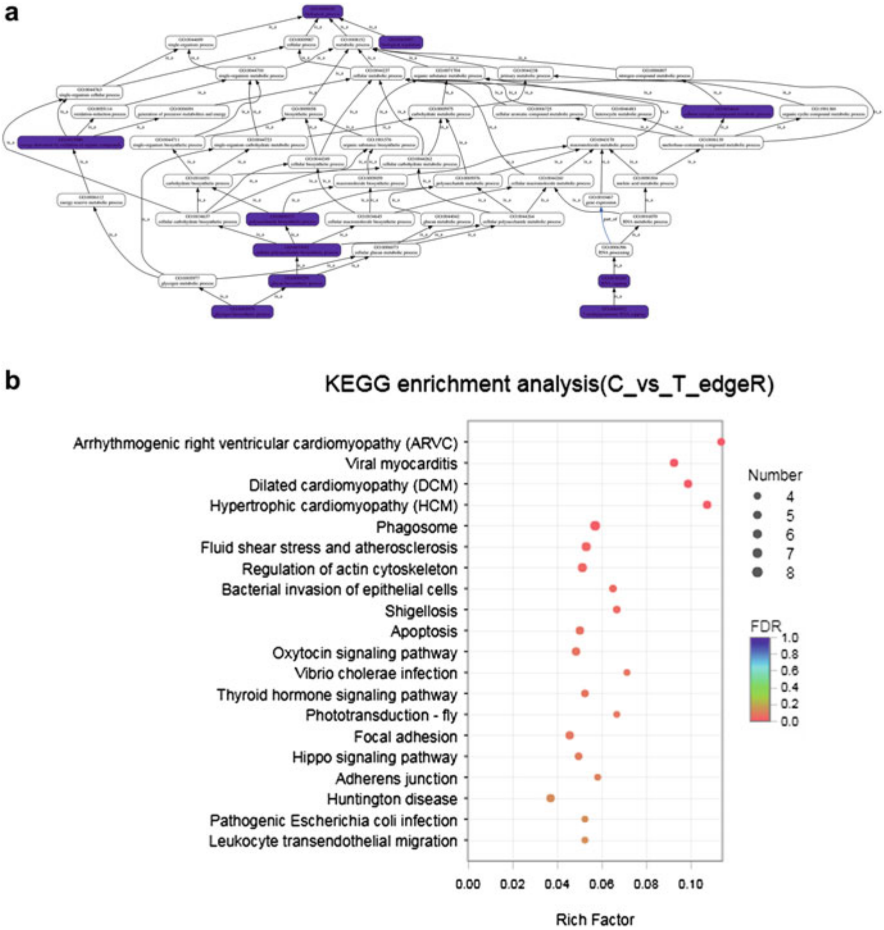

Fig. 15.2 Gene Ontology (GO) and KEGG enrichment disclose the toxicity pathways after exposure to polystyrene MPs. (**a**) The hierarchy enrichment of GO terms highlights the annotations of biological processes with red boxes. (**b**) KEGG enrichment analysis of DEGs after exposure to polystyrene MPs

cytoskeleton were significantly affected by regulating the activities of ROS and apoptosis (Fig. 15.2b).

Moreover, MPs can disrupt cell surface structures or other extracellular matrix components at moderate levels and inhibit the cell signaling processes of extracellular receptor interactions between the ligand and the cell surface [18]. Besides, MPs may disrupt endocytic activity and activate the cellular innate immune system [19]. In the gastrointestinal tract, MPs were reported to be accumulated and caused inflammatory responses and oxidation [20, 21]. Moreover, MPs may produce reactive oxygen species (ROS), activate antioxidant-related enzymes, and boost glutathione S-transferase (GST) activity and MAPK signaling pathways.

4 Combined Toxicity of MPs with Other Environmental Pollutants

Because of their chemical and physical characteristics, MPs are easy to adsorb environmental pollutants, such as heavy metals [22] and organic pollutants [23], and show combined toxicity effects on organisms. The combined toxicity is related to the properties of adsorbates, particle size, and the composition of plastics [24]. When considering the interaction between particles and organisms, particle size is an important characteristic [25]. Because MPs are small and possess large hydrophobic surface areas, they are prone to serve as vehicles for microorganisms or chemicals. After exposure to these MPs mixed with other toxic substances, combined toxic effects were exhibited in organisms, including synergistic, additive, or antagonistic effects [26].

In our lab, Wang et al. [27] reported the combined toxicity of MPs and three concomitant heavy metals (Cd, Pb, and Zn) in the environment. We revealed the enrichment and distribution of heavy metals and MPs in marine medaka. Moreover, the individual and combined effects on intestinal toxicity and gonadal development were systematically investigated. As far as we know, this is the first report about the individual and combined effects of heavy metals (Cd, Pb, and Zn) and polystyrene MPs on intestinal toxicity and gonadal development of marine medaka.

MPs can adsorb polyfluoroalkyl substances (PFASs), polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), antibiotics, and other organic pollutants [28]. The adsorption types mainly include surface adsorption, pore filling, and distribution [29]. Low-density polyethylene (LDPE) MPs in San Diego Bay were found to adsorb PAHs, PCBs, and polybrominated diphenyl ethers (PBDEs), and these compounds could be biomagnified in medaka (*Oryzias latipes*) and caused liver injuries, such as glycogen depletion, fat vacuolization, and cell necrosis [30]. Another study investigated the combined toxic effects of 5 μm PS-MPs and F-53B. After their exposure in zebrafish, the bioavailability and bioaccumulation of F-53B were reduced due to the strong adsorption capacity of PS-MPs to F-53B. However, co-exposure to PS-MPs and F-53B induced severe oxidative stress and inflammation in zebrafish [31].

Environmental monitoring results showed that plastic chips can enrich metals in the ambient environment [32]. The concentrations of Al, Fe, Mn, Cu, Pb, and Zn reached 180 $\mu g \cdot g^{-1}$ in the plastic particles collected on the beach, and the concentrations of Cd, Cr, Co, and Ni reached 0.92 ng·g-1, which are close to or higher than those in the surrounding environment [33]. PE plastic microspheres (2–4 μm) were reported to enrich Cd in seawater and caused serious reproductive toxicity to *Moina mongolica*, because the adsorbed Cd on PE plastic microspheres was released in the acidic digestive or gastrointestinal tract and subsequently transported to other tissues. Thus, stronger combined toxicity was generated than that of PE microspheres alone [34]. The surface area enhances as the particle size decreases, implying that micro-sized particles may achieve stronger adsorption of pollutants [24]. MPs in the environment have a coarser structure with a bigger surface area and possess stronger sorption abilities [25]. The combined toxicity of MPs is highly dependent on the plastic and absorbed compound types, the number of particles ingested, the release rate of contaminants, *etc*.

Except for the combined toxicity of plastics with other environmental pollutants, the toxicity of plasticizers cannot be ignored for their wide use to increase plasticity in the process of plastic synthesis [29, 35]. Different plasticizers are widely used globally and often exhibit ecological toxicity as environmental hormones [35, 36]. Organisms may be directly exposed to leached additives after MPs are ingested [29]. Such additives and monomers may interfere with the signaling pathways of estrogen and testosterone synthesis, resulting in endocrine disruption [37]. The most common plasticizers are phthalate esters (PAEs), which are primarily used in the manufacturing of PVC products, such as upholstery, shower curtains, flooring, and food containers [38]. PAEs as plasticizers are not covalently bonded to the polymer matrix and easily leach from plastics, especially at a high temperature and low pH [35]. Exposure to PAEs activates the CYP450 detoxification system and disturbs endocrine system, resulting in metabolic disorders, oxidative stress, endocrine disorders, and immunodepression [39].

5 AOPs Relevance to MPs Toxicity

AOPs may recognize the specific toxicity pathways and biological key events (KEs) occurring at different levels of the organism with adverse outcomes. Thus, the AOPs may indicate biological processes happening at several levels of the organization from the molecular to the cellular, organism, and tissue [40], which can be used to pinpoint potential threats and outline the chain of KEs that led to the risk effects. As a result, they explain how stressors might start the crucial sequence of KEs needed for the commencement of an adverse outcome. The connections of KEs describe the quantitative connection between two consecutive KEs to trigger the next KEs. The toxicological threshold, temporal dynamics, and dose–response connections are all described by KEs relationships [41, 42]. Individual KEs may be used to determine whether experimental endpoints and assays are the most important for measuring MPs toxicity.

AOPs relevance to MPs toxicity has not yet been fully known. Physical, structural (size and form), and chemical characteristics, such as the chemical composition of MPs and adsorbed substances on their surface, may contribute to MPs' toxicity. MPs can initiate biocorona and cell contact by interacting with lipids, proteins, and other small molecules in the cell membrane and/or biomolecules in the surrounding media. Chemical, physical, receptor-mediated, and mechanical interactions are all possible with the cellular microenvironment. When it comes to MPs, interactions between cells can be established by biocorona components to define the eventual AOPs [43]. Pattern recognition receptors play a crucial role in interactions by identifying agonists of the innate immune response. ROS risks can be amplified by photochemical weathering when the plastic is exposed to UV light [44]. Proinflammatory responses may be initiated by ROS as a result of an oxidative stress response.

Oxidative stress has been reported in *Artemia salina* after exposure to polystyrene MPs [17], however, there is no clear evidence to suggest that oxidative stress occurs in humans after exposure to plastic dust. Oxidative stress and inflammation are proposed to be the primary mechanisms of MPs-induced toxicity [19]. Although AOPs induced by MPs may not elucidate MPs' physical, chemical, and structural identity, some existing AOPs can certainly contribute to the prioritization of toxicity endpoints and assays for targeted investigation.

6 Conclusion

This chapter summarizes the reported effect of MPs and molecular toxicity mechanisms in the reservoir. According to the published literature, MPs are a threat to aquatic biota. The formation of ROS that has recently been identified as the molecular initiating event *via* AOPs, which is a more general phenomenon of the cellular stress response. However, few studies have been conducted on the toxicological mechanisms of MPs and the results are not sufficiently conclusive. To fill these gaps, more detailed toxicology research on MPs is required.

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Conflicts of Interest The authors declare no conflict of interest.

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Chapter 16
Molecular Toxicity Mechanism of Plasticizers in the Reservoir

Naima Hamid and De-Sheng Pei

Abstract Plasticizers, due to their robustness, flexibility, and low production costs, have broad industrial applications. However, they are released into the reservoir environment because of inappropriate disposal discharge and cause adverse health effects. This chapter mainly focuses on the molecular mechanism of the plasticizers, particularly phthalates (PAEs) and bisphenol A (BPA), when they are exposed to or interacted with organisms. Previously, various studies have reported that plasticizers affect growth development by altering the thyroid and estrogen axis, leading to infertility. Furthermore, these chemicals can disturb reproductive capacity and decrease egg production *via* reducing steroidogenesis, activating peroxisome proliferator receptors (PPAR), and enhancing oxidative stress levels. In a nutshell, plasticizer pollution in the reservoir should be deeply concerned, and more studies on the adverse health effects of plasticizers on aquatic species are critically needed.

Keywords Peroxisome proliferator-activated receptors (PPAR) pathway · Endocrine disruption · Reproductive toxicity · Phthalates

1 Introduction

Plasticizers are chemicals including flame retardants and surfactants that are combined with polymers to increase flexibility in industrial products. They are not permanently covalently bound to the plastics. Therefore, they can slowly diffuse into the reservoir environment and cause environmental pollution. Plasticizers are ubiquitous existence, and their metabolites were found in the air, soil, water, and biota [1–3]. Bisphenol A (BPA) and phthalates (PAEs) are the most essential plasticizers due to their inducing endocrine disruption abilities, particularly in mammals [4]. Presently, more than 20 types of PAEs have been found in the reservoir environment, of which six di-methyl phthalate (DMP), di-ethyl phthalate (DEP), di-butyl phthalate (DBP), benzyl-butyl phthalate (BBP), di-(2-ethylhexyl) phthalate (DEHP), and di-n-octyl phthalate (DNOP) are considered a priority chemical classified according to the United States Environmental Protection Agency (US EPA) [5]. Despite their restriction, their worldwide usage has rapidly escalated with a rate of 1.8 million tons to 8 million tons from the years 1975–2011 [6]. In

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Europe, about 1,150,000 metric tons of high-volume plastic monomer were produced in 2005–2006 [7]. Structurally, PAEs have a central ring with two esters, which vary with molecular weight. Longer chains have high molecular weight and possess a higher residence rate in the reservoir environment [4].

From 2010 to 2015, PAEs usage increased at an annual rate of 7.70% [8, 9]. Among all PAEs, only DEHP holds 80% of production in China [10]. Considering the high percentage prevalence in the reservoir environment, various species are exposed to and interacted with PAEs mixtures [10, 11]. In terms of toxicity, DEHP is the most widely studied and considered a toxic chemical due to its significant reproductive effects [12]. From the published studies, it was found that DBP, DEHP, and DNOP can disturb developmental growth, increase reactive oxygen species (ROS), and elevate oxidative stress that can cause oocyte apoptosis [12]. Moreover, it may also disrupt the peroxisome proliferator-activated receptors (PPARs) functions, perturb follicle production, and suppress vitellogenin (VTG) protein and estrogen receptor (ER) genes, which are considered the biomarkers of endocrine disruption [9, 13].

2 DEHP Mechanistic Toxicity in the Three Gorges Reservoir Area (TGRA)

Due to DEHP being known as the most toxic chemical congener of PAEs, a recently published study investigated the in-depth toxicity of DEHP exposed at environment-relevant concentrations (ERC) in the TGRA [14]. This study using cell lines and zebrafish as the model species elucidated the underlying mechanisms for DEHP toxicity and its associated ecological risks, which is the holistic approach for integrated toxicological assessment. In the TGRA, the levels of DEHP were considerably higher than that previously prescribed by the US EPA [14]. However, the general trend revealed a decrease in the DEHP levels from the upper, middle, and lower reaches of the TGRA. Further, *in vitro* toxicity of DEHP was determined by using a cell line. At the exposure levels of 100–800 μg/L, a significant decrease in cell viability was observed [14]. Short-term exposure to 400 μg/L DEHP in zebrafish embryos can activate the PI3K-AKT-mTOR pathway [14]. Moreover, a long-term (3 months) exposure to 10–33 μg/L DEHP disturbed the hypothalamus–pituitary–thyroid (HPT) axis pathway. Thus, DEHP levels in the TGRA showed an enhanced potential to induce reproductive toxicity *in vivo* and DNA damage *in vitro* [14].

3 Plasticizers' Toxic Effect on Peroxisome Proliferator-Activated Receptors (PPARs)

PAEs can induce the transcriptional changes of PPAR and cause reproductive toxicity. PPARs are nuclear receptor proteins, which can bind to particular DNA sequences and affect the transcription levels of DNA [12]. They are categorized into three types: PPARα participates in fatty acid degradation; PPARβ and PPARγ control fatty acid metabolism and glucose levels, respectively [15]. It is confirmed that PAEs and BPA can disrupt the PPARs levels in mammals [16]. Similarly, a study was reported in mouse fibroblasts and observed the elevated expression levels of pparγ2 after exposure to BPA (18 mg/L) [17]. Similarly, Deng et al. [18] found that *pparα* and *pparβ* transcription levels were also increased when rats were exposed to DEHP [18]. Furthermore, different structural phthalates have different metabolism abilities that may alter the PPARs and peroxisome proliferation [19]. In mice, an *in vitro* study investigated the toxic effects of BPA and found that two days of exposure elevated the PPARγ2 expression level [17]. Moreover, PPARα and PPARβ transcript levels were also enhanced after DEHP exposure in rats [7]. PPAR protein levels were also altered in rats after exposure to monoester phthalates [20]. PAEs binding with PPARs can interact with retinoid X receptor (RXR), induce the change of hormones, and participate in the carbohydrate and lipid metabolism [17]. Notably, PAEs can alter the normal functioning of PPARs, and long-chain PAEs were more significantly bound than single-chain PAEs [19]. The detailed toxicity pathway of PAEs affected PPARs function and induce reproductive effects is presented in Fig. 16.1.

4 The Impairment of Thyroid and Estrogen Axes by Phthalates and BPA

If the hormonal axes are disturbed by plasticizers, the hypothalamic–pituitary–gonadal (HPG) axis and thyroid axis balance are also affected [21]. They are responsible to regulate metabolic, developmental, and reproductive functions [22]. Thyrotropin-releasing hormone (TRH) is released first from the hypothalamus and then converts into triiodothyronine (T_3) and thyroxine (T_4). Furthermore, these hormones are responsible for regulating the level of thyroid-stimulating hormone (TSH), which is released from the pituitary gland [23]. TSH mainly involves the synthesis of T_4 in the thyroid gland, converts them to T_3, and then further degrades it into T_2 [24]. The physiological effects of thyroid hormones mainly including T_3 and T_4 are mediated *via* binding to thyroid nuclear receptors α, β, and γ [16].

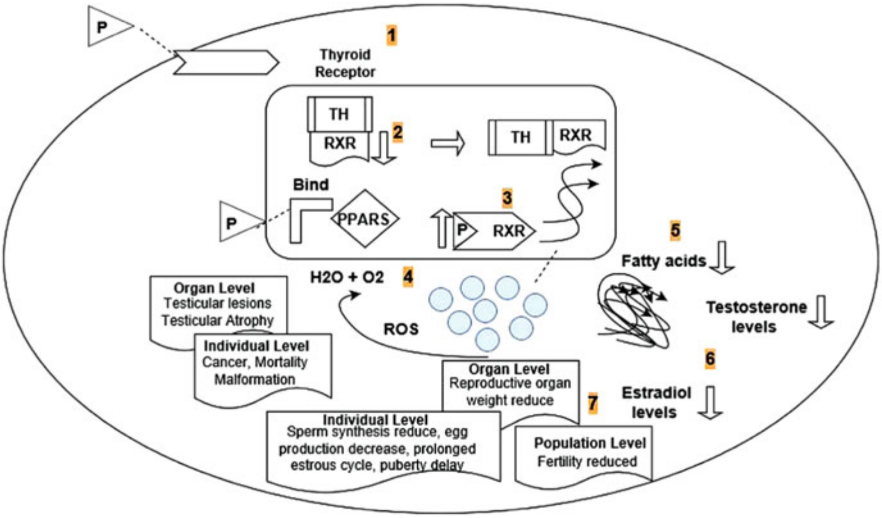

Fig. 16.1 The toxicity mechanism of PAEs (P) at different levels. 1—PAEs bind with thyroid hormone receptor (TR); 2—the formation of retinoid X receptor (RXR) complex; 3—PPARs gene and protein expression level is upregulated, and PAEs activate PPARs; 4—Fatty acid oxidation leads to the formation of ROS; 5—PAEs reduce the transport of fatty acids, decrease the testosterone levels (6), and reduce the estradiol levels (7), which will have deleterious effects at the organ, individual, and population levels

4.1 *Phthalates and BPA Effects on Thyroid Axis*

In previous studies, the effects of phthalates and BPA on thyroid hormone (TH) have been reported in various aquatic species and mammals. Among the PAEs, DEHP is the potent inhibitor that specifically binds to the TR with the string ligand binding energy values [25]. A study conducted on African frogs (tadpoles) and treated with DBP at a low ERC of 2 mg/L showed an altered expression level of four TH-related genes [26]. Gayathri et al. [27] discussed that PAEs caused thyroid gland hyperactivity [27], whereas Wenzel et al. [28] suggested that PAEs amplified the iodide uptake and elevated the iodide symporter activity [28]. However, it is unknown whether PAEs can either modify or alter the TH levels in aquatic species. In amphibians, BPA alters the T_3 function resulting in shortened interocular distances in African tadpoles [29]. Furthermore, exposure to BPA perturbs the hatching ratio in zebrafish [30] because of the upregulated expression levels of the vitellogenin (Vtg) gene and the suppressed growth of somatic cells to vitellogenesis.

Studies have evidenced that PAEs and BPA can disturb the normal transcriptional levels of the genes involved in cholesterol transport and steroidogenesis [16, 31]. In steroidogenesis, the first step includes the conversion of cholesterol into pregnenolone with the help of an enzyme cytochrome P450 (CYP11A1) [11]. When DBP, DEHP, and DMP were exposed to rats, the gene expression level of CYP11A1 has been reduced significantly [32]. In addition, progestogens are changed into androgens *via* CYP17A1, which are responsible for the mediation of both 17a-hydroxylase and 17, 20-lyase activities [29].

4.2 Phthalates and BPA Effects on Estrogen Axis

Many studies have been conducted and the results indicated that the estrogenic activity was altered after exposure to PAEs and BPA congeners in mammals, amphibians, fish, and human cell lines [23]. PAEs can easily bind with the estrogen receptor (ER) of humans, rats, rainbow trout, and zebrafish [7, 33]. When ERs bind to PAEs, they disturb the Vtg expression levels, particularly when exposed to DEHP in zebrafish, exposure to BBP in rainbow trout, and exposure to DBP in marine medaka [16, 34, 35]. PAEs exposure also affected the reproduction ability of female fish and rats. Previously, Xu et al. [31] reported that chronic exposure to DEHP disturbs the estrous cycle in rats [31]. Moreover, in aquatic species, egg ovulation has been delayed after exposure to DEHP. For example, 40 mg/L of DEHP exposure to zebrafish for three weeks impaired zebrafish reproduction, particularly the oogenesis. They further demonstrated that lower egg production might be due to the downregulated expression levels of ptgs2, which is considered an essential enzyme for zebrafish ovulation [36]. Similarly, like PAEs, BPA also possesses estrogenic effects, particularly on ERα and Erβ. For example, exposure to a low concentration of BPA (0.23 mg/L) upregulated the ERα expression level in amphibians [37]. Besides, BPA may reduce the production of luteinizing hormone (LH), which could be the reason for disrupting the estrous cycle [25].

5 Combined Reproductive Effects of Plasticizers

Plasticizer mixtures also exhibited augmented high estrogenic activity, compared to individual chemical toxicity [38]. For the evaluation of plasticizer toxic mixture effect in animal models, previous studies on fish were enormously published, especially screening the combined toxicity of chemicals on various organs in zebrafish (*Danio rerio*). Other commonly used animal models encompassed mice (*Mus musculus*), *Caenorhabditis elegans*, *Carassius auratus, Daphnia magna,* and marine medaka (*Oryzias melastigma*) [39–41]. In contrast, *in vitro* models are commonly used to determine mixture toxicity, including *Vibro qinghaiensis sp-Q67, Vibrio fischeri, Photobacterium phosphoreum, Microtox, Folsomia fimetaria, Scenedesmus vacuolatus, Lemna minor, Escherichia coli, Bacillus subtilis, Photobacterium leiognathi, Anabaena CPB4337, Aliivibrio fischeri,* and H295R cells. Xu et al. [42] conducted a study to elucidate the joint toxic effects of the binary mixtures of DBP + EE2 on developmental growth, reproduction, and histological alterations using male zebrafish [42]. It was found that Vtg and AOX levels were significantly decreased in all binary mixtures, compared to individual

EE2 and DBP exposed groups [42]. It can be concluded that EE2 and DBP may act synergistically to enhance endocrine-disrupting effects in male zebrafish. Similar results were observed from the exposure to the binary combinations of anti-inflammatory antibiotics (CBZ, PHO, SMX, and TMP), where male zebrafish showed high Vtg gene expression levels with an increase in hepatic mass [43]. Likewise, CBZ, DIC, EE2, and MET binary mixtures of antibiotics also induced reproductive abnormalities in progenies [44]. Dong et al. (2018) investigated the combined effects of DEHP+DEP on fetal Leydig cells in male rats [45]. Nevertheless, DBP + DEHP cocktails have shown synergistic and dose-additive effects to reduce sperm count, epididymis, and liver weight in male rats [46]. Besides, cystic ovaries disrupted the estrous cycle and reduced fertility when female rats were exposed to the quinary mixtures of phthalates (DEP, DEHP, DBP, DNOP, DIBP, and BBP) [47]. Furthermore, phthalates combinations with parabens also altered or modified the enzymatic activities for reproduction (estrogen metabolism) [48]. Antioxidant enzymatic actions, such as SOD, CAT, and GPx, were elevated in binary mixtures of phthalates (DBP + DEP) [46, 49]. The combined mixture effects of PAEs with Glycerin monostearate (GMS) resulted in a reduction in testosterone (T2) level; moreover, metabolomics and western blot analysis also confirmed the down-regulation of the expression of steroidogenic proteins in male rats (Table 16.1) [52]. Further, DEP + DEHP binary mixture effects were examined using *Caenorhabditis elegans*, and found the unregulated expression of genes linked with lipid metabolism and reproduction [41]. Some stress response genes were also upregulated, while the heat shock proteins were significantly downregulated [41]. In conclusion, alterations in the transcription levels or histological and antioxidant levels were more sensitive to mixture toxicities (Table 16.1).

6 Conclusion

When plasticizers are released into the reservoir, they remain persistent and interact with various aquatic organisms. Plasticizers mainly include phthalates (PAEs) and Bisphenol A (BPA). When exposed to aquatic species, plasticizers can disrupt the thyroid axis, estrogen axis, and various developmental and growth effects. Notably, plasticizers may reduce the cholesterol transported to mitochondria and decrease the cholesterol levels, which leads to disturbing the steroidogenesis and thyroid axis. The reproductive impact on both sexes (male and female) is predominant in the production of lower egg and sperm, which decreases the gene expression responsible for maintaining the estrogen or thyroid cycle. Taken together, the release of plasticizers in the reservoir should be strictly banned, so that these adverse effects can be avoided and ecosystem sustainability can be ensured.

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Table 16.1 Mixture effects of the plasticizers on reproductive toxicity using *in vivo* assays at environmentally relevant concentrations (ERC)

Compound name	Mixture	Species exposure	Observation	Exposure regime	Endpoint/mixture effects	Reference
Phthalates, metal	DBP, DEHP, Cu	*Carassius auratus*	Antioxidant response, SOD, CAT, MDA, GSH	7, 21 days	Antagonistic effects using the integrated biomarker response index, alterations in antioxidant status in fish liver	[39]
Phthalates, PAH	DBP, BaP	Adult male rats	SOD, GSH-Px, CAT, and MDA	Time (9:30–11:30 a.m.) for 4, 8, and 12 weeks	Effect oxidative stress state in the testis, decrease the SOD and GSH-Px activities, increase CAT activity, testes damaged due to production of ROS	[40]
Phthalates	DEHP, DEP	*Caenorhabditis elegans*	Gene expression, lipid staining, fecundity assay	48 h	fasn-1, pod-2, fat-5, acs-6 and sbp-1, vtg. Upregulated. Stress response genes, ced-1 wah-1, daf-21, gst-4 upregulated; ctl-1, cdf-2, hsp-16.1, hsp-16.48, sip-1 were downregulated	[41]
Phthalates	DEHP, DEP	Male rats	Combined effect on fetal Leydig cell	Postnatal day 1	Gestational (GD12–20) exposure of DEP + DEHP resulted in synergistic and/or dose-additive effects on the development of fetal Leydig cell	[50]
Phthalates, PCBs	DEHP, Aroclor 1254	Adult rats	Metabolic responses	12 days	Fourteen lysoPCs, PC (18:4/18:1), lysoPE (18:2/0:0), phenylalanine, and tryptophan were identified as potential biomarkers for the DEHP +PCBs. RNA expressions indicate hepatic lipid accumulation	[51]
Phthalates, GMS	DEHP, DEP, DMP, BBP, DEP, DNOP, glycerin monostearate (GMS)	Adult rats	Reproductive toxicity	15 weeks	Steroidogenic proteins (StAR, P450scc, CYP17A1, 17β-HSD, and P450arom) indicated that MIXPs	[52]

(continued)

Table 16.1 (continued)

Compound name	Mixture	Species exposure	Observation	Exposure regime	Endpoint/mixture effects	Reference
					exposure downregulated the expression of steroidogenic proteins and altered androgen metabolism.	
Phthalates, EE2	DBP, 17-α-ethynylestradiol (EE2)	Zebrafish (*Danio rerio*)	VTG induction, AOX protein level	21 days	Decreased VTG levels as compared to EE2-only groups; EE2 and DBP may act additively on VTG and antagonistically on AOX induction in males	[42]
Synthetic estrogen EE2, ZM	Ethynylestradiol (EE2) Antiestrogen ZM	*Pimephales promelas* gonads	Individual treatment-related expression increase/decrease	2 days, semi-static	Mixture exposure provides a distinction between different modes of estrogenic action	[53]
Plasticizers	DEHP, DBP, ATBC	Zebrafish (*Danio rerio*)	Reproductive toxicity	3 months	Decrease in GSI, impairment of fecundity, disruption of oogenesis, spermatogenesis. Male zebrafish were more sensitive to the combined exposure	[9]
Phthalate, parabens	PP, DEHP, BP, triclosan, TBBPA	Adult rats	Influences of multiple EDCs, using ERCs	1 h	Results showed that EDCs interact *in vivo*, magnifying one another's effects, consistent with inhibition of enzymes that are critical for estrogen metabolism	[48]
Antibiotics	CBZ, F, PHO, SMX, TMP,	Zebrafish (*Danio rerio*)	liver Cytohistological parameters, in zebrafish	21 days	Males zebrafish showed an increase of Vtg immunostaining, increased hepatic mass	[43]
Phthalates	BBP, DBP, DEHP, DIDP, DINP, DNOP	*Oryzias melastigma, Danio rerio*	Developmental toxicity and EDR estrogenic of phthalates	72 h	The mixture of the six phthalates exhibited enhanced estrogenic activity	[38]

Phthalates, metal	DBP, DEHP, Cu	*Daphnia magna*, *Photobacterium phosphoreum*	Combined effects of Cu and PAEs	14 h	Antagonism effects were found in the joint toxicity of Cu(II) combined with DBP or DEHP using the toxic unit method	[54]
Phthalates	DBP, DEP	Zebrafish embryos (*Danio rerio*)	Oxidative stress, innate immune response	12 h	Enhance the production of ROS and lipid peroxidation (LPO). The activity of antioxidant enzymes including SOD, CAT, and GPx was increased.	[49]

Conflicts of Interest The authors declare no conflict of interest.

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Chapter 17
Molecular Toxicity Mechanism of PPCPs in the Reservoir

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Abstract Due to the ubiquitous environmental occurrence and associated health risks, pharmaceuticals and personal care products (PPCPs) as emerging contaminants widely exist in the reservoir. This chapter particularly emphasizes the mechanistic toxicity pathways of the PPCPs interacting with aquatic species. Previous studies revealed that these emerging chemicals mainly disrupted the reproductive system by decreasing the estrous cycle and reducing weight and sperm production. Besides, some pharmaceutical groups like sulfonamides (SAs) and tetracycline (TC) particularly affect the detoxification metabolism pathway. The cytotoxicity and genetic toxicity with increased apoptosis levels and DNA damage were also observed even at low exposure doses of PPCPs. In summary, the fate of PPCPs and their health risks to humans should be paid great attention to, and we must ensure their safe disposal to protect the ecosystem integrity in the reservoir.

Keywords Reproductive toxicity · PPCPs · Detoxification pathway · Zebrafish

1 Introduction

Over the past years, pharmaceuticals and personal care products (PPCPs) are recognized as emerging global pollutants owing to their increasing prevalence in the environment [1]. PPCPs possess unique bioaccumulative properties in the reservoir. However, even at lower exposure doses, PPCPs create alarming risks to aquatic species. The United States Environmental Protection Agency (US EPA) recognized numerous PPCPs predominance in the wastewaters. Therefore, it may threaten the receiving aquatic species from the surface water [2]. In 2000, the first list of 33 priority compounds was strictly banned. Later, in 2007, compounds such as diclofenac, carbamazepine, ibuprofen, triclosan, parabens, musk xylene, bisphenol, tetracycline, erythromycin, bezafibrate, indomethacin, and sulfonamides are also included in the prohibition list [3]. Previous studies reported that some of these compounds are endocrine disruptors [4, 5]. Another important reason of concern is the increase of antibiotic-resistant bacteria that have become ineffective for the treatment of numerous diseases caused by antibiotic-resistant bacteria [6].

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Table 17.1 General classification of the PPCPs as an emerging pollutant

Classification	Sub-groups	Chemical name	Adverse effects
Pharmaceuticals	Antibiotics, anti-inflammatory, antibacterial, antimalarial, estrogen, steroids, antiepileptic, lipid regulators, antifungal, antiseptics, antidepressants, analgesics, veterinary antibiotics, and growth hormones	Penicillin, amoxicillin, tetracycline, ibuprofen, paracetamol, naproxen, diclofenac, bezafibrate sulfonamides, indomethacin, carbamazepine, erythromycin, 17β estradiol, progesterone, testosterone, zeranol	Endocrine disruption, disrupt metabolic pathway, cytotoxicity
Personal care products (PCPs)	Disinfectants, fragrances, cosmetics, sunscreens, conservation agents	Musk xylol, parabens, musk xylene, tetracycline, triclosan, phthalates, benzophenone-3, octyl dimethyl-PABA, camphor, methylisothiazolinone, 1,4-dioxane formaldehyde, paraformaldehyde, benzalkonium chloride, methyldibromo glutaronitrile	Endocrine disruption, disrupt metabolic pathway, apoptosis, and DNA damage

Many PPCPs are toxic and are frequently used in personal care products, such as cosmetic formulations, sunscreens, paints, and both veterinary and human medicines [7–11]. The use of these chemicals can cause toxic severe implications including cytotoxicity, mutagenicity, neurotoxicity, genetic toxicity, and estrogenicity (Table 17.1). However, studies are elusive in terms of the mechanistic toxicity of these emerging contaminants. Next-generation toxicity testing in the twenty-first century (TOX_{21}) tools may elucidate the toxicology mechanism of prioritizing PPCPs.

2 PPCPs Mechanistic Toxicity in the Three Gorges Reservoir Area (TGRA)

In the TGRA, PPCPs are of great public concern due to their elevated ecological risks. In a recent study, selected PPCPs and non-steroidal anti-inflammatory drugs (NSAIDs) were quantified in the TGRA, and then their toxicities were determined using bioassays and confirmed *via* predicted TOX_{21} techniques [12]. Results showed that NSAIDs possess high ecological risks for *in vitro/in vivo* toxicity, which agrees with the predicted results. Furthermore, experimental results revealed that musk xylene from PPCPs and diclofenac from pharmaceuticals activate the PI3K-AKT-mTOR pathway. Thus, musk xylene and diclofenac could be ranked as the priority pollutants that depressed the detoxification pathway.

Similarly, in three pharmaceuticals, the mechanistic toxicity was ranked as tetracycline hydrochloride (TH) > indomethacin, (IM) > bezafibrate (BF) both *in vitro* and *in vivo*. Contrarily, TOX_{21} tools confirmed that IM is considered the most toxic pharmaceutical with the highest LC_{50} value, while TH appeared to be the highest LC_{50} value experimentally but the lowest LC_{50} estimated value in the PI3K-AKT-mTOR pathway [13]. IM significantly disrupted hepatogenesis and hematopoiesis with the downregulation of genetic biomarkers (*drl, mpx*, and *gata2a*) [12].

The sulfonamides (SAs) class also belongs to pharmaceuticals, particularly antibiotics, which pose higher ecological risks in the TGRA [14]. Among sulfonamide congeners, sulfamethoxazole (SMX) appeared as the individual chemical with elevated environmental impacts in the TGRA and increased *in vitro* or *in vivo* toxicity. Hamid et al. [14] applied various predicted models to confirm the toxicity potential among individual and combined sulfonamide mixtures in the TGRA. Besides, after SAs binary mixture treatment on zebrafish embryos, the average developmental growth was decreased with the suppression of the detoxification pathway [14]. The mathematical combination index (CI) and independent action (IA) model indicated maximum synergistic effects of the SAs mixtures with the population affected level fa = 0.9 [14]. In contrast, the IA model found the mixture effects ranked as additive > antagonistic > synergistic effects [14].

3 Mechanism of Detoxification Metabolism Pathway

It is believed that SAs induced transcriptional changes in the detoxification metabolism pathway resulting in hepatogenesis and hematopoiesis toxicity [14]. Generally, the detoxification mechanism of any chemicals involved various metabolic pathways, which are composed of three phases. Phase I consists of cytochrome p450 (CYP450) family genes. Phase II includes sulfotransferases (SULTs) and glucuronosyltransferases (UGTs). Lastly, phase III comprises numerous transmembrane proteins (TPs), which allow the pollutant to excrete out from the body [15]. Nevertheless, the comprehensive detoxification pathways studies are limited, particularly for aquatic species in the reservoir [7–11]. The detailed mechanism of SAs disrupting detoxifying metabolism pathway is presented in Fig. 17.1.

The single and joint chemical exposure of the SAs chemical group significantly disturbs the detoxification metabolism pathway in zebrafish (Fig. 17.1). Recently, a study reported by Hamid et al. [14] suggested that SMX as a single chemical treatment upregulated the phase I genes, implying its high toxicity potential. Moreover, the transcriptional results were consistent with developmental toxicity (elevated pericardial edema) [14]. After exposure to SDZ, SMR, ST, and SPY, the gene transcriptional levels of phase I were suppressed in a concentration-dependent manner, which is consistent with the previous report by Liu et al. [13].

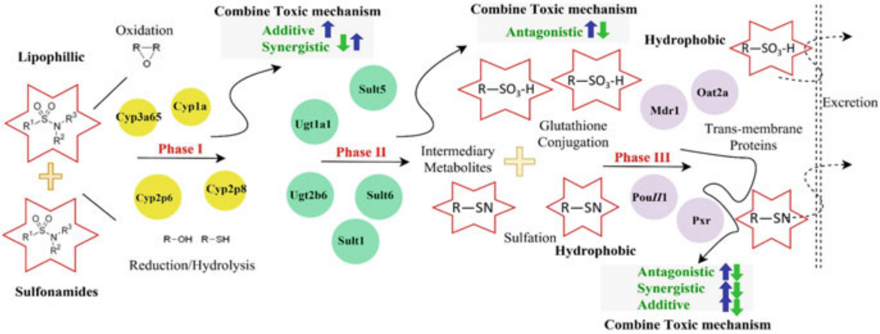

Fig. 17.1 The detailed description of the detoxification pathway of the sulfonamides (individual and mixtures) was modified from the previous report [14]

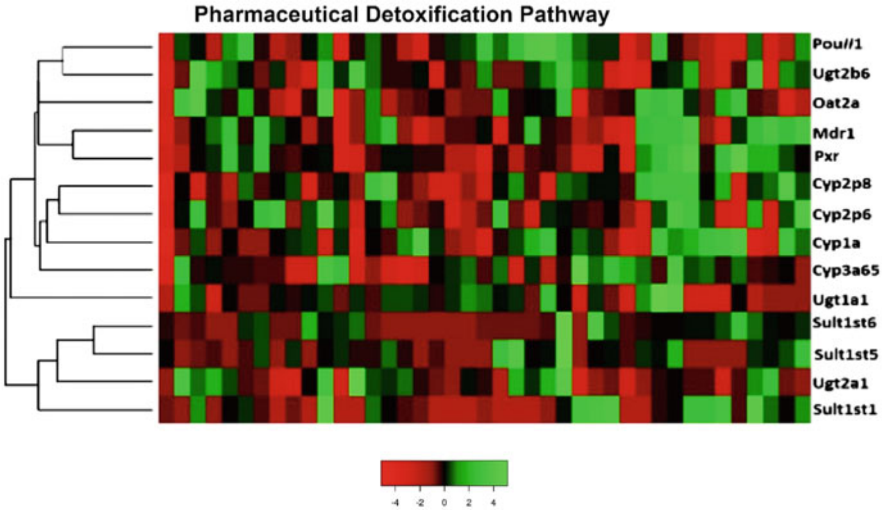

Fig. 17.2 Transcriptomic expression levels of the detoxification pathway genes after SAs treatment (single and joint binary mixtures). This figure is modified from our previous study [14]

Regarding the detoxification pathway of Phase II, the single SAs behave as the suppressors of the *sult* family genes in all treated groups [14]. Generally, SULTs are used in sulfation by forming intermediate complexes of the drugs [16–18]. However, UGTs formulate glutathione intermediatory complexes that make the chemical water-soluble or increase its hydrophobicity [17]. Phase III comprises the transmembrane proteins that help the chemical to remove from the cell membrane. Notably, all the genes in phases I and II were reported to behave synergistically to disturb the detoxification pathway in zebrafish (Fig. 17.2) [14].

4 PPCPs Toxicogenetic Endpoints *In vitro* or *In vivo*

Toxicologists have taken up various technological approaches, such as transcriptomics (qRT-PCR, RNA-seq, and microarrays), proteomics (western blot, ELISA, 2D-PAGE, and mass spectrometry), and metabolomics (nuclear magnetic resonance spectroscopy-NMR, lipotoxicity test, Matador-luciferase cytotoxicity assay, and liquid chromatography-mass spectroscopic analysis-LC-MS) to reveal the mechanistic toxicological mechanisms of the PPCPs. Here, a summary of numerous *in vivo* studies on mixture toxicity is presented in Table 17.2. The majority of the published studies have focused on determining developmental toxicity *in vivo*, such as mortality, deformity, and oxidative stress [6, 19]. Likewise, non-steroidal anti-inflammatory drugs (NSAIDs) like naproxen severely decrease the body weight and length of the zebrafish at environmentally relevant concentrations (ERC) [35]. Specifically, most PPCPs possess endocrine disruption properties mainly by perturbing the hypothalamic–gonadal axis (HPG) pathway [33]. Previously, a study reported by Yu et al. [20] investigated the transgenerational reproductive effects of sulfamethoxazole antibiotics, in which the nematode lifespan and the HPG transcription levels were markedly affected [20]. Further, various antibiotics increased hepatic mass with an elevated *vtg* transcription level in adult male zebrafish [26]. Interestingly, some pharmaceuticals also disrupt the detoxification pathway by affecting the transcript levels even at lower ERCs. A recent study highlighted that sulfonamides, tetracycline, and indomethacin disturb the metabolism pathway even at low exposure concentrations [12, 14].

Except for the mixture effects at the protein level, phthalates and PPCPs mixtures downregulate the expression levels of the steroidogenic proteins in adult rats after being treated with the quinary mixtures of DMP, DEP, DBP, BBP, DEHP, DNOP, and GMS [36]. Similarly, atenolol, carbamazepine, diclofenac, and gemfibrozil joint exposure increased the cytotoxicity in *Dreissena polymorpha* [1]. Furthermore, binary mixtures of sulfonamides also disturb the metabolism detoxification pathway with the maximum synergistic effects [14]. Similarly, the ERC mixture of steroidal pharmaceuticals affects the transcript levels of the reproductive genes in rainbow trout [37]. Thus, PPCPs may disturb the HPG axis and participate in the detoxification pathway with prominent cytotoxic effects even at lower exposure doses.

5 Computational Approaches for Toxicological Screening

Numerous predictive modeling techniques have been used for assessing chemical toxicity [38]. Ligand descriptors are the individual chemical information of the compounds that are simulated by software programs [39]. Different modeling tools, such as molecular docking *via* quantitative structure–activity relationship and chemical index (CI) are the most widely used to determine the chemical

Table 17.2 Toxicogenetic endpoints of PPCPs in various bioassays exposed at the ERCs

Chemical	Species	Exposure time	Toxic endpoints	Reference
Sulfamethoxazole	*D. rerio*	8 hpf (hours per fertilization)–96 hpf	8 hpf = yolk sac damaged, 24 hpf = eyespot delayed development 48 hpf = anomaly in the ovum and spinal cord flexure/bent spine 96 hpf = bending tail	[19]
Sulfadiazine	*D. rerio*	8hpf–96 hpf	8 hpf = yolk sack greatly convex, 24 hpf = mutation in zebrafish embryos, 48 hpf = spinal cord flexure/bent spine, 96 hpf = swim bladder deletion with temporary blood clot	[19]
Sulfamethoxazole	*C. elegans*	(F0–F6) generations	Lifespan was severely affected at embryo exposure (F1) and reproduction was affected following germline exposure (F2). In non-exposed F3–F6 generations, nematode lifespan, and reproduction showed significant inhibitions.	[20]
Bezafibrate	Human sperm cells	15, 30, 45 min	DNA damage ↑ at 30 min Apoptotic cells ↑ at 45 min	[21]
Indomethacin	KB, Saos-2, 1321 N, U-87MG cell lines	72 h	Cell viability ↓ 50.31% only for U-87MG cells.	[22]
Tetracycline	*D. rerio*	6–120 h	Hatching rate↓ (72-hpf) EC50 = 31.05 μM malformation rate (EC50): 120 hpf = 36.92 μM Mortality Rate (LC50): 120 hpf = 41.2 μM Heartbeat ↓ 20% at 337.5 μM; acta1a, myl7, and gle1b significantly altered at 202.5 μM	[23]
Bezafibrate	Human embryo cells	24 h	PPARα mRNA level ↑ at 300 and 1000 μM Inhibit the phosphorylation of Akt	[24]
Sulfadiazine	*D. rerio*	8hpf–96 hpf	Malformation, such as hemorrhage, blood	[25]

(continued)

Table 17.2 (continued)

Chemical	Species	Exposure time	Toxic endpoints	Reference
			coagulation, tail bending, pericardial edema, and swim bladder deletion	
Carbamazepine, Fenofibric acid, Propranolol, Sulfamethoxazole, Trimethoprim	*D. rerio*	21 days	Male zebrafish showed an increase of Vtg, increased hepatic mass	[26]
Indomethacin	Gastric cells MGC-803	12, 24, 48 h	Cell viability ↓ with increasing dose and duration. Gastric cancer cell apoptosis through Akt/GSK3β/NAG-1 pathway	[27]
Sulfamethoxazole, Sulfadiazine, Sulfamerazine, Sulfapyridine, Sulfameter	*D. rerio*	8–96 hpf	Disrupt the detoxification metabolism pathway	[14]
Atenolol, Carbamazepine, Diclofenac, Gemfibrozil	*Dreissena polymorpha*	8–96 hpf	Elevated cytotoxicity of diclofenac and gemfibrozil.	[1]
Tetracycline	*D. rerio*	96 h	EC50 = 0.0071 μM CYP1A ↑ at 0.045 μM and 0.45 μM LC_{10} = 1241.52 μM, LC_{50} = 134.35 μM	[28]
Indomethacin	*R. subcapitata*	72 h	NOEC (No observed effect concentration) = 2.35 μM EC10 = 12.86 μM	[29]
Sulfadiazine	*D. rerio*	8hpf–96 hpf	8 hpf = yolk sack greatly convex, 24 hpf = mutation in zebrafish embryos, 48 hpf = spinal cord flexure/bent spine, 96 hpf = swim bladder deletion with temporary blood clot	[19]
Oral contraceptives (EE2, NOR)	*Daphnia magna*	25 days	Synergistic effects were observed with the 57% decrease in the offspring number, inhibited swimming	[30]
Parabens, phthalates	Adult rats	1 h	Results showed that EDCs interact *in vivo*,	[31]

(continued)

Table 17.2 (continued)

Chemical	Species	Exposure time	Toxic endpoints	Reference
			magnifying one another's effects, consistent with inhibition of enzymes that are critical for estrogen metabolism	
Carbamazepine, Diclofenac, EE2, Mefenamic acid	*Daphnia magna*	48 h	The drug mixture reduced the age at first reproduction of daphnid in the F0 and F2 generation and increased the body length at first reproduction in the generations F0 and F3.	[32]
Diclofenac, Mefenamic acid, Naproxen, Acetylsalicylic acid, Ibuprofen	*D. rerio*	21 days	NSAIDs affect the gene transcription of the HPG axis pathway with the significant upregulation of the *fshβ, lhβ, fshr*, and *lhr* genes.	[33]
Tetracycline	*C. pyrenoidosa* (CP) *S. obliquus* (SO)	96 h	CP:$EC_{20–96h} = 5.96$, $EC_{50–96h}$: 15.44, $EC_{80–96h}$:40.01 μM SO: $EC_{20–96h} = 1.87$, $EC_{50–96h}$: 7.36 $EC_{80–96h}$: 28.96 μM	[34]

interaction with particular proteins [38]. These predictive models are extensively applied in bioinformatics studies for *in silico* toxicity prediction [39].

6 Conclusion

In recent years, PPCPs have emerged as emerging pollutants because of their harmful effects on the reservoir. Previously, published studies revealed that despite the lower concentrations in the reservoir, the endocrine disruption effects in the aquatic species become most prevalent. Furthermore, the body length and weight of most of the species have been reduced with elevated apoptosis levels and DNA damage. Recently, some studies also highlighted that sulfonamides and tetracycline might disturb the detoxification pathway. Moreover, prioritizing the PPCPs and pharmaceuticals toxicity, indomethacin, sulfamethoxazole, diclofenac, naproxen, and tetracycline have been categorized as the most toxic chemicals. Thus, more detailed studies are required to determine the exact mechanistic toxicity of these emerging contaminants.

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Conflicts of Interest The authors declare no conflict of interest.

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Chapter 18
Adverse Outcome Pathways (AOPs) of Pollutants in the Reservoir

Marriya Sultan and De-Sheng Pei

Abstract This chapter explains the adverse outcome pathways (AOPs) for evaluating risks caused by pollutants found in the reservoir. Different kinds of pollutants exist in the reservoir at the same time, and these mixture contaminants pose a significant challenge in evaluating possible biological effects and risk assessment. Therefore, the AOP framework assists in deciphering the molecular or biochemical data from field-sampled organisms that are exposed to a combined mixture of contaminants. The resulting endpoints are later utilized to infer the possible risk. Despite being simple, the major shortcoming of traditional AOP is the poor replication of complex toxicological processes. A more advanced AOP is developed, called quantitative adverse outcome pathway (qAOP), which contains one or more biology-based computational models delineating key event (KE) correlations and combining a molecular initiating event (MIE) with an adverse outcome (AO).

Keywords Quantitative adverse outcome pathway (qAOP) · Molecular initiating event (MIE) · Key events (KEs) · Toxicodynamics · Endpoints

1 Introduction

For reservoir water quality monitoring, a quantitative assessment related to the fate of pollutants in water bodies is required, which assists in the remediation of the contaminants. If only quantification in terms of concentrations is analyzed, then the analytically undetected contaminants may produce transformation products that are toxicologically substantial and elicit mixture effects. Thus, the approach of adverse outcome pathways (AOPs) is suggested, which may improve the environmental impact assessments [1]. The concurrent exposure of organisms to a variety of contaminants does not essentially mean that joint effects are aroused at measurable levels [2]. A single contaminant may act differently, and the association between the dose of contaminant and the concentrations found in the mixtures may not contribute to the detectable effect [3]. The AOPs framework assists in the interpretation, communication, and translation of pathway-specific systematic data into responses related to the assessment of risks of chemicals to the environment and human health [4].

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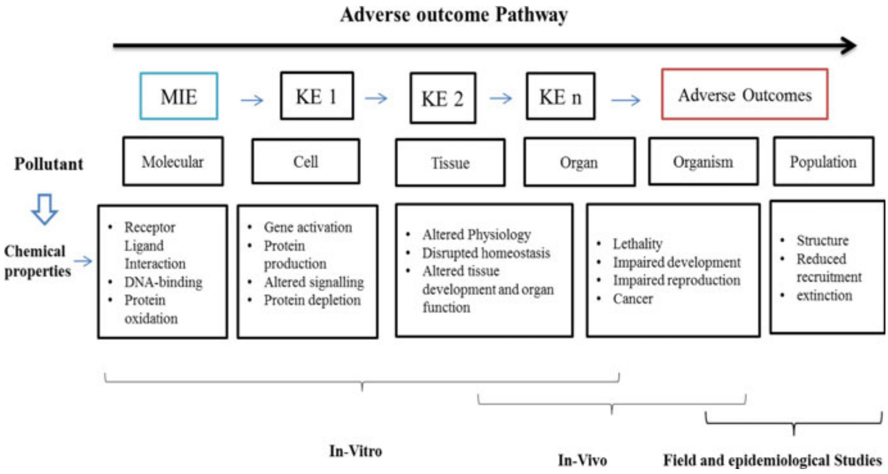

Fig. 18.1 AOPs framework for reservoir water pollutants

The AOPs model was promoted by National Research Council in the early 1980s as an approach to anticipate diseases in humans developed from the concept of biomarkers [5]. AOPs model explains the toxicity mechanisms starting from the initiation of the molecular event to some other key events (KEs) that take place when a specific chemical interacts with the environment to the adverse outcome (AO) and is detected at the individual level [6]. AOPs have gained considerable approachability as a systematic framework for toxicological information. Associations like the Organization for Economic Cooperation and Development (OECD) with a workgroup of international experts have developed guidance for the assessment and technical review of AOPs and accepted data related to toxicology by various regulatory experts [4].

An AOP comprises molecular initiating events (MIEs), KEs, key event relationships (KERs), and AOs. The starting point of AOP is MIE, which involves the interaction of chemicals at the molecular level, such as the binding of ligand-receptor. The KEs are at the cell, tissue, or higher levels of biological organization. The MIE triggers the biological changes resulting in a typical endpoint of biologic dysfunction known as an AO that is further weighed up in the risk assessment [7].

Figure 18.1 explains the overall concept of AOPs across various scales of biological organization including the MIEs caused by most pollutants, such as receptor-ligand interaction, DNA binding, and protein oxidation at the molecular level. Similarly, at the cellular and tissue level, KE1 contains gene activation, protein production, and altered signaling, and other KEs include effects at the organ level (such as impaired development) and reproduction leading to population-level impacts (such as species extinction).

For impact assessment of various chemical contaminants in the reservoir, water bioanalytical tools are usually designed for apprehending KEs of biological reactions caused by exposure to contaminants and observed MIEs at the cell and organism, which may lead to AOs at the population level [3]. AOPs are characterized as linear events, however, parallel flows and crossing pathways could also be involved. They are deliberated as flexible configurations that ought to be incessantly refined by replenishing old and novel data. These repetitive refinement drills involve the explanation and calculation of the toxicodynamic interactions between contiguous KEs and the description of toxicokinetic settings leading to the activation of an AOP [8].

2 Development of AOPs for Assessment of Water Pollutants

To assess the AOs of pollutants in the reservoir ecosystem, the water pollution status is determined first by analyzing all groups of contaminants. The data of contaminants found in specific sites are then clustered hierarchically according to detected levels, such as low, moderate, and high concentration levels. Site-specific information could also be remarked to relate it to commercial, public, or agricultural activities, such as cultivation and the presence of meadows or factories, for predicting in advance what kind of pollutants could be detected in the reservoir and the river basin [9]. The complementary information on toxicity caused by pollutants detected in the reservoir water is also useful for examining the spatial and temporal distribution. Next, the dose-response relationship information of pollutants is added to component-based modeling approaches [2].

For the development of AOPs, selective aquatic vertebrates and invertebrate species are used as model organisms, because they are appropriate bioindicator species with a short lifespan that can be observed over a short duration. Moreover, they could be considered for in-depth studies at the molecular and population levels. However, the interspecific differences are an integral part of the natural environment between aquatic organisms. Thus, they need to be adapted to an AOP framework. Pollutant-mediated AOP enables an improved understanding of the environmental effects of pollutants in varied ecosystem communities [10]. Organisms model (such as zebrafish and other aquatic fish species) and toxicodynamic studies of single or combined pollutants are measured [3].

AOP cannot elucidate the toxicokinetics of pollutants. Consequently, no risk could be assessed directly, because AOP doesn't describe the specific chemical or contaminant. Therefore, absorption, distribution, metabolism, and elimination properties are not interpreted for specific chemicals, and the risk assessment couldn't be performed. However, AOP can explain the potential hazard caused by a specific pollutant at a concentration, which can trigger the MIE and further activate the AOP [11]. The overall schematic of AOP development for risk assessment of pollutants found in the reservoir is presented in Fig. 18.2. It requires the information from the exposure assessment of the contaminants and then is integrated with the AOP as a preliminary exposure assessment to provide the basis for AOP development. If an MIE occurs, then an effect at an equal or higher level of organization may take place and can be defined as a KE. Examples might be the subsequent phosphorylation of a

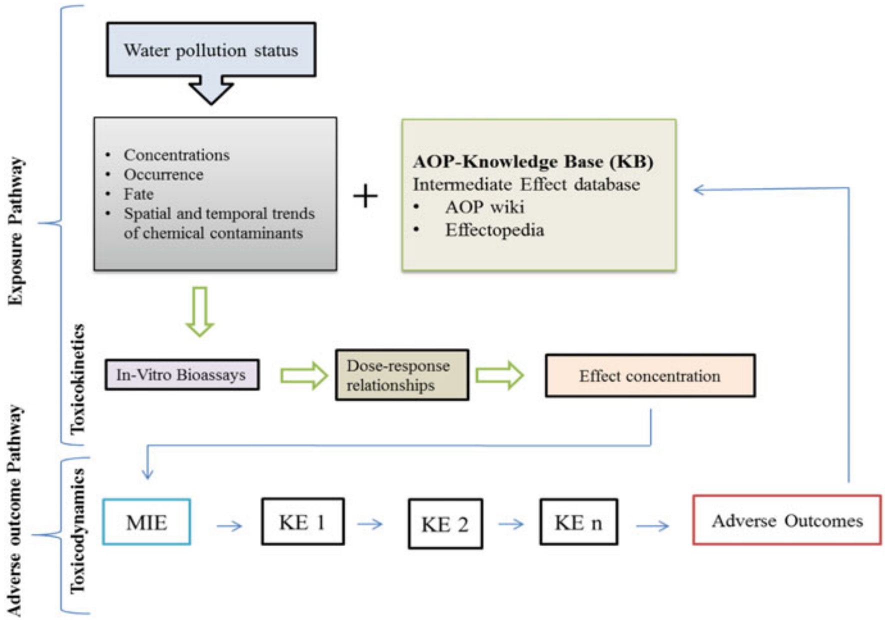

Fig. 18.2 Schematic of AOP development for reservoir pollutants by integrating exposure pathway information as preliminary evidence

transcription factor activated as an MIE causing the upregulation of an enzyme that synthesizes a hormone. Upregulation of the enzyme would exist as one module that would be sequentially linked to another module of hormone increase, each of which would exist as KEs. In some cases, a linkage or relationship to a specific MIE may not be known [12]. The linkage between the KE modules is defined as a KE relationship (KER). In most cases, the KER may be inferred or determined between KEs or MIEs through hypothesis testing and literature reviews. The resulting information can be loaded into databases (i.e., AOP-wiki), where the KER can be reviewed, categorized, and confirmed through additional studies [5].

2.1 *Bioassays Prioritization for AOPs*

In common, a chemical contaminant could trigger different AOPs at different exposure levels. Like at low concentrations of phthalates, a direction may be reversed for some developmental effects [13]. Thus, for this purpose, a quantitative understanding of associations between the KEs and the AO is necessary to match the exposure of the target site with a relevant response of AOP. Such in vitro assays are required to match the KEs for explaining the link between the exposure concentration of chemicals and the predicted AOs [14]. While addressing complex pollutant mixtures including emerging contaminants and natural toxicants, the chemical exposure scenarios of the contaminants and inherent innate biological characteristics (such as the reproduction and neurophysiology of organisms in the reservoir) are firstly taken into account. Based on the AOP concept, the bioassay prioritization approach is applied to predict a particular class of pollutants that is likely to be toxic for all organisms of the same species. Although the toxicity mechanism might not be clear for a given class of pollutant, the selection of a bioassay acute and chronic toxicity experiments could offer qualitative instruction, considering the mechanism of absorption, distribution, metabolism, excretion, and target sites [15]. Zebrafish, echinoderms, and molluscs are common organism models. Moreover, in silico studies and *in vitro* or *in vivo* bioassays are also being utilized to adapt the AOP approach [16]. According to the water directive framework, chemical indicators and ecosystem quality are not related to each other [17]. Regular environmental monitoring analysis of contaminants and biological examinations are regarded as distinct activities. Contaminant exposure and associated ecological impacts are studied for the single cause and effect relation of individual pollutants rather than taking into consideration the mixture toxicity of several contaminants together [9].

2.2 Design of Mechanistic Test Approaches

Systematic pre-tests act as filters for toxicological decisions and had more than 50 years of history in specific fields of toxicology [18]. In previous decades, numerous unnecessary in vivo studies related to carcinogenicity were waived. More commonly, when the results of animal and bacterial cell mutagenicity were positive, it was not considered necessary to execute carcinogenicity tests for a longer span in rats or mice because of the high likelihood of getting a positive result [19]. For the prediction of risks associated with exposure to pollutants, it is important to delineate a comprehensive set of KEs that are quantifiable *in vitro*, but under *in vivo* conditions, it displays adverse effects. In many AOPs, genotoxicity tests including chromosome breaks and DNA mutation are selected as KEs [11]. Mitochondrial toxicity is another example of pre-tests for the KE, as it is associated with several AOs [20]. Adverse effects appear when a chemical contaminant invades the target cells in tissue at concentrations that affect mitochondrial function, leading to cell or organ dysfunction [11]. Similarly, the predictive systems of developmental toxicity facilitated by contaminants impede neural crest cell migration, block the growth of neurite, and affect gene regulation during embryonic development [21]. Moreover, it can interfere with developmental signaling pathways leading to oxidative stress and endoplasmic reticulum stress [22, 23].

3 Endpoints in the AOP Framework for Risk Assessment

AOP is a vital part of the risk assessment conceptual model, because it provides the vital framework, through which ecotoxicological information is acquired, applied, and integrated at different levels of biological organization to efficiently evaluate outcomes. Information in AOPs consists of a chain of events starting from MIEs to AOs. They may also include such endpoints, whose mechanistic importance is ambiguous, but they are informative as a particular biomarker of the activation pathway [24].

One example is vitellogenin production in male fish, which indicates the activation of the ER signaling pathway related to reproductive dysfunction. With increased mechanistic details in AOPs, endpoints related to an adverse effect may contribute to identifying the responsible initiating event. The down-regulation of VTG expression in female fish collected from water could be deduced from the perspective of potential effects on reproductive ability, which could be utilized to identify particular chemicals responsible for this outcome [15]. For quantitative risk assessment of a single chemical contaminant, molecular and biochemical endpoints generally do not deliver adequate data. For such chemicals with little information, chemicals are placed within an established AOP and endorsed with an evaluation of marginal data. For example, the well-developed knowledge of potent ER agonists like EE2 could be utilized to understand the behavior of other potential estrogenic compounds in water [25].

Sometimes, the effects triggered by different chemical mixtures act *via* the same MIE. Thus, the AOP frameworks can elucidate such effects. Moreover, contaminants mixture may affect the pathways that later converge at shared intermediate steps, and could also be summed up for similar risks [24]. AOP framework also explicates the problem of interspecies extrapolation by identifying convergence or divergence spots within the pathways. The AOP is also an influential concept for the extrapolation of such species, which have conserved function mechanisms and were secured to endpoints pertinent to a particular risk problem. Like ecological receptors, the endpoints are fertility, reproduction, development, growth, and survival rate [23].

4 Use of the AOP for Risk Assessment of Pollutants

AOP has been applied in many studies for the assessment of chemical risk because of its promising characteristics [26]. In a previous study [27], an AOP was applied to a population model for white suckers in Great Lakes, and predicted the population based on the data on sex steroids in fish exposed to pulp and paper mill effluent. The chemicals consistently affected an early KE, such as enzyme inhibition, the activation of receptors, and the suppressive production of steroids, which resulted in decreased egg production [4]. In a previous study [26], the AOPs of microplastics based on their mechanism of ecotoxicity and human health toxicity were discussed.

The key MIE was a generation of ROS, and AOs were growth reduction, reproductive failure, and increased mortality. Similarly, a recent study was reported to monitor the San Francisco Bay-Delta watershed [5]. The AOPs were added to the preliminary monitoring list of contaminants using AOP-driven tools. Based on AOP principles, bioinformatics pathway software was developed, which served as a suitable tool to predict endpoints like behavioral changes and developmental lethality that influence wildlife populations. Recently, Corsi et al. performed an AOP analysis to evaluate pollutants from 57 great lake tributaries for identifying the contaminants of biological relevance [28].

San Francisco Bay-Delta has been studied widely over the years for effect-based analysis of the estrogenic activity of alkylphenols (Aps), alkylphenol ethoxylates (APEs), pyrethroid insecticide (bifenthrin), and persistent herbicides [29]. Estrogenic effects were not prominent when a single contaminant was assessed at environmentally relevant concentrations. However, exposure to mixture contaminants resulted in significant estrogenic activity, which is consistent with a recent study that estrogenic activity of Diuron is increased in the presence of APEs and Aps in male fathead minnows [30].

5 Outlook and Prospects

AOP has proved to be an important tool for evaluating the effects of chemical mixtures in the field [31]. AOP-based risk profiling and ranking based on relative toxicity are of great use for chemical prioritization. In the last few decades, AOP has been used to evaluate the negative impacts of contaminants found in water bodies on various biological scales. Currently, the use of quantitative adverse outcome pathways (qAOPs) has become a new approach in the AOP system. A qAOPs model including the KEs and their relationships within an AOP is developed based on scientific evidence that could be acquired from the AOP database, a resource that registers the AOPs and collects the essential mechanistic information [32]. Notably, the AOP knowledge base (https://aopkb.oecd.org/) is a crowd-based resource that catalogs AOPs and aggregates essential mechanistic information, such as auxiliary weight of evidence for adjoining KERs. An overall qAOP model elucidates the linkages between KEs and narrates the dose-response-based stimulation of an MIE to the response dynamics of KEs and AOs [33].

The overall AOPs description is well organized, which is followed by well-defined assistance. Although the AOPs applications are developing, regulatory gaps are required to be attained for replacing the currently used toxicological approaches with the AOP-based risk assessment [8]. However, it is still expected that the conceptual framework of AOP would undergo a mechanistic change in the risk assessment using the approach of weight evidence approach for the mechanistic testing of apical endpoints [34].

6 Conclusion

This chapter explains the development of a conceptual AOP model for reservoir pollutants. The pollutants trigger the initiating events at the molecular to the cellular level and later to the organ, organism, and population scale. The overall approach includes observational information, field experimental studies of contaminants, pollutants occurrence and concentrations in the environment, and their spatial distribution. This information is integrated with the AOP that assists in the development of mechanistic tests for assessing the adverse effects at different levels. This approach offers a platform to incorporate data of molecular and cellular biomarker that predicts the substantial endpoints.

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Chapter 19
Invasive Alien Species Problem in the Reservoir

Marriya Sultan and De-Sheng Pei

Abstract This chapter discusses the issue of invasive alien species in the Three Gorges Reservoir (TGR), highlights different natural and anthropogenic variables in the propagation of different exotic plants and fish species, and assesses their impacts on the reservoir ecosystem. After the construction of the TGR, many exotic plant and fish species made their way to the reservoir region; most of the invasive species were the result of factors, such as impoundments, international trade *via* water, road access, and recreational activities like aquaculture. Besides, these factors including natural climatic factors also influenced the proliferation of exotic species. The most common and problematic exotic plant and fish species in the reservoir were water hyacinth (*Eichhornia crassipes*) and red swamp crayfish, which could result in the deterioration of water quality, impede the growth of native species, influence the food chain, and cause geological hazards. Hence, we should properly monitor the reservoir threats caused by invasive species and the conservation of native species.

Keywords Three Gorges Reservoir (TGR) · Invasive alien species · Exotic plant species · Reservoir ecosystem · Species conservation

1 Introduction

On a national and international scale, the Three Gorges Reservoir (TGR) region is recognized as one of the most important biodiversity hotspots in China [1, 2]. The unique biodiversity is accredited to its distinct geographical locality, climate, and topography. The flora of the TGR consists of a range of species that evolved from temperate, tropical, and subtropical zones, whereas the fauna of this region is characterized by both aquatic and terrestrial animals [3]. The region is home to about 6400 plant species with 20% of the overall seed plants in China. Moreover, the vegetation of this area also includes about 36 endemic species and various rare and ancient species [1]. This area also contains 3400 species of insects and around 500 terrestrial vertebrate species, making up 8.5% and 22% of China's total insects and vertebrates, respectively [4].

The Three Gorges Dam (TGD) and the adjoining region of the reservoir with an overall area of around 58,000 km^2 are recognized as the TGR region [4]. Since the

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completion of its construction in 2009, various environmental problems have been identified in the region [5]. The problem of alien species invasion became a threat to the native biodiversity of the TGR region [6]. Biological invasions are a threat to biodiversity and ecosystem functions that further inflict excessive economic and social damage [7]. The drawdown zone and riparian zone areas in the TGR are more susceptible to invasion, because they are exposed to the changes caused by human activities. The hydrological alterations assist the transport of non-native invaders, which later colonize the area and offer strong competition to the native species for their survival [1].

According to the report by Xiong et al. [6], about 42 non-native species (one alga, one crayfish, one mollusk, one frog, one freshwater turtle, 23 fishes, and 14 higher plants) were observed in the TGR region, among which 15 species originate from North American, 13 from Asia, 7 from Central and South America, 5 from Europe, and 2 from African origin. Further, the non-native aquatic species observed in the TGR region was 3.5 species year^{-1} from the year 2003–2015 [8].

In this chapter, we focus on the spread of non-native (exotic) flora and fauna around the TGR region and elucidate their effect on the reservoir ecosystem and management strategies of alien species in the TGR.

2 Impact of Local and Landscape Variables on Exotic Species Invasion

Natural, landscape, and dam operational factors significantly influence the type of flora and fauna of the region surrounded or inundated by the reservoir. Local and landscape variables and dam operations-related factors result in the reduction of fish productivity, the discharge of toxic contaminants into the water, the initiation of sediment build-up, and the alteration of downstream habitats, such as loss of floodplains, river deltas, and riparian zones [9–11]. Human impact is also considered an imperative aspect, as the rivers flow through developed agricultural and populated areas surrounding the TGR region with around 40% cultivated land. The land characterized by patchy and disturbed natural parts is an increased occurrence of exotic species [5]. The aquatic species of the natural and regular flow patterns are disrupted by dam operations. The spawning grounds are also blocked, and aquatic populations are fragmented [3]. As a result, alien species successfully invade the vacant ecological niches. According to a previous study [12], 23 invasive fish species were found in the upper reaches of the Yangtze River and the main reservoir area. The population of some species is likely to increase, when some of them are in the population outbreak stage. The reason for their high reproduction is high nutrient inputs and increased primary productivity after the impoundment. The variables inducing the alien species invasion in the TGR region are presented in Fig. 19.1.

The TGD area was a closed region and had restricted the size of the waterway, therefore, ships had no such access to the water before the construction of the dam

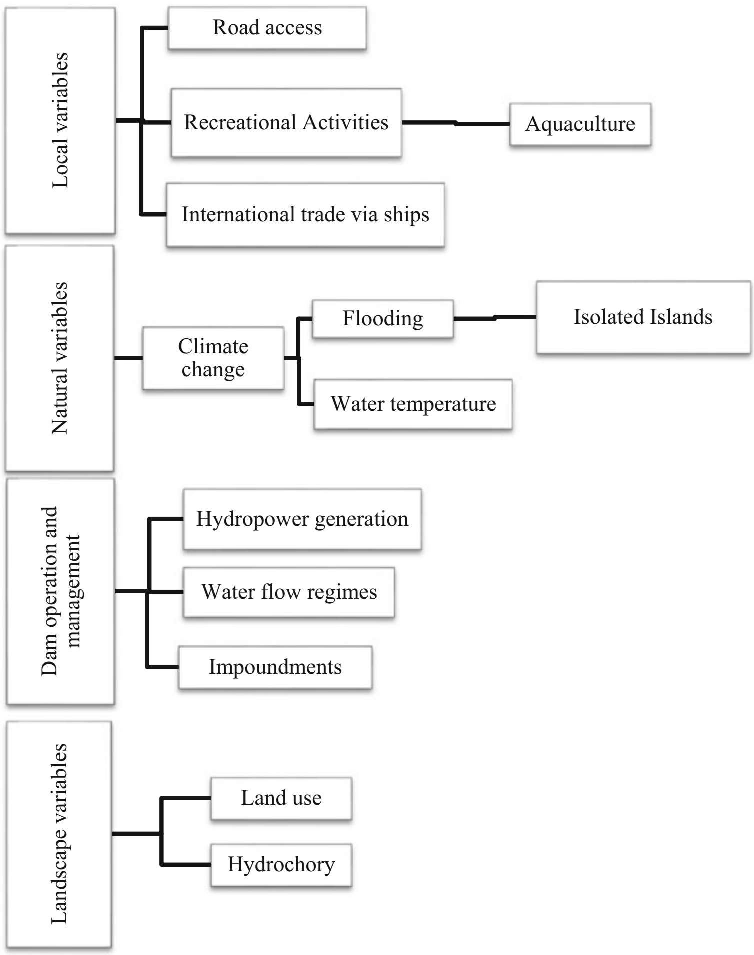

Fig. 19.1 Variables influencing the exotic species invasion

[13]. Thus, non-native aquatic species were not observed in the TGD region. For the opening of ship locks for navigation purposes, many exotic species of various origins, such as North America, Central America, Europe, Asia (95% species), and Africa (5%), were reported in the reservoir region [8]. Among the local variables, international trade is an important vector that may escalate the number of non-exotic species. Due to an increasing number of vessels passing through the dam and carrying the imported goods from different continents to Chongqing, the number of non-native species may increase in the TGD region [6]. Several dam operation and management factors, such as impoundments, hydropower generation, and water flow regimes, also influence the introduction of exotic species in the reservoir, because they cause human disturbances, which may decrease the population of native species, thus providing an opportunity for non-native species to invade

[14]. Reservoir flooding and water flow regimes are also important sources of dispersion of exotic species, as water fluctuation is linked with the spread of exotic species. In the TGR region, when the water level is pushed up to 20 m or above from October to January, many exotic species from adjacent uplands are entrained and moved to drawdown areas. Similarly, during summer when the water recedes, nutrient content increases sustaining the decomposition process and reassuring the invasion of exotic species in the absence of competitors [5]. Besides, hydropower projects require road facilities, which act as an important vector for the spread of non-natives in the inland regions of the dam. Moreover, reservoirs linked to the roads had human access that likely increases recreational activities, such as sight-seeing [14]. According to the earlier studies, regional and landscape factors are also considered better predictors of the community structure, compared to the local scale variables. In the vicinity of the TGR, agricultural activities were not allowed in the area between 175–275 m, and the former agricultural land was transformed into an artificial forest, in which the weeds colonized and thus exotic species invaded this area [5]. Habitat fragmentation because of flooding can lead to the formation of isolated islands, where the native species may decline, and exotic species make their way and establish their colonies [3, 15]. Natural conditions, such as cold-water environments, prevent the entrainment of non-native (exotic) fishes [16]. However, under the scenario of increasing temperature because of global warming, the establishment of non-native species could be enhanced in these regions that were formerly not suitable for their survival [14].

3 Exotic Plant Species in the TGR Region

Various exotic plant species were observed near the dam wall after the completion of the TGD project [17]. The most common was the South American native alien fleabane *Conyza sumatrensis* (*Asteraceae*) [18], an annual or biennial tall herbaceous plant, which reproduces by self-pollination. Because of high seed production, it is widely spread around the vicinity of the dam [15]. In the drawdown zone of TGD, plant community structure is also evolving, and the most troublesome alien plant species were *Eupatorium adenophorum* and *Alternanthera philoxeroides* [19]. Similarly, the exotic annual weed *Echinochloa crus-galli* had now become prevalent in many parts of the drawdown area [20]. According to a previous report [21], more or less 145 exotic plant species were found in the post-dam foliage in the Yangtze River basin between Chongqing and Yichang City, but only a few of them could form the dominant plant populations, such as *Alternanthera philoxeroides*, *Aster subulatus*, *Erigeron acer*, *Phytolacca Americana*, and *Xanthium sibiricum*.

In the Pengxi River area of the TGR region, about 186 plant species were detected, of which 21 were identified as invasive herbs and the most common were *Bidens pilosa*, *Conyza canadensis*, *Setaria viridis*, and *Xanthium sibericum*. Besides these *Chenopodium ambrosioides*, *Pistia stratiotes*, *Talinum paniculatum*, and *Canna indica* were found below 175 m or above 175 m. Some of the species were found to spread in patches with less coverage like *Alternanthera philoxeroides*. *Conyza canadensis* was the most common species in the TGR area, whereas some of the species were rare, such as *Pistia stratiotes* with one or two occurrences in this area [5]. For restoration and reconstruction of vegetation, some aquatic plants were also introduced in the water level fluctuation zone of the TGD region. It also included non-native plants, such as *Chrysopogon zizanioides*. Moreover, *Canna indica*, *Cyperus alternifolius*, and *Elodea nuttallii* were also suggested to be introduced for ecological restoration [6]. In the Yangtze River of the TGD, more or less 24 non-native species were found with 5 species being observed in point bars and 13 and 4 in mid-channel bars of the downstream region. The non-native species occupied the areas that were disrupted by humans. Thus, the mid-channel bars had a high risk of species invasion, and the species richness of native plants was also less because of reachability *via* water flow and wind seed plants. Consequently, the number of short-lived herbs proliferated in this region [22]. A list of some common alien plant species around the vicinity of the TGR region is shown in Table 19.1.

4 Exotic Fish Species Along the TGR Region

According to a previous report [23], the biological invasion has put 27.7% of fish species at risk in the Yangtze River. Around eighteen exotic fish species belonging to 10 families were identified in the upper Yangtze River, of which *Ictalurus punctatus, Protosalanx hyalocranius, Megalobrama amblycephala*, and *tilapia* were widespread in the TGR. These exotic fish records were linked with the impoundment of the TGR, because no exotic fish were observed in the river before the impoundment. The highest number of exotic fish species was reported in Zigui. The widespread species in the lacustrine zone were *Ameiurus melas, Cirrhinus molitorella, Hemisalanx brachyrostralis,* and *Polyodon spathula* [24]. According to the previous report [25], alien species, such *as Ameiurus melas, Tinca tinca,* and *Ictalurus punctatus*, were observed in the TGR region. Moreover, in the Wanzhou section of the Yangtze River, exotic piscivorous species, such as largemouth bass (*Micropterus salmoides*), were also observed.

Exotic fish including *Gambusia affinis* (western mosquitofish), *Megalobrama amblycephala* (bluntnose black bream), and *Pseudorasbora parva* (topmouth gudgeon) had invaded the Yangtze River. Among these fish species, the western mosquitofish (*Gambusia affinis)* population is more in the central Yangtze River because of its short life span, faster growth, and lower fecundity, which affects the presence of native species like medaka (*Oryzias latipes*) [26]. Non-native fish species are also introduced into the dams via aquaculture to increase the import of aquatic fish [27]. Non-native fish species like *Micropterus salmoides*, *Clarias batrachus*, and *Ictalurus punctatus* were introduced into the TGR to improve fisheries. Similarly, fish species, such as *Gambusia affinis* and *Oreochromis niloticus* in the TGR, may explain the reason for the decline of some endemic fish species [6]. As shown in Table 19.2, some of the exotic fish species were observed in the TGR region.

Table 19.1 Common non-native plant species found in the TGR region

Location	Exotic Specie	Reference
Three Gorges Dam	*Conyza sumatrensis (Asteraceae)*	[15]
Drawdown zone of the TGR	*Eupatorium adenophorum* *Alternanthera philoxeroides*	[19]
Yangtze River basin Chongqing and Yichang City	*Alternanthera philoxeroides* *Aster subulatus* *Erigeron acer* *Phytolacca Americana* *Xanthium sibiricum*	[21]
Drawdown zone of the TGR	*Echinochloa crus-galli*	[20]
Pengxi River	*Bidens pilosa* *Conyza Canadensis* *Setaria viridis* *Xanthium sibiricum.* *Chenopodium ambrosioides,* *Pistia stratiotes* *Talinum paniculatum* *Oxalis corymbosa* *Alternanthera philoxeroides* *Crassocephalum crepidioides* *Equisetum ramosissimum* *Euphorbia maculate* *Solanum aculeatissimum* *Sonchus oleraceus* *Veronica polita* *Abutilon theophrasti* *Senecio scandens* *Amaranthus retroflexus* *Euphorbia hirta* *Canna indica*	[5]
The TGR region	*E. crassipes*, *A. philoxeroides* *Chrysopogon zizanioides* *Elodea nuttallii*, *Canna indica* *Cyperus alternifolius*	[6]
Yangtze river	*Salix variegata Franch.* *Myricaria laxiflora* *Ranunculus sceleratus*	[22]

5 Effect of Exotic Species on the Reservoir Ecosystem

The invasions of some exotic species have worsened the quality of water in the TGD region. Water hyacinth (*Eichhornia crassipes*), the foulest invasive aquatic weed has become widely dispersed in the TGD region forming dense coverings on the surface water [18]. It inhibits air diffusion into the water and reduces the dissolved oxygen content in water, resulting in hypolimnion conditions. As we know, the organic

Table 19.2 Common non-native fish species in the TGR region

Location	Exotic Specie	Reference
Yangtze River	*Protosalanx hyalocranius* *Salangichthys tangkahkeii* *Hemisalanx brachyrostralis* *Neosalanx taihuensis* *Acipenser schrenckii* *Polyodon spathula* *Huso dauricus,* *Acipenser schrenckii* *Tinca tinca* *Megalobrama amblycephala* *Cirrhinus molitorella* *Ietalurus punetaus* *Micropterus salmoides* *tilapia* *Lucioperca lucioperca* *Colossoma brachypomus* *Ameiurus melas* *Gambusia affinis*	[24]
Yangtze River	*Ameiurus melas,* *Tinca tinca,* *Ictalurus punctatus*	[25]
The TGR region	*Gambusia affinis* *Oreochromis niloticus* *Micropterus salmoides* *Claria batrachus* *Ictalurus punctatus*	[6]
Yangtze River	*Gambusia affinis* (western mosquitofish) *Megalobrama amblycephala* (bluntnose black bream) *Pseudorasbora parva* (topmouth gudgeon)	[26]

matter content is increased and water quality is deteriorated [28]. Exotic fishes also cause the displacement of native fishes, leading to interspecific competition and the spread of pathogens [26]. Besides, the introduction of red swamp crayfish (*Procambarus clarkii*) into the TGD region has resulted in its widespread in the watershed [29]. During the reproductive period of crayfish, intense burrowing is carried out to prevent desiccation, which could result in the collapse of the bank [6].

6 Management of Alien Species and Implications for the Reservoir Conservation

To cope with new invasions, ecological monitoring is required to improve [30]. In the TGR region, such efforts related to biodiversity conservation and tackling the issue of threats by alien species invasion have been not strongly fulfilled. To determine the dispersal patterns and spatial distribution of the alien species, a landscape-level monitoring system is needed [5]. The regional native species are required to be conserved and enriched by establishing nature reserves, national parks, and gene banks adjacent to the reservoir, so that the movement of invasive species is impeded. Target planting of native species should be carried out in the towns bordering the reservoir and stringent actions should be taken regarding the plantation of exotic species [1]. In narrow corridors, higher proportions of alien plants are observed. When the edge effect is at maximum with the corridor perimeter to area ratio, then the possibility of invasion is increased. Thus, the broad corridor can function as an effective "buffer," and this barrier must be maintained and established with native species [1]. For controlling the invasive alien species, mechanical, biological, and physical methods have been adopted, but the effects were not as much successful as predicted. Besides, mechanical and physical methods are also costly and chemical methods sometimes cause the problem of water pollution, but biological agents for controlling the alien species could threaten the non-target species [6].

7 Conclusion

The environmental changes in the TGR could directly or indirectly affect the reservoir ecosystem and the diversity of flora and fauna. Natural, local, landscape, and dam management-related variables influence the exotic species invasion in the TGR. In literature, very limited research related to the spread of exotic in the TGR region and different habitats of alien species has been documented. However, the available literature has addressed the negative impacts of invasive species on water quality, native species richness, and the spread of pathogens. Therefore, it is vital to pay attention to the spread of non-native alien species in the reservoir region with a special focus on monitoring and management programs for reservoir water quality and endemic species conservation.

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Part V
Conclusions

Chapter 20
Final Thoughts and Concluding Remarks

De-Sheng Pei and Naima Hamid

Abstract Although reservoir ecotoxicology as a modern scientific discipline is proposed by us, there are many ecotoxicology issues in the reservoir that deserve thorough consideration. In several chapters of this book, we take the Three Gorges Reservoir as an example to describe the reservoir ecosystem (Chap. 2), distribution of pollutants (Chap. 3), migration of pollutants (Chap. 4), and microorganisms diversity (Chap. 7), etc., implying that different patterns may occur in other types of reservoirs. Thus, more individual case analyses may enrich our understanding of reservoir ecotoxicology. Taken together, in this book, we highlight the environmental characteristics in the reservoir system, discuss the study methods that are used to elucidate the ecotoxicology in the reservoir, and explain the detailed ecotoxicological effects and molecular mechanism of pollutants in the reservoir.

Keywords Reservoir ecotoxicology · Pollution load · Toxicity assessment · Toxicological effects · Mechanistic genetic toxicity

1 Summarized Viewpoint and Conclusion

Water resources are very precious and vital for human life. To protect and use the water efficiently, water reservoir plays an important role in both natural and artificial environment [1]. Over the past years, many reservoirs have been created primarily due to a high-water requirement for irrigation and energy demand [2]. Thus, reservoir ecotoxicology is an emerging academic discipline to protect reservoir native flora and fauna at the population, community, and ecosystem levels. We propose this discipline to appeal to more researchers to participate in this subject, because reservoir ecotoxicology is a multidisciplinary study, including ecology, ecotoxicology, environmental science, environmental hygiene, reservoir management, and water resources engineering. Although the Three Gorges reservoir in China is not the largest in the world, the Three Gorges Dam is the world's largest hydroelectric facility now [3]. Thus, the research results of the Three Gorges reservoir may be instructive for reservoir ecological health and environmental safety.

Among different types of reservoirs in the world, human anthropogenic disturbances affect the water quality of the reservoir so badly that it causes changes in the

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species composition of flora and fauna [4]. Different environmental stressors, including environmental pollutants, hydrologic alterations, sedimentation flux, nutrient loading, and erosion, also affected the ecological characteristics of the reservoir.

Various sources and distribution of environmental contaminants polluted the reservoir water. Heavy metals were mostly mainly reported among all the pollutants [5–7]. Similarly, polycyclic aromatic hydrocarbons (PAHs) distribution was attributed to the area's extensive pyrogenic and petrogenic combustion activities. The pollution of phthalate esters (PAEs) should be considered in most reservoirs because their levels exceeded the prescribed limits by World Health Organization (WHO) and the United States Environmental Protection Agency (US EPA) with high ecological risks [8, 9]. Pharmaceuticals and personal care products (PPCPs) and microplastic and nanoplastic pollution as emerging pollutants in the reservoirs attract more attention worldwide [10, 11]. Reservoir water-level fluctuation zone (WLFZ) increased the complexity of contaminant migration and conversion.

Ecological risk assessment using animal models is a vital approach to checking reservoir ecotoxicity levels. Many animal models, including zebrafish, mice, medaka, *Caenorhabditis elegans*, *Carassius auratus,* and *Daphnia magna,* can be used for elucidating the molecular mechanism of reservoir ecotoxicology. Moreover, bioconcentration and bioaccumulation toxicity tests are imperative, because bioconcentration can reveal the accumulation of a water-borne chemical by an aquatic organism [12]. However, bioaccumulation explains the uptake from all environmental sources including water, food, and sediment [13]. A simple aquatic food chain (green algae-*Daphnia magna*-fish) could provide insights into possible human health risks of chemicals at environmental relevant concentrations (ERCs) [14].

Detailed reservoir ecotoxicological effects of pollutants and their genetic mechanisms are fundamental for identifying their health risks. Elevated levels of chemicals in the ecosystem can result in adverse impacts on wildlife. Previous studies showed that heavy metals might induce oxidative stress in freshwater species [15, 16]. However, parts of persistent organic pollutants (POPs) could activate the aryl hydrocarbon receptor (AhR) signaling pathway [17], cause neurodevelopmental disorders [18], and possess endocrine disruption properties [19]. Besides, many PPCPs also exhibited endocrine disruption properties with elevated apoptosis levels and DNA damage [20]. For reservoir ecotoxicity study, the adverse outcome pathway (AOP) also is a vital approach for assessing the negative effects of reservoir pollutants at different levels [21].

With the development of reservoir ecotoxicity, the government will profoundly understand the toxicological mechanism of chemical mixture and formulate effective measures to decrease the pollution load in the reservoir.

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