Environmental Science and Engineering

Aravind Jeyaseelan
Kamaraj Murugesan
Karthikeyan Sivashanmugam *Editors*

Sustainable and Cleaner Technologies for Environmental Remediation

Avenues in Nano and Biotechnology

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Chapter 1
Catalytic Degradation of 4-Nitrophenol and Organic Dyes Using Silver and Platinum Nanoparticles Synthesized by Plant Extracts

M. Karthikeyan, V. Blessy, and A. Thirumurugan

Abstract For a decade, nanotechnology research has been one of the most attractive field of wastewater treatment. In the present study, green route synthesized noble metallic nanoparticles of silver and platinum catalytic activity were assessed for degradation of 4-Nitrophenol as a model by UV−visible spectroscopy. Then, catalytic activity of typical organic dyes of Alizarin red S and Brilliant blue G were also performed and monitored by UV−visible spectroscopy. The efficiency of silver and platinum nanoparticles in reducing the 4-Nitrophenol and dyes were compared. The catalytic activities of both nanoparticles were also quantified by analyzing the sodium Borohydride reduction of 4-Nitrophenol. As a result, it has been observed that the silver nanoparticles show more reducing ability than platinum nanoparticles.

Keywords Silver NP's · Platinum NP's · Green synthesis · Catalytic activity · 4-Nitrophenol · Organic dyes

1.1 Introduction

The need for the production of nano materials has increased due to their various applications in the field of electronics, catalysis, medicine etc., Since the materials are at nano scale have different and unique properties when compared with bulk materials, they gain more attention in various applications (Akilandaeaswari and Muthu 2021). Mostly the nano materials are prepared from noble metals such as gold, silver, platinum etc., (Khan et al. 2022). Silver is one of the most used metals which are widely used as a disinfecting agent (Lee and Park 2020). Silver nanoparticles have effective antibacterial activity when compared with other nanoparticles and hence it is widely used in the field of dentistry, catheters, and making burn wounds to control bacterial growth (Wan et al. 2021). The platinum nanoparticles also have

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wide application in the field of catalysis, biomedicine, sensing, cancer therapy etc., but the production of platinum nanoparticles was not much explored in the field of nanotechnology (Joseph and Vijayanandan 2021).

Organic dyes are the major pollutants of water source which are widely used in textile industries, research activities etc., and hence the removal of such dyes are major concern (Haverkamp and Marshall 2009). Alizarin red S is the most used dyes in textile industry. It is 1, 2-dihydroxy anthraquinone sulfonic acid sodium salt which is synthesized by sulfonation of alizarin. Alizarin Red S also gains applications in various fields such as staining in microscopy, acid base indicator, determination of fluorine etc., (Kavitha et al. 2013). Coomassie brilliant blue which is a non-azo dye find its wide application in research laboratories especially in protein estimations. It is also widely used for staining in electrophoresis, Bradford assay etc., (Akhtar et al. 2013). Since these dyes heavily contaminate the water, they should be treated before discharged into the environment (Banerjee et al. 2014) various physical and chemical methods such as adsorption, coagulation, flocculation, photo catalysis etc., are employed to treat such dye containing water (Sharma et al. 2014). Nanoparticles synthesized from various noble metals have ability to catalyze various chemical interactions like hydrocarbons oxidation, hydrogen bonding, as well as redox reactions. As they have more surface area compared with their bulk materials, they have more and new catalytic properties (Dash et al. 2015). Because of these advantages, nanoparticles play a major role in catalyzing the reduction of organic dyes. The sodium borohydride reduction of 4-Nitrophenol to aminophenol group is the well-known chemical reaction. Hence it is widely used to quantify the catalytic activity of nanoparticles (Abou-Gamra 2014).

Therefore, in the present study, we have made an attempt on previously synthesized silver and platinum nanoparticles by plant extracts was used to determine the catalytic reduction of 4-Nitrophenol and assessment of catalytic reduction of organic dyes.

1.2 Materials and Methods

1.2.1 Catalytic Activity in the Sodium Borohydride Reduction of 4-Nitrophenol

An aliquot of 4-Nitrophenol (0.2 and 1 mM) was treated with freshly prepared sodium borohydride solution (3.6 and 16.5 mM) and 0.2 mL freshly prepared colloidal Ag NPs and Pt NPs in a 1 cm quartz cuvette. The reaction mixture was shaken thoroughly, and the UV–visible spectrum was recorded at room temperature (23–27 ºC). The progress of the reaction was monitored by recording the absorption intensity of 4-nitrophenolate ion and the intensity of 4-aminophenol with certain time intervals, and the spectrum was recorded.

1.2.2 Assessment of Catalytic Activity in Reduction of Typical Dyes

The organic dyes such as Alizarin Red 1 ml of 7×10^{-4} M and Brilliant Blue-G 1 ml of 3×10^{-4} M was prepared. Similarly, 0.001 M $NaBH_4$ and 1 M H_2O_2 was prepared. Six test tubes were collected, washed thoroughly and labeled as D + NB, D + NB + SNP's, D + HP, D + HP + SNP, D + NB + Pt NP's, D + HP + Pt NP. (D−Dye, NB−Sodium borohydride, SNP's−Silver nanoparticles suspension, Pt NP's –Platinum nanoparticles suspension, HP – Hydrogen peroxide). In each test tube was added 1 ml of labeled material and made the volume up to 4 ml in all test tubes. The degradation of Alizarin Red and Brilliant Blue–G dyes by $NaBH_4$ and H_2O_2 was monitored in the presence or absence of platinum and silver nanoparticles spectrophotometrically.

1.3 Results and Discussion

1.3.1 Catalytic Reduction of 4-Nitrophenol

In this study, green route synthesized silver and platinum nanoparticles by plant extracts was used to determine the catalytic reduction of 4-Nitrophenol as model and assessment of catalytic reduction of typical organic dyes. The Fig. 1.1 shows the UV−visible spectrum of 4-nitrophenol mixed with deionized water. As compared with Fig. 1.1a the UV−visible spectrum at Fig. 1.1b indicates the formation of nitrophenolate ion while sodium borohydride was mixed with nitro phenol. When the sodium borohydride was added with aqueous solution of 4-nitrophenol, the absorption maxima of the solution was shifted from 319−401.5 nm due to the formation of more phenolate ions. Without the addition of nanoparticles there will be no reduction of nitro group to amino group (Rauf et al. 2005). Once the nanoparticles were added the intensity of peak at 401 nm was decreased and there was increase in intensity at 319 nm which indicates the formation of aminophenol group (Fig. 1.2a and b).

1.3.2 Assessment of Catalytic Activity in Reduction of Typical Organic Dyes

The rate of degradation of alizarin red-S dye by sodium borohydride and hydrogen peroxide were monitored in the presence and absence of nanoparticles spectrophotometrically. The Fig. 1.3a and b shows the catalytic degradation of organic dye Alizarin red-S by sodium borohydride and hydrogen peroxide respectively in the presence and absence of nanoparticles. In both the reduction profiles, the addition of nanoparticles

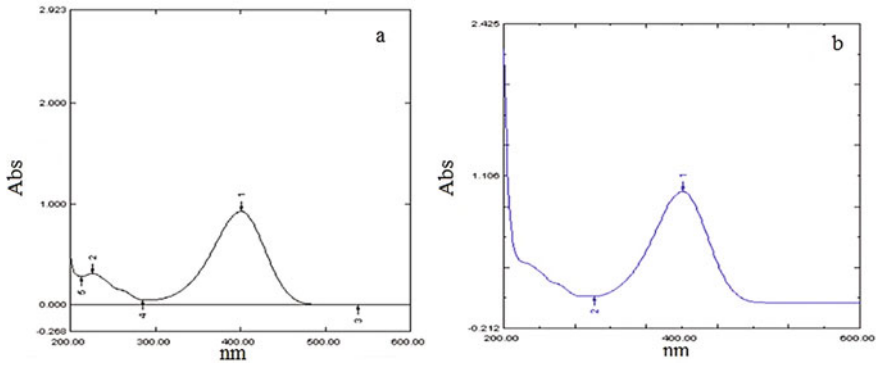

Fig. 1.1 UV−visible spectrum showing the peak for **a** 4-Nitrophenol **b** 4-Nitrophenol mixed with sodium borohydride (nitrophenolate ion)

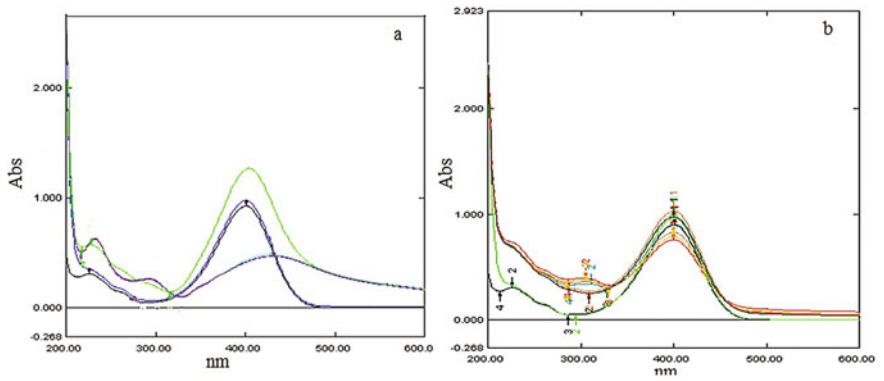

Fig. 1.2 Overlay of UV–visible spectrum at various time intervals using **a** SNP's **b** Pt NP's during catalytic reduction

enhanced the degradation of dye and have desirable difference between the initial and final dye concentration. From the reduction profile, the platinum nanoparticles have more catalytic activity in degradation of alizarin red – S compared to the silver nanoparticles.

Similarly, the rate of degradation of brilliant blue−G dye by sodium borohydride and hydrogen peroxide were monitored in the presence and absence of nanoparticles spectrophotometrically. The Fig. 1.4a and b shows the catalytic degradation of organic dye Brilliant blue−G by sodium borohydride and hydrogen peroxide respectively in the presence and absence of nanoparticles. In both the reduction profiles, the addition of nanoparticles enhanced the degradation of dye and have desirable difference between the initial and final dye concentration. When compared to alizarin red degradation, the silver nanoparticles show enhanced catalytic activity for the reduction of brilliant blue–G in combination with both reducing agents.

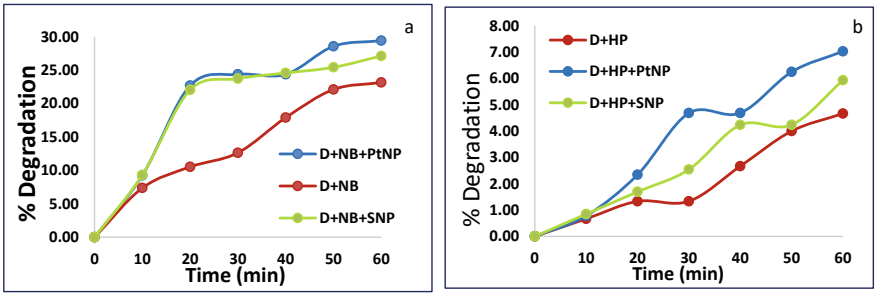

Fig. 1.3 **a** Sodium borohydride (NB) and **b** Hydrogen peroxide (HP) reduction profile of alizarin red-S in presence and absence of nanoparticles (Pt NP's, SNP's)

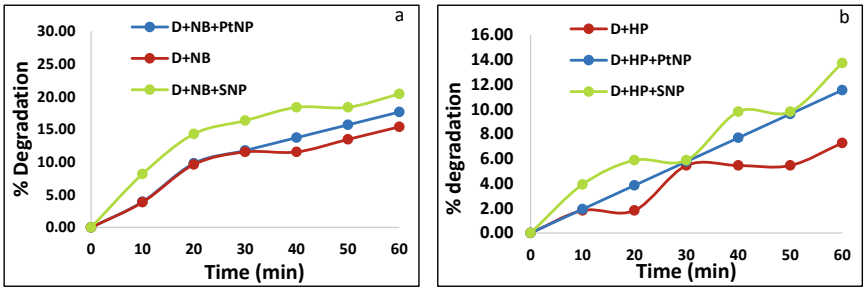

Fig. 1.4 **a** Sodium borohydride (NB) and **b** Hydrogen peroxide (HP) reduction profile of Brilliant blue-G in presence and absence of nanoparticles (Pt NP's, SNP's)

1.4 Conclusion

In this study, previously synthesized silver and platinum nanoparticles were successfully used for reduction of 4−Nitrophenol and assessment of catalytic activity for alizarin red-S and brilliant blue−G. The obtained results show that the nanoparticles have significant ability to catalyze the reduction reactions. Hence, these nanomaterials can be used as catalyst for various industrial dye degradation.

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References

Abou-Gamra ZM (2014) Kinetics of decolorization of Alizarin Red S in aqueous media by Fenton-like mechanism. Eur Chem Bull 3(1):108–112

Akhtar MS, Panwar J, Yun YS (2013) Biogenic synthesis of metallic nanoparticles by plant extracts. ACS Sustain Chem & Eng 1(6):591–602

Akilandaeaswari B, Muthu K (2021) One-pot green synthesis of Au-Ag bimetallic nanoparticles from Lawsonia inermis seed extract and its catalytic reduction of environmental polluted methyl orange and 4-Nitrophenol. J Taiwan Inst Chem Eng 127:292–301

Banerjee P, Satapathy M, Mukhopahayay A, Das P (2014) Leaf extract mediated green synthesis of silver nanoparticles from widely available Indian plants: synthesis, characterization, antimicrobial property and toxicity analysis. Bioresour Bioprocess 1(1):1–10

Dash SS, Bag BG, Hota P (2015) Lantana camara Linn leaf extract mediated green synthesis of gold nanoparticles and study of its catalytic activity. Appl Nanosci 5(3):343–350

Haverkamp RG, Marshall AT (2009) The mechanism of metal nanoparticle formation in plants: limits on accumulation. J Nanopart Res 11(6):1453–1463

Joseph A, Vijayanandan A (2021) Photocatalysts synthesized via plant mediated extracts for degradation of organic compounds: a review of formation mechanisms and application in wastewater treatment. Sustain Chem Pharm 22:100453

Kavitha KS, Baker S, Rakshith D, Kavitha HU, Yashwantha Rao HC, Harini BP, Satish S (2013) Plants as green source towards synthesis of nanoparticles. Int Res J Biol Sci 2(6): 66−76

Khan W, Khan N, Jamila N, Masood R, Minhaz A, Amin F, Nishan U (2022) Antioxidant, antibacterial, and catalytic performance of biosynthesized silver nanoparticles of Rhus javanica, Rumex hastatus, and Callistemon viminalis. Saudi J Biol Sci 29(2):894–904

Lee YJ, Park Y (2020) Graphene oxide grafted gold nanoparticles and silver/silver chloride nanoparticles green-synthesized by a Portulaca oleracea extract: assessment of catalytic activity. Colloids Surf, A 607:125527

Rauf MA, Ashraf S, Alhadrami SN (2005) Photolytic oxidation of coomassie brilliant blue with H2O2. Dyes Pigm 66(3):197–200

Sharma VC, Gaherwar S, Thakur M (2014) Microwave-mediated green synthesis of silver nanoparticles by using vinca rosea & its application in air pollution control. Indian J Sci Res 4(1):100–103

Wan H, Li C, Mahmud S, Liu H (2021) Kappa carrageenan reduced-stabilized colloidal silver nanoparticles for the degradation of toxic azo compounds. Colloids Surf, A 616:126325

Chapter 2
Biological and Eco-Friendly Cost-Effective Measure for Remediation

Anil Kumar Moola, Selvam Sathish, S Mari Selvam, Balasubramanian Paramasivan, Sujatha Peela, Harish Kumar Seenivasan, and Dhandapani Gurusamy

Abstract The natural environment is being spoiled with harmful chemicals from tannery effluent, industrial effluent, and domestic wastewater by human uses. Therefore, the avoidance of pollution and the degradation of harmful chemical compounds are essential for the future mankind. Several microorganisms are involved in the biodegradation of harmful contaminants in the soil including bacteria, fungi, and algal species. Phytoremediation is the best choice for many developing and developed nations to as it is an eco-friendly process for the decontamination of several environmental pollutants. Green plants absorb the wastes materials in the soil through their roots. Nowadays, *in-vitro* studies on the phytoremediation of emerging pollutants are giving better results. This chapter focuses on the recent studies on phytoremediation through various roots, including hairy roots, adventitious roots, and normal roots. It also reports the mechanism of biodegradation and enzymatic reactions that

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trigger the phytoremediation process. This information is essential for assessing the feasibility of a plant for the phytoremediation process before its application in the target site.

Keywords Biodegradation · Harmful chemicals · Microorganisms · Plant · Phytoremediation · Soil

2.1 Introduction

Nowadays, the global nations were having a challenging problem with the need for groundwater. But, the groundwater is rapidly polluted by urbanization, use of pesticides, heavy metals (HMs), tannery effluents, phenols, organic matter, explosives, azo dyes, and industrial wastes (Carolin et al. 2017). From the industrial wastewater, large quantities of heavy metals are released, such as Hg (II), Pb (II), Cd (II), Ag (I), Ni (II), and Cr (VI). These are highly toxic, non-degradable, and have infinite lifetimes (Mokarram et al. 2020). The harmful chemical compounds are synthesized in two different ways, including natural elements and industrial wastewater. Natural compounds can easily be degraded by external factors than industrial wastewater. In this sense, many biological methods can remove these harmful substances from industrial wastewater and contaminated wastewater (Crini and Lichtfouse 2019). In removing toxic compounds from soil and water, various methods are followed like adsorption on activated carbon, chemical oxidation, incineration, and solvent extraction. These methods have several disadvantages, including low efficiency, high cost, or by-product generation. Therefore, phytoremediation is the most valuable method to remove harmful chemical compounds from soil and wastewater (González et al. 2006; Al-Baldawi et al. 2018). In this technology, the root system of the green plants absorbs the harmful chemicals, while emerging pollutants are also removed from the wastewater simultaneously. Phytoremediation is classified based on the mechanism of action employed by the plants as follows: phytoextraction (accumulation of toxic chemicals and elimination of toxicity), rhizofiltration (root system for removing toxic chemicals from polluted water), phytostabilization (removal of bioavailability of toxic chemicals in the soil), phytovolatilization (evaporation of several toxic chemical compounds) (Antoniadis et al. 2017). Various strategies and processes are involved in phytoremediation for improving the uptake of heavy metals, such as genetic strategies (genetic modification of plants) and chelate-assisted strategies (Sharma 2021). Hairy root culture is one of the most valuable phytoremediation processes to uptake the toxic compounds from soil and water. It can grow faster in a toxic environment than the regular root system (Gujarathi et al. 2005; Perotti et al. 2020). Microorganisms also help in the process of phytoremediation as bacterial and fungal pathogens in hyperaccumulator plants. Based on the 'literary reports, the review on comprehensive discussion on synergistic mechanism of plants, microbes and other additives has been rarely reported.

Hence in this chapter, features and the enzymatic reactions that take place in plants and microbes during the process of phytoremediation has been described. The key concepts of plant, microbe and biochar assisted remediation has been annotated. This chapter helps the researchers to understand the benefits of phytoremediation and its significance on synergistic relation with bacteria and other additives.

2.2 Bio-based Technologies

In recent days, the world faces various problems owing to pollution of oil, air, water, and land. These problems are temporarily solved with several bio-based technologies like bioaugmentation, biostimulation, composting, bioventing, and biosparging. Bioremediation is the process of removing contaminants from polluted soil or water with the help of several processes including bioaugmentation and biostimulation (Tyagi et al. 2011; Lellis et al. 2019). The types of bioremediation techniques has been illustrated in Fig. 2.1 (Sharma 2020). Biostimulation is one of the bio-based technologies to promote the degrading rate of hazardous chemical compounds from contaminated soil. In this technology, low-energy lasers are used to stimulate the rate of wound healing and degradation of contaminants (Wasewar 2022). Bioaugmentation is another most important method in bio-based technology for bioremediation of polluted environments and it is one of the best methods to degrade pollutants than other methods. Two different processes are involved in this method including the addition of a pre-adapted pure bacterial strain and the addition of a transformed gene vector into indigenous microorganisms (Li et al. 2018; Huang et al. 2020). Bioaugmentation is successful in agricultural land, forestry, and wastewater treatment with the help of indigenous microorganisms. Bioaugmentation has been reported significant achievement in anaerobic digestion of lignocellulosic residues (Tsapekos et al. 2017), waste activated sludge (Cayetano et al. 2021), food waste (Jiang et al. 2020) to enhance the process with the help of microbial enrichment culture. In bioaugmentation, microbial communities play a major role in remediation. Microbial strain selection and their subsequent survival and activity determine the success rate of bioaugmentation. Hence the basic or initial and most important step of bioaugmentation is strain selection due to the involvement of microorganisms in fulfilling the whole process. For instance, Yang et al. (2019) reported that both hydrogenotrophic and aceticlastic methanogenic pathway has to be accounted for bioaugmentation where the former and latter showed 71.1 and 59.7% higher efficiency than control. In addition to microbial strains, the efficiency of the plant in bioaugmentation is determined based on several factors including the chemical nature and concentration of pollutants, reactor setup and physicochemical characteristics of the environment (El Fantroussi and Agathos 2005; Yang et al. 2019). Several new approaches were developed in bioaugmentation including bioaugmentation with nanomaterials comprehensively defined as nanobiohybrids (Guo et al. 2020), cells encapsulated/immobilized performing more efficiently compared to free system, gene modified bioaugmentation (Nzila et al. 2016), rhizosphere and plant assisted augmentation

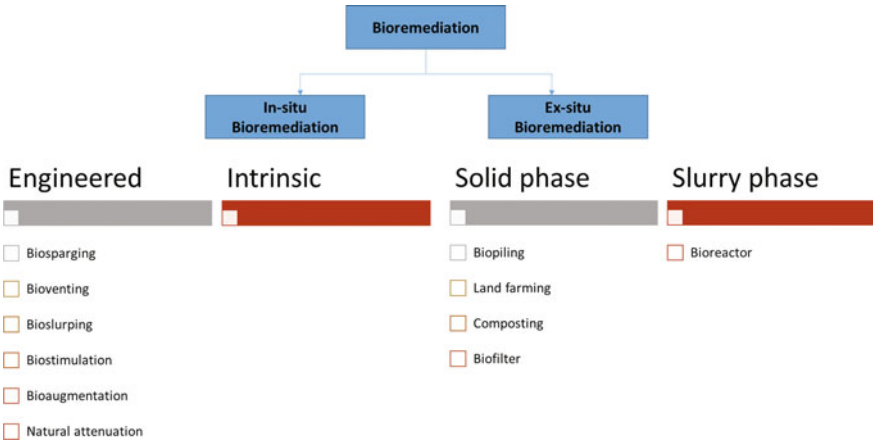

Fig. 2.1 Types of bioremediation techniques

(Itusha et al. 2019; Viji et al. 2022). Phytoremediation is one of the best techniques in bioaugmentation to remove pollutants from green plants with help of microorganisms. Microbial-enhanced sugarcane (*Saccharum officinarum*) was successfully eliminated the lindane from doped soil (Salam et al. 2017). Phytoremediation has various applications including degrading the pollutants, stabilizing the soil condition, increasing the biomass, decreasing the carbon dioxide level, and increasing the oxygen level. In the phytoremediation process, the plant roots thoroughly uptake the pollutants with the help of microorganisms in the rhizosphere. The pollutants then translocate into the upper regions of the plant for nutrient utilization and the remaining pollutants evaporate into the atmosphere through transpiration (Weyens et al. 2009; Ekta and Modi 2018).

2.3 Phytoremediation—An Overview

The term phytoremediation (Latin word) indicates the process of removal of the toxic contaminants by plant roots from the environment (Etim 2012). Generally, phytoremediation is defined as cleaning the polluted environment with natural plants. In phytoremediation, the green plants reduce the number of toxic compounds from the environment with root-associated soil microbes (Ali et al. 2013). Phytoremediation checks out the pollution of the contaminated environment through a series of processes namely phytofiltration, phytostabilization, phytoextraction, phytovolatilization, and phytotransformation, rhizosecretion. The detailed mechanism has been represented schematically in Fig. 2.2. Its mechanism includes various processes such as heavy metal uptake, metal translocation to shoots, heavy metal tolerance, exclusion of toxic compounds, vacuolar compartmentalization, production of phytochelatins, and metallothioneins (Sharma 2021). In phytoremediation,

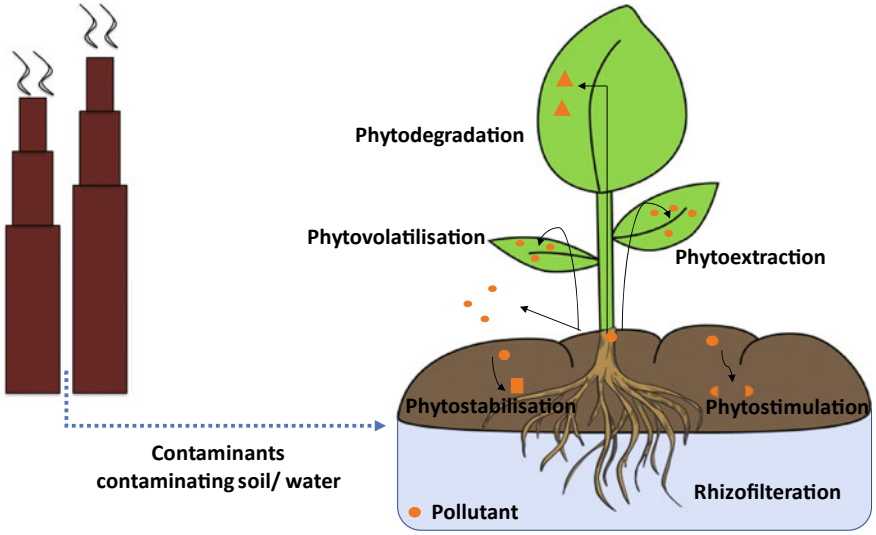

Fig. 2.2 General mechanism involved in phytoremediation by using plant roots

bioabsorption has several significant steps, including chemisorption, complexation, adsorption on surface and pore, ion exchange, micro precipitation, hydroxide condensation onto the surface (Shinomol & Bhanu 2021). Several factors including redox potential, pH, soil organic matter, clay content, nutrient balance, cation exchange capacity (CEC), concentrations of other trace elements in soil, soil moisture and temperature influences the uptake of metal from the contaminated soil (Neilson and Rajakaruna 2015). In translocating pollutants, the plant system has different tolerance processes including sequestration/ compartmentalization, binding/ chelation, excretion from aerial plant components, enzymatic and non-enzymatic antioxidants, and protection, stress recovery, and damaged protein repair (Antoniadis et al. 2017). Particularly, persistent organic pollutants have gradually become one of the most critical compound pollutants in the world due to their long-term bioavailability. Specific attention is paid to polycyclic aromatic hydrocarbons, polybrominated diphenyl ethers, organochlorine pesticides, and halo hydrocarbons.

The phytostimulation is the process of promoting microbial biodegradation in the rhizosphere. Phytotransformation is one of the most important metal absorption processes in the degradation of harmful pollutants comprising the degradation, decolourization and transformation to less toxic compounds. The phytotransformation of methylene blue from water using *Azolla pinnata* has been found to show decolourization efficiency of 85% removal validating the fact of ability of plants to transfrom organic compounds (Al-Baldawi et al. 2018). The taller plants have two different strategies for adsorbing heavy metals including exclusion (uptake of pollutants from soil to shoot through root) and accumulation of toxic compounds (detoxification of toxic compounds) (Luo et al. 2016). The accumulators can accumulate the harmful chemical compounds in the aerial parts of plants and exclude

the accumulation of toxic compounds in plants' biomass. Metal uptake and translocation of toxic compounds in the plant cell membrane also involves proton pumps (ATPases that consume energy and generate electrochemical gradients), co- and anti-transporters (proteins that use the electrochemical gradients generated by ATPases to drive the active uptake of ions), and channels (proteins that facilitate the active uptake of ions). Some organic acids also enhance the uptake of metal ions from contaminated soil. Heavy metal contaminated soil treated with citric acid assisted *Brassica napus* in hydroponic method reduced significant amount of cadmium, hydrogen peroxide (H_2O_2), electrolyte leakage, malondialdehyde (MDA) accumulated in plant roots (Ehsan et al. 2014).

Nowadays, air pollution is a primary challenge for all developed nations due to urbanization, vehicles, and industrialization. This makes the ozone in the surrounding environment depleted. Various methods were discovered to remove the toxic gases (including carbon monoxide, carbon dioxide, and greenhouse gases) from the natural air. Phytoremediation is one of the best methods to remove toxic gases from a contaminated environment. *Sida hermaphrodita* is one example of the phytoremediation of toxic gas through absorption from fossil-fuel combustion (Uchman et al. 2017).

2.3.1 Plant Roots in Phytoremediation

The harmful contaminants in the environment include heavy metals and complex chemicals that the plants directly uptake by their roots and few specific plants limit the uptake amount of heavy metals such as arsenic since its toxicity limit is 40 and 200 mg /kg in sandy and clay soils. The plants involved in phytoremediation adopt different mechanisms including passive uptake through the apoplast, direct transcellular transport from the environment to the plant vascular system, and active uptake through the symplast by plants during heavy metal uptake. The uptake of trace metal elements is controlled by root factors, including soil acidification by root exudates, translocator activity and selectivity, root membrane activity, avoidance strategy mechanisms, the release of redactors or oxidants, chelator root excretion, phytosiderophores, acids, and hydrogen ions (Vithanage et al. 2012).

Rhizosecretion is one of the essential roles of plant roots involved in phytoremediation. In this process, the valuable natural products, including enzymes, alcoholic compounds, and other substances, were secreted by plant roots (Takkar et al. 2022). The plant roots exudate compounds with specific biological activity in low quantity. Plant exudates are in multiple forms of sugar, alcohol, and acids. The exudates are annually produced ranging from 10−20% by photosynthetic plants. The exudates level is identified by the chemical or chromatographic method due to the low amount of secreted compound. The secretion of exudates is affected by various factors, including plant species, age of the plant, temperature, light, plant nutrition, soil microorganism, medium composition, soil moisture, and root damage. The primary source of exudates is the tip of the root (Antoniadis et al. 2017). Some of the essential

enzymes including laccases, dehalogenase, nitroreductases, nitrilases, and peroxidases were secreted by the plant for degrading the organic chemical compounds. The xenobiotic compounds are also degraded by the exudates of plants (Hussain et al. 2018). The rhizospheric microorganisms help to improve the uptake of metal by their enzymatic action on these metals (Kumar et al. 2017). With root exudates, microbial biodegradation is stimulated to minimize the toxicity of the chemicals. The microbial interaction of plants was highly influenced in phytoremediation to remove the pollution from contaminated soil and water. The plant growth-promoting rhizobacteria promote nitrogen fixation, phytohormone production, specific enzymatic activity, and plant protection (Moola et al. 2021). The energy is more important for uptake of the base metals (Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+}) through the active transport mechanism. However, organic chemicals can easily be diffused into the plant root cells by a passive transport mechanism. If the substrate concentration is low, the degradation can be explained by Michaelis−Menten kinetics. The first-order reaction is considered, while the higher substrate concentrations are limited. According to Monod kinetics, bacteria can be used as a substrate for development, and the growth of substrates is better at higher availability. However, it was noted that bacteria have limited degradation capability if the substrate's concentration is minimal, which can prohibit the biodegradation of contaminated sites. Combining with the phytoremediation potential of plants, bacteria can therefore be used to overcome problems with environmental pollutants. (Hussain et al. 2018). Examples of some plant species and their root system utilized for enhanced remediation of inorganic and organic pollutants are depicted in Table 2.1.

2.3.2 Adventitious Roots in Phytoremediation

The adventitious roots develop from various tissues, cells, and neighboring cells of vascular tissues. Besides, they develop in the postembryonic stage of plant growth (Geiss et al. 2018). Plants generally adapt to their native natural environment, including water availability and micro-and macronutrient availability, concentration, and localization. Hence, the plants change their organ and tissues depending upon the environmental conditions. The adventitious roots are also a modification of the shoot by the plant's adaptation mechanism (Bellini et al. 2014). The growth of adventitious roots is inhibited when an excess amount of heavy metal is accumulated in the surrounding soil environment. The inhibition is high when the solute potential of the external medium is high that may deny the plant from nutrient uptake. Direct inhibition of enzymes that are of physiological importance, and inhibition of mitotic division of the meristematic cells may also occur (Odjegba and Fasidi 2004). *ThMT3* gene expression in the transgenic *Salix matsudana* increases the tolerance of copper stress and nitric oxide (NO) production (Yang et al. 2015). A high concentration of copper (5−500 mM) causes leaf toxicity, strongly affects adventitious root formation and produces ethylene at higher levels in *Populus alba*'s shoot culture (Franchin et al. 2007). The aquatic adventitious roots have the photosynthetic ability

Table 2.1 Examples of the plant species and their root system utilized for enhanced remediation of inorganic and organic pollutants

S.no	Plant species	Type of root system	Uptake elements	References
1.	*Armoracia rusticana*	Hairy root system	Uranium	Soudek et al. (2011)
2.	*Pteris vittata*	Hairy root system	Arsenic and chromium	Kalve et al. (2011)
3.	*Eleocharis acicularis*	Rhizome	Cu, Zn, As, and Cd	Sakakibara et al. (2011)
4.	*Brassica juncea* L	Hairy root system	Zinc and nickel	Ismail et al. (2012)
5.	*Nicotiana tabacum*	Hairy root system	Arsenic	Talano et al. (2014)
6.	*Brassica napus HR and Pantoea sp. FC 1*	Hairy root system	Chromium	Ontanon et al. (2014)
7.	*Sorghum bicolor* × *sudanense*	Adventitious roots	As, Cu, Pb and Zn	Babu et al. (2014)
8.	*Ipomoea aquatica*	Adventitious roots	Copper	Chanu and Gupta (2014)
9.	*Nicotiana tabacum*	Hairy root system	2,4-Dichlorophenol	Angelini et al. (2014)
10.	*Nicotiana tabacum*	Hairy root system	Copper	Perez-Palacios et al. (2015)
11.	*Solanum nigrum L*	Tap-root	Cadmium	Ji et al. (2015)
12.	*Alyssum bertolonii*	Hairy root system	Nickel	Ibañez et al. (2016)
13.	*Hyptis capitate*	Hairy root system	Copper	Malik et al. (2016)
14.	*Adenophora lobophylla A. potaninii*	Hairy root system	Cadmium	Malik et al. (2016)
15.	*Moringa oleifera Typha latifolia Cymbopogon proxmus*	Hairy root system	Cadmium	Ghada et al. (2017)
16.	*V. zizanioides*	Hairy root system	Arsenic	Moogouei (2018)
17.	*Vetiveria zizanioides*	Hairy root system	Nitrate	Moogouei (2018)
18.	*Amaranthus chlorostachys*	Hairy root system	Metformin and cesium	Moogouei (2018)
19.	*Elodea canadensis*	Adventitious roots	Cobalt	Mosoarca et al. (2018)
21.	*Lemna minuta Kunth*	Hairy root system	Cr(VI)	Paisio et al. (2018)
22.	*Brassica napus*	Hairy root system	Chromium	Perotti et al. (2020)

through the uptake of oxygen microelectrode and CO_2. The adventitious root system of *Meionectes brownie* has the maximum rate of photosynthesis (0.38 μmol O_2 $m^{-2}s^{-1}$) (Rich et al. 2011). The function of the adventitious root system is based on the location of adventitious roots in plants. If the adventitious root generates from the stem, they help in tolerating the biotic stresses (Steffens and Rasmussen 2016). When there is flooding, it affects the plant that produces the aquatic adventitious roots due to the absorption of air (Ayi et al. 2016).

2.3.3 Hairy Roots in Phytoremediation

The hairy root (HR) system of the plants plays a major role in phytoremediation during the metal uptake. Generally, HRs have fast growth and development than normal root systems in natural plants. It have potential energy in the phytoremediation process (Majumder and Jha 2012). The root system has several processes to remove the pollutants, including adsorption, transport, translocation, hyperaccumulation or transformation, and mineralization. The carrot's hairy root culture was eliminating more than 90% of exogenous phenolic compounds from contaminated culture medium within 120 h (Zhou et al. 2013). The hairy root culture of *Brassica napus* removed 97–98% of 2, 4-dichlorophenol within 1hr treatment (Agostini et al. 2003). The root system of *Vetiveria zizanioides* has reached a high amount of Pb (2280 mg kg^{-1} DW) in 5000 mg Pb kg^{-1} of contaminated soil (Chen et al. 2004). *Agrobacterium rhizogenes* strain ATCC 15,834 infected *Helianthus annuus* has fast-growing hairy root culture and that catalyzes the disappearances of antibiotics including tetracycline (TC) and oxytetracycline (OTC) from the aqueous medium (Gujarathi et al. 2005). The hairy root culture induced by *A. rhizogenes* (NCIM 5140) in explants of *Sesuvium portulacastrum,* removed the harmful chemicals (Reactive green 19A HE4BD) from textile effluents within 5 days incubation periods (Lokhande et al. 2015). The normal root system of *Canna indica Linn* removed 41–55% of triazophos (O, O-diethyl-O-(1-phenyl-1, 2, 4-triazole-3-base) sulfur phosphate, TAP) in a hydroponic system (Cheng et al. 2007). *Vetiveria zizanoides* removed the phenolic content*s* through hydroponics technology (Singh et al. 2008).

2.3.4 Remediation with Microorganisms

Few microorganisms could act as biofertilizers in polluted soil and water. Naturally, plants interact with microorganisms for improved nitrogen fixation. Recent developments in bioremediation approaches have been made during the last 20 years with the intention of restoring damaged areas rapidly and inexpensively (Balloi et al. 2010; Leong and Chang 2020). Examples of some microbes involved in heavy metal bioremediation has been summarized in Table 2.2. Remediation through bioaugmentation with *Bacillus* sp., *Lysinibacillus* sp., or *Rhodococcus* sp. diminished

Table 2.2 Example of microbes are mostly involved in degrading pollutants

S.no	Microbes	Pollutant	References
1.	*Kocuria flava*	Copper	Achal et al. (2012)
2.	*Stenotrophomonas sp.*	Copper	Zaki and Farag (2010)
3.	*Enterobacter sp.*	Copper	Bestawy et al. (2013)
4.	*Desulfovibrio desulfuricans*	Nickel	Ockjoo et al. (2015)
5.	*Enterobacter cloacae*	Nickel	Banerjee et al. (2015)
6.	*Acinetobacter sp.*	Zinc	Tabaraki et al. (2013)
7.	*Desulfovibrio desulfuricans*	Cadmium	Ockjoo et al. (2015)
8.	*Enterobacter cloacae*	Lead	Banerjee et al. (2015)
9.	*Pseudomonas aeruginosa*	Lead	Ahmady-Asbchin et al. (2015)
10.	*Termitomyces clypeatus*	Chromium	Fathima et al. (2015)
11.	*Trametes versicolor*	Zinc	Sahan et al. (2015)
12.	*Pleurotus mutilus*	Uranium	Mezaguer et al. (2013)
13.	*Pleurotus ostreatus*	Lead	Zhang et al. (2016)
14.	*Aspergillus lentulus*	Lead	Mishra and Malik (2012)
15.	*Candida parapsilosis*	Mercury	Muneer et al. (2013)
16.	*Bacillus cereus*	Cadmium	Jan et al. (2015)
17.	*Cupriavidus metallidurans CH34*	Chromium	Alviz-Gazitua et al. (2019)
18.	*Pseudomonas aeruginosa and Bacillus cereus*	Cadmium	Nath et al. (2018)
19.	*Pseudomonas aeruginosa*	Cadmium	Chellaiah (2018)
20.	*Bacillus megaterium*	Boron, lead, and cadmium	Esringu et al. (2014)
21.	*Bacillus licheniformis*	Mercury	Muneer et al. (2013)
22.	*Enterobacter cloacae*	Mercury	Al-Garni et al. (2010)
23.	*Enterobacter, Klebsiella, Leifsonia, Bacillus*	Zinc	Jain et al. (2020)
24.	*Rhizophagus clarus*	Mercury	Gontia-Mishra et al. (2016)
25.	*Trichoderma virens*	Cadmium	Khanna et al. (2019)

the metal contents of the HM-induced contaminated soils, according to Fauziah et al. (2017). The soil microbes have the efficiency of remediation to degrade the harmful chemical compounds. *Burkholderia fungorum* DBT1 is a soil microorganism that transforms dibenzothiophene, phenanthrene, naphthalene, and fluorine (Andreolli et al. 2013). *Lolium multiflorum* plant system degrades the petroleum hydrocarbons (Arabian medium crude oil) with the help of petroleum-degrading microorganisms including *Glomus intraradices* (AMF), *Sphingomonas paucimobilis* (bacterium),

and *Cunninghamella echinulata* (filamentous fungi). Phytoremediation of metals in maize plants is successful with the addition of *Bacillus licheniformis* and *Rhodotorula dairenensis* (García et al. 2013). The plant's endophytic microorganisms produce economically valuable substances and plant growth-promoting substances such as phytohormones, plant growth regulators, and host protecting molecules. Various endophytic genera were found in plants, including *Serratia, Enterobacter, Acinetobacter, Agrobacterium, Bacillus, Herbaspirillum,* and *Klebsiella* strains. The endophytic microorganisms can improve plant growth by enhancing nutrient acquisition, water uptake, and decreased oxidative stress enzyme activities in host plants grown in contaminated soils (Feng et al. 2017). The bacteria's resistance to heavy metals like zinc and copper will be evaluated based on biochemical investigations of the bacteria. Some biodegradative bacteria were degrading the toxic chemical substances like herbicides, pesticides, refrigerants, solvents, and other organic compounds to beneficial plant growth substances (Zhang et al. 2020). The soil microbes participate in various remediation processes, including soil formation, energy transfer, and nutrient cycling. Through these processes, the microorganisms can promote revegetation and increase the stability of polluted ecosystems. But a high concentration of pollutants reduces biomass, population number, and diversity (Li et al. 2012). Soil microorganisms and plant root-associated bacteria were enhancing the production of plant root exudates, including organic molecules. Heavy metal phytotoxicity can be decreased by altering the bioavailability of metals in soil and preventing metal translocation through phytoremediation (Ma et al. 2016). The plant-associated microorganisms have the capacity of absorbing pollutants and promote plant growth through several direct and indirect mechanisms. The plant-associated microorganisms can produce plant growth regulators, including auxins, cytokinins, and gibberellins (Weyens et al. 2015). Bacterial and fugal strains degrade petroleum hydrocarbons from contaminated soil. From this study, the maximum phytoremediation rate was reported as 0.51 mg of TPH g^{-1} of dry plant d^{-1} at 60 days of culture and 90% of hydrocarbon was removed from the contaminated soil (Escalante-Espinosa et al. 2005). *Lolium perenne* plant was inoculated with microbial strains of *Bacillus subtilis, Sphingobacterium multivolume, Acinetobacter radioresistance, Rhodococcus erythropolis,* and *Pseudomonas fluorescens* for remediation of petroleum-contaminated soil. From this study, the maximum degradation rate was achieved up to 58% after 162 days from inoculation (Tang et al. 2010).

2.4 Biochar Mediated Remediation

Biochar is gaining remarkable attention in bioremediation techniques over years owing to its significant physicochemical characteristics. The porous surface, functional groups, surface area and charge properties of biochar enhances it use as a stand-alone bioadsorbent for various pollutants viz organic, petroleum, dyes, heavy metals, etc. (Cheng et al. 2021). Being produced from biomass like agricultural residues, biosolids, municipal solid wastes, it is carbon rich product possessing the

heterogeneous characteristics mainly depending on feedstock and reaction conditions (Selvam and Paramasivan 2022). As concerned to the scope of this chapter, literary reports on biochar for bioremediation validate it to be cost-effective and sustainable bioadsorbent compared to commercial adsorbents. The effectiveness of its adsorption has been proved for antibiotics, hydrocarbons, pesticides and metal contaminants. The proposed mechanism for the biosorption is primarily upsurging the microbial community to degrade the contaminants in amended soils. In addition to that, biochar efficiently adsorbs contaminants like heavy metals and decreases its bioavailability and toxicity (Karppinen et al. 2017). Song et al. (2021) reported that bacteria immobilized on biochar in polycyclic aromatic hydrocarbon contaminated soil increased the removal rate of the contaminant compared with the individual applications. Bioremediation efficiency is improved significantly by the biochemical material especially the biochar being immbolized with plant growth promoting bacteria drives soil micro-ecology and enhances the remediation pathway with this accumulator system (Wu et al. 2019). The studies on utilizing biochar with phytoremediation report that amendment of biochar reduces heavy metal and metalloid bioavailability. However, few plants need elevated doses to accumulate the bioavailable metals and metalloids (Narayanan and Ma 2022). Summarizing these entries, advantages of biochar for bioremediation involve improved physicochemical properties of contaminated soil, enhanced microbial activities and population, and improved agricultural productivity.

2.5 Conclusion and Future Perspective

Using phytoremediation technology, heavy metal uptake by roots seems to be a prosperous way to remediate the polluted environment. Microbial remediation and phytoremediation are reliable, cost-effective, efficient, eco-feasible alternatives. When compared with other conventional methods, these methods are better biological tools to remediate polluted conditions. However, several factors must be considered to accomplish better remediation results. The future perspectives to enhance the research includes the following.

- The technologies providing low-cost solution and eco-friendly to remediate contaminates has to be explored further.
- Also, focus on developing agricultural techniques to enhance remediation efficiency to reduce the time and cost utilized in the removal of heavy metals from soil to a considerable level for the well-being of mankind and nature has to be enhanced.
- Exploring the plants that has higher biomass, environmental affordability and high heavy metal accumulation potential for overcoming the associated limitations of phytoremediation.
- Eco-friendly additives with phytoremediation for enhancing the process efficiency and simultaneous enhancement of soil structure has to be explored more.

- Most of studies so far reported is based on laboratory scale. Translative research on field demonstration and its execution has to be implemented.

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References

Achal V, Pan X, Fu Q, Zhang D (2012) Biomineralization based remediation of As(III) contaminated soil by Sporosarcina ginsengisoli. J Hazard Mater 201–202:178–184

Agostini E, Coniglio MS, Milrad SR, Tigier HA, Giulietti AM (2003) Phytoremediation of 2,4-dichlorophenol by Brassica napus hairy root cultures. Biotechnol Appl Biochem 37:139–144

Ahmady-Asbchin S, Safari M, Tabaraki R (2015) Biosorption of Zn (II) by Pseudomonas aeruginosa isolated from a site contaminated with petroleum. Desalin Water Treat 54(12):3372–3379

Al-Baldawi IA, Abdullah SRS, Anuar N, Hasan HA (2018) Phytotransformation of methylene blue from water using aquatic plant (Azolla pinnata). Environ Technol Inno 11:15–22

Al-Garni SM, Ghanem KM, Ibrahim AS (2010) Biosorption of mercury by capsulated and slime layerforming Gram-ve bacilli from an aqueous solution. Afr J Biotechnol 9(38):6413–6421

Ali H, Khan E, Sajad MA (2013) Phytoremediation of heavy metals-concepts and applications. Chemosphere 91:869–881

Alviz-GazituaP F-A, Rojas LA, Turner RJ, Guiliani N, Seeger M (2019) The response of cupriavidus metallidurans CH34 to cadmium involves inhibition of the Initiation of biofilm formation, decrease in intracellular c-di-GMP levels, and a novel metal regulated phosphodiesterase. Front Microbiol 10:1499

Andreolli M, Lampis S, Poli M, Gullner G, Biró B, Vallini G (2013) Endophytic burkholderia fungorum DBT1 can improve phytoremediation efficiency of polycyclic aromatic hydrocarbons. Chemosphere 92:688–694

Angelini VA, Agostini E, Medina MI, González PS (2014) Use of hairy roots extracts for 2, 4-DCP removal and toxicity evaluation by Lactuca sativa test. Environ Sci Pollut Res 21:2531–2539

Antoniadis V, Levizou E, Shaheen SM, Ok YS, Sebastian A, Baum C, Prasad MN, Wenzel WW, Rinklebe J (2017) Trace elements in the soil-plant interface: phytoavailability, translocation, and phytoremediation–a review. Earth Sci Rev 171:621–645

Ayi Q, Zeng B, Liu J, Li S, van Bodegom PM, Cornelissen JH (2016) Oxygen absorption by adventitious roots promotes the survival of completely submerged terrestrial plants. Ann Bot 118:675–683

Babu AG, Shim J, Shea PJ, Oh BT (2014) Penicillium aculeatum PDR-4 and trichoderma sp. PDR-16 promotes phytoremediation of mine tailing soil and bioenergy production with sorghum-sudangrass. Ecol Eng 69:186–191

Balloi A, Rolli E, Marasco R, Mapelli F, Tamagnini I, Cappitelli F, Borin S, Daffonchio D (2010) The role of microorganisms in bioremediation and phytoremediation of polluted and stressed soils. Agrochimica 54:353–369

Banerjee G, Pandey S, Ray AK, Kumar R (2015) Bioremediation of heavy metals by a novel bacterial strain enterobacter cloacae and its antioxidant enzyme activity, flocculant production, and protein expression in presence of lead, cadmium, and nickel. Water Air Soil Pollut 226(4):1–9

Bellini C, Pacurar DI, Perrone I (2014) Adventitious roots and lateral roots: similarities and differences. Annu Rev Plant Biol 65:639–666

Bestawy EE, Helmy S, Hussien H, Fahmy M, Amer R (2013) Bioremediation of heavy metal-contaminated effluent using optimized activated sludge bacteria. Appl Water Sci 3(1):181–192

Carolin CF, Kumar PS, Saravanan A, Joshiba GJ, Naushad M (2017) Efficient techniques for the removal of toxic heavy metals from the aquatic environment: a review. J Environ Chem Eng 5:2782–2799

Cayetano RDA, Park J, Kim GB, Jung JH, Kim SH (2021) Enhanced anaerobic digestion of waste-activated sludge via bioaugmentation strategy—phylogenetic investigation of communities by reconstruction of unobserved states (PICRUSt2) analysis through hydrolytic enzymes and possible linkage to system performance. Bioresour Technol 332:125014

Chanu HK, Gupta A (2014) Necrosis as an adaptive response to copper toxicity in Ipomoea aquatica forsk. and its possible application in phytoremediation. Acta Physiol Plant 36:3275–3281

Chellaiah E (2018) Cadmium (heavy metals) bioremediation by Pseudomonas aeruginosa: a minireview. Appl Water Sci 8:154

Chen Y, Shen Z, Li X (2004) The use of vetiver grass (Vetiveria zizanioides) in the phytoremediation of soils contaminated with heavy metals. Appl Geochem 19:1553–1565

Cheng S, Xiao J, Xiao H, Zhang L, Wu Z (2007) Phytoremediation of triazophos by Canna indica Linn. in a hydroponic system. Int J Phytoremediation 9:453–463

Cheng N, Wang B, Wu P, Lee X, Xing Y, Chen M, Gao B (2021) Adsorption of emerging contaminants from water and wastewater by modified biochar: a review. Environ Pollut 273:116448

Crini G, Lichtfouse E (2019) Advantages and disadvantages of techniques used for wastewater treatment. Environ Chem Lett 17(1):145–155

Ekta P, Modi NR (2018) A review of phytoremediation. J Pharmacogn Phytochem 7(4):1485–1489

El Fantroussi S, Agathos SN (2005) Is bioaugmentation a feasible strategy for pollutant removal and site remediation? Curr Opin Microbiol 8:268–275

Ehsan S, Ali S, Noureen S, Mahmood K, Farid M, Ishaque W, Rizwan M (2014) Citric acid assisted phytoremediation of cadmium by Brassica napus L. Ecotoxicol envi safety 106:164–172

Escalante-Espinosa E, Gallegos-Martínez ME, Favela-Torres E, Gutiérrez-Rojas M (2005) Improvement of the hydrocarbon phytoremediation rate by Cyperus laxus Lam. inoculated with a microbial consortium in a model system. Chemosphere 59:405–413

Esringu A, Tarun M, Gunes A, Karaman MR (2014) Roles of Bacillus megaterium in remediation of boron, lead, and cadmium from contaminated soil. Commun Soil Sci Plan Anal 45:1741–1759

Etim EE (2012) Phytoremediation and its mechanisms: a review. Int J Env Bio 2:120–136

Fathima A, Aravindhan R, Rao JR, Nair BU (2015) Biomass of Termitomyces clypeatus for chromium (III) removal from chrome tanning wastewater. Clean Technol Environ Policy 17(2):541–547

Fauziah SH, Agamuthu P, Hashim R, Izyani AK, Emenike CU (2017) Assessing the bioaugmentation potentials of individual isolates from landfill on metal-polluted soil. Environ Earth Sci 76:401

Feng NX, Yu J, Zhao HM, Cheng YT, Mo CH, Cai QY, Li YW, Li H, Wong MH (2017) Efficient phytoremediation of organic contaminants in soils using plant–endophyte partnerships. Sci Total Environ 583:352–368

Franchin C, Fossati T, Pasquini E, Lingua G, Castiglione S, Torrigiani P, Biondi S (2007) High concentrations of zinc and copper induce differential polyamine responses in micropropagated white poplar (Populus alba). Physiol Plant 130:77–90

García JAL, Grijalbo L, Ramos B, Fernández-Piñas F, Rodea-Palomares I, Gutierrez-Mañero FJ (2013) Combined phytoremediation of metal-working fluids with maize plants inoculated with different microorganisms and toxicity assessment of the phytoremediated waste. Chemosphere 90: 2654−2661

Geiss G, Gutierrez L, Bellini C (2018) Adventitious root formation: new insights and perspectives. Annu Plant Rev online: 127–156

Ghada M, Najla AI, Sajad MH, Fida A, Eltohami MS, Abdurahman HN, Hajo EE, Tilal E (2017) Efficacy of some sudanese medicinal plants extracts to remove heavy metals from water. Aust J Basic Appl Sci 11:51–55

Gontia-Mishra I, Sapre S, Sharma A, Tiwari S (2016) Alleviation of mercury toxicity in wheat by the interaction of mercury-tolerant plant growth-promoting rhizobacteria. J Plant Growth Regul 35(4):1000–1012

González PS, Capozucca CE, Tigier HA, Milrad SR, Agostini E (2006) Phytoremediation of phenol from wastewater, by peroxidases of tomato hairy root cultures. Enzyme Microb Technol 39:647–653

Gujarathi NP, Haney BJ, Park HJ, Wickramasinghe SR, Linden JC (2005) Hairy roots of Helianthus annuus: a model system to study phytoremediation of tetracycline and oxytetracycline. Biotechnol Progr 21:775–780

Guo Z, Richardson JJ, Kong B, Liang K (2020) Nanobiohybrids: materials approaches for bioaugmentation. Sci Adv, 6(12): eaaz0330

Huang C, Liu Q, Chen X, Nan J, Li Z, Wang A (2020) Bioaugmentation with Thiobacillus sp. H1 in an autotrophic denitrification desulfurization microbial reactor: microbial community changes and relationship. Environ Res 189: 109927.

Hussain I, Aleti G, Naidu R, Puschenreiter M, Mahmood Q, Rahman MM, Reichenauer TG (2018) Microbe and plant assisted-remediation of organic xenobiotics and its enhancement by genetically modified organisms and recombinant technology: a review. Sci Tot Environ 628:1582–1599

Ibañez S, Talano M, Ontañon O, Suman J, Medina MI, Macek T, Agostini E (2016) Transgenic plants and hairy roots: exploiting the potential of plant species to remediate contaminants. N Biotechnol 33:625–635

Ismail AM, Theodor PA (2012) The effect of heavy metals Zn and Ni on the growth of in vitro hairy root cultures of Indian mustard Brassica juncea L. Int J Adv Biotechnol Res 3:688–697

Itusha A, Osborne WJ, Vaithilingam M (2019) Enhanced uptake of Cd by biofilm forming Cd resistant plant growth promoting bacteria bioaugmented to the rhizosphere of Vetiveria zizanioides. Int J Phyto 21(5):487–495

Jain D, Kour R, Bhojiya AA, Meena RH, Singh A, Mohanty SR, Rajpurohit D, Ameta KD (2020) Zinc tolerant plant growth promoting bacteria alleviates phytotoxic effects of zinc on maize through zinc immobilization. Sci Rep 10:13865

Jan AT, Azam M, Siddiqui K, Ali A, Choi I, Haq QMR (2015) Heavy metals and human health: mechanistic insight into toxicity and counter defense system of antioxidants. Int J Mol Sci 16(12):29592–29630

Ji P, Jiang Y, Tang X, Nguyen TH, Tong YA, Gao P, Han W (2015) Enhancing of phytoremediation efficiency using indole-3-acetic acid (IAA). Soil Sediment Contam 24:909–916

Jiang J, Li L, Li Y, He Y, Wang C, Sun Y (2020) Bioaugmentation to enhance anaerobic digestion of food waste: dosage, frequency and economic analysis. Bioresour Technol 307:123256

Kalve S, Sarangi BK, Pandey RA, Chakrabarti T (2011) Arsenic and chromium hyperaccumulation by an ecotype of Pteris vittata–prospective for phytoextraction from contaminated water and soil. Curr Sci: 888–894

Karppinen EM, Stewart KJ, Farrell RE, Siciliano SD (2017) Petroleum hydrocarbon remediation in frozen soil using a meat and bonemeal biochar plus fertilizer. Chemosphere 173:330–339

Khanna K, Jamwal VL, Gandhi SG, Ohri P, Bhardwaj R (2019) Metal resistant PGPR lowered Cd uptake and expression of metal transporter genes with improved growth and photosynthetic pigments in Lycopersicon esculentum under metal toxicity. Sci Rep 9:5855

Kumar SS, Kadier A, Malyan SK, Ahmad A, Bishnoi NR (2017) Phytoremediation and rhizoremediation: uptake, mobilization, and sequestration of heavy metals by plants. Plant-microbe interactions in agroecological perspectives. Springer, Singapore, pp 367–394

Lellis B, Fávaro-Polonio CZ, Pamphile JA, Polonio JC (2019) Effects of textile dyes on health and the environment and bioremediation potential of living organisms. Biotechnol Res Innov 3(2):275–290

Leong YK, Chang JS (2020) Bioremediation of heavy metals using microalgae: recent advances and mechanisms. Bioresour Technol 303:122886
Li HY, Wei DQ, Shen M, Zhou ZP (2012) Endophytes and their role in phytoremediation. Fungal Diversity 54:11–18
Li Y, Yang G, Li L, Sun Y (2018) Bioaugmentation for overloaded anaerobic digestion recovery with acid-tolerant methanogenic enrichment. Waste Manag 79:744–751
Lokhande VH, Kudale S, Nikalje G, Desai N, Suprasanna P (2015) Hairy root induction and phytoremediation of textile dye, Reactive green 19A-HE4BD, in a halophyte, Sesuvium portulacastrum (L.) L. Biotechnol Rep 8:56–63
Luo ZB, He J, Polle A, Rennenberg H (2016) Heavy metal accumulation and signal transduction in herbaceous and woody plants: paving the way for enhancing phytoremediation efficiency. Biotechnol Adv 34(6):1131–1148
Ma Y, Rajkumar M, Zhang C, Freitas H (2016) Beneficial role of bacterial endophytes in heavy metal phytoremediation. J Env Management 174:14–25
Majumder A, Jha S (2012) Hairy roots: a promising tool for phytoremediation. Microorganisms in Environmental Management. Springer, Dordrecht, pp 607–629
Malik S, Andrade SA, Mirjalili MH, Arroo RR, Bonfill M, Mazzafera P (2016) Biotechnological approaches for bioremediation: in vitro hairy root culture. In Transgenesis and Secondary Metabolism Springer International Publishing, Cham, pp 1–23
Mezaguer M, El Hayet Kamel N, Lounici H, Kamel Z (2013) Characterization and properties of Pleurotus mutilus fungal biomass as adsorbent of the removal of uranium (VI) from uranium leachate. J Radioanal Nucl Chem 295(1):393–403
Mishra A, Malik A (2012) Simultaneous bioaccumulation of multiple metals from electroplating effluent using Aspergillus lentulus. Water Res 46(16):4991–4998
Mokarram M, Saber A, Sheykhi V (2020) Effects of heavy metal contamination on river water quality due to release of industrial effluents. J Clean Prod 277:123380
Moogouei R (2018) Use of terrestrial plants for phytoremediation of pollutants from solutions. Iran J Sci Technol 42:1753–1759
Moola AK, Balasubramanian P, Satish L, Shamili S, Ramesh M, Kumar TS, Kumari BD (2021) Hairy roots as a source for phytoremediation. In: Strategies and Tools for Pollutant Mitigation. Springer, Cham, pp. 29–47.
Mosoarca G, Vancea C, Popa S, Boran S (2018) Adsorption, bioaccumulation and kinetics parameters of the phytoremediation of cobalt from wastewater using Elodea Canadensis. Bull Environ Contam Toxicol 100:733–739
Muneer B, Iqbal MJ, Shakoori FR, Shakoori AR (2013) Tolerance and biosorption of mercury by microbial consortia: potential use in bioremediation of wastewater. Pak J Zool 45:247–254
Narayanan M, Ma Y (2022) Influences of biochar on bioremediation/phytoremediation potential of metal-contaminated soils. Front Microbiol 13.
Nath S, Deb B, Sharma I (2018) Isolation of toxic metal-tolerant bacteria from soil and examination of their bioaugmentation potentiality by pot studies in cadmium- and lead-contaminated soil. Int Microbiol 21:35
Neilson S, Rajakaruna N (2015) Phytoremediation of agricultural soils: using plants to clean metal-contaminated arable land. Phytoremediation. Springer, Cham, pp 159–168
Nzila A, Razzak SA, Zhu J (2016) Bioaugmentation: an emerging strategy of industrial wastewater treatment for reuse and discharge. Int J Environ Res Public Health 13(9):846
Ockjoo J, Choi JH, Kim IH, Kim YK, Oh BK (2015) Effective bioremediation of cadmium (II), nickel (II), and chromium (VI) in a marine environment by using Desulfovibrio desulfuricans. Biotechnol Bioprocess Eng 20(5):937–941
Odjegba VJ, Fasidi IO (2004) Accumulation of trace elements by Pistia stratiotes: implications for phytoremediation. Ecotoxicol 13:637–646
Ontanon OM, González PS, Ambrosio LF, Paisio CE, Agostini E (2014) Rhizoremediation of phenol and chromium by the synergistic combination of a native bacterial strain and Brassica napus hairy roots. Int Biodeterior Biodegradation 88:192–198

Paisio CE, Fernandez M, González PS, Talano MA, Medina MI, Agostini E (2018) Simultaneous phytoremediation of chromium and phenol by Lemna minuta Kunth: a promising biotechnological tool. Int J Env Sci Technol 15:37–48

Perez-Palacios P (2015) Environmental biotechnology, development of biotechnological strategies for the bioremediation of heavy metals. Doctoral Thesis, Spain

Perotti R, Paisio CE, Agostini E, Fernandez MI, González PS (2020) CR (VI) phytoremediation by hairy roots of Brassica napus: assessing efficiency, mechanisms involved, and post-removal toxicity. Env Sci Pollut Res: 1–10.

Rich SM, Ludwig M, Pedersen O, Colmer TD (2011) Aquatic adventitious roots of the wetland plant meionectes brownii can photosynthesize implications for root function during flooding. New Phytol 190:311–319

Sahan T, Ceylan H, Akta N, (2015) Optimization of biosorption of Zn (II) ions from aqueous solutions with low-cost biomass Trametes versicolor and the evaluation of kinetic and thermodynamic parameters. Desalin Water Treat 57:1−12

Sakakibara M, Ohmori Y, Ha NTH, Sano S, Sera K (2011) Phytoremediation of heavy metal contaminated water and sediment by Eleocharis acicularis. CLEAN-Soil Air Water 39:735–741

Salam JA, Hatha MA, Das N (2017) Microbial-enhanced lindane removal by sugarcane (Saccharum officinarum) in doped soil-applications in phytoremediation and bioaugmentation. J Environ Manage 193:394–399

Selvam SM, Paramasivan B (2022) Microwave assisted carbonization and activation of biochar for energy-environment nexus: a review. Chemosphere 286:131631

Sharma P (2021) Efficiency of bacteria and bacterial assisted phytoremediation of heavy metals: an update. Biores Technol 328:124835

Sharma I (2020) Bioremediation techniques for polluted environment: concept, advantages, limitations, and prospects. In: Trace Metals in the Environment-New Approaches and Recent Advances. IntechOpen

Shinomol GK, Bhanu RK (2021) Phytoremediation and the way forward: challenges and opportunities. Appl Water Sci: Remediat Technol 2:405–436

Singh S, Melo JS, Eapen S, D'souza SF (2008) Potential of vetiver (Vetiveria zizanoides L. Nash) for phytoremediation of phenol. Ecotoxicol Environ Saf 71:671–676

Song L, Niu X, Zhang N, Li T (2021) Effect of biochar-immobilized Sphingomonas sp. PJ2 on bioremediation of PAHs and bacterial community composition in saline soil. Chemosphere, 279: 130427

Soudek P, Petrová Š, Benešová D, Vaněk T (2011) Uranium uptake and stress responses of in vitro cultivated hairy root culture of Armoracia rusticana. Agrochimica 55:15–28

Steffens B, Rasmussen A (2016) The physiology of adventitious roots. Plant Physiol 170:603–617

Tabaraki R, Ahmady-Asbchin S, Abdi O (2013) Biosorption of Zn (II) from aqueous solutions by Acinetobacter sp. isolated from petroleum spilled soil. J Environ Chem Eng 1 (3): 604–608.

Takkar S, Shandilya C, Agrahari R, Chaurasia A, Vishwakarma K, Mohapatra S, Mishra A (2022) Green technology: phytoremediation for pesticide pollution. In Phytoremediation Technology for the Removal of Heavy Metals and Other Contaminants from Soil and Water. pp 353–375.

Talano MA, Oller ALW, González P, González SO, Agostini E (2014) Effects of arsenate on tobacco hairy root and seedling growth, and its removal. In Vitro Cell Dev Biol Plant 50:217–225

Tang J, Wang R, Niu X, Zhou Q (2010) Enhancement of soil petroleum remediation by using a combination of ryegrass (Lolium perenne) and different microorganisms. Soil Tillage Res 110:87–93

Tsapekos P, Kougias PG, Vasileiou SA, Treu L, Campanaro S, Lyberatos G, Angelidaki I (2017) Bioaugmentation with hydrolytic microbes to improve the anaerobic biodegradability of lignocellulosic agricultural residues. Bioresour Technol 234:350–359

Tyagi M, da Fonseca MMR, de Carvalho CC (2011) Bioaugmentation and biostimulation strategies to improve the effectiveness of bioremediation processes. Biodegradation 22:231–241

Uchman W, Skorek-Osikowska A, Werle S (2017) Evaluation of the potential of the production of electricity and heat using energy crops with phytoremediation features. Appl Therm Eng 126:194–203

Viji AS, Antony BT, Wagh MS, Osborne WJ (2022) Bioremoval of cadmium by co-cultivated bacterial strains, bacillus paramycoides and bacillus subtilis, in a pilot-scale phyto-and rhizoremediation approach. Int J Environ Sci and Technol 19(8):7565–7574

Vithanage M, Dabrowska BB, Mukherjee AB, Sandhi A, Bhattacharya P (2012) Arsenic uptake by plants and possible phytoremediation applications: a brief overview. Environ Chem Lett 10:217–224

Wasewar KL (2022) Process intensification in bio-based approaches for environmental remediation. In: Innovative Bio-Based Technologies for Environmental Remediation. CRC Press, pp 109–124

Weyens N, Van Der Lelie D, Artois T, Smeets K, Taghavi S, Newman L, Carleer R, Vangronsveld J (2009) Bioaugmentation with engineered endophytic bacteria improves contaminant fate in phytoremediation. Environ Sci Technol 43:9413–9418

Weyens N, Thijs S, Popek R, Witters N, Przybysz A, Espenshade J, Gawronska H, Vangronsveld J, Gawronski SW (2015) The role of plant-microbe interactions and their exploitation for phytoremediation of air pollutants. Int J Mol Sci 16:25576–25604

Wu B, Wang Z, Zhao Y, Gu Y, Wang Y, Yu J, Xu H (2019) The performance of biochar-microbe multiple biochemical material on bioremediation and soil micro-ecology in the cadmium aged soil. Sci Tot Environ 686:719–728

Yang J, Chen Z, Wu S, Cui Y, Zhang L, Dong H, Yang C, Li C (2015) Overexpression of the tamarix hispida ThMT3 gene increases copper tolerance and adventitious root induction in salix matsudana Koidz. Plant Cell, Tissue Organ Cul 121:469–479

Yang Z, Wang W, Liu C, Zhang R, Liu G (2019) Mitigation of ammonia inhibition through bioaugmentation with different microorganisms during anaerobic digestion: selection of strains and reactor performance evaluation. Water Res 155:214–224

Zaki S, Farag S (2010) Isolation and molecular characterization of some copper biosorped strains. Int J Environ Sci Technol 7(3):553–560

Zhang S, Zhang X, Chang C, Yuan Z, Wang T, Zhao Y et al (2016) Improvement of tolerance to lead by filamentous fungus Pleurotus ostreatus HAU-2 and its oxidative responses. Chemosphere 150:33–39

Zhang H, Yuan X, Xiong T, Wang H, Jiang L (2020) Bioremediation of co-contaminated soil with heavy metals and pesticides: influence factors, mechanisms and evaluation methods. Chem Eng J 398:125657

Zhou ML, Tang YX, Wu YM (2013) Plant hairy roots for remediation of aqueous pollutants. Plant Mol Biol Report 31:1–8

Chapter 3
Performance of Lead (Pb) Resistant *Staphylococcus aureus* from Tannery Waste Discharge Site

P. Muthukumaran and S. Karthika

Abstract Heavy metal resistant bacteria were identified from tannery effluent wastewater in and around Ezar Tanneries in Vaniyambadi, Vellore district, South India. In the first step, eleven bacterial isolates were collected from tannery effluent wastewater and tested on Luria Bertani (LB) agar plates enriched with 5 mg/l concentrations of five distinct heavy metal ions, including Cr, Cu, Ni, Pb, and Zn. Heavy metal resistance was a primary factor in choosing these two isolates. Staphylococcus aureus was determined to be the causative agent based on morphological and biochemical characteristics of the isolates. All of the detected isolates showed resistance to both copper and lead. Maximum growth of Staphylococcus aureus, a sewage isolate, occurred at 30 °C and pH 6.5. Bioremediation of heavy metal-tainted sewage and wastewater may benefit from the use of the discovered Staphylococcus aureus strain that is resistant to the metals in question.

Keywords Tannery effluent · Lead (Pd) · *Staphylococcus aureus* · Bioremediation

3.1 Introduction

Raw tannery effluent waste water contain huge amount of heavy metals that are not degraded by the conventional process of wastewater treatment and removal of these heavy metals from waste water needs advance chemical technology and it's expensive too. Presence of high concentration of toxic heavy metals in wastewater directly leads to both contamination of receiving water bodies and deleterious impact on aquatic life. Particularly, the mining industry generates residues with high levels of heavy metals (Ni, Cd, Pb, Mn, Cu, and Zn) that are deposited in open areas presenting an ecological and human health risk. Lead (Pb) a major pollutant that is found in soil, water and air is a hazardous waste and is highly toxic to human, animals, plants and

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microbes (Low et al. 2000; Elahi et al. 2019). The microorganisms respond to these heavy metals by several processes; including transport across the cell membrane, biosorption to the cell walls and entrapment in extracellular capsules, precipitation, complexation and oxidation–reduction reactions (Rai et al. 1981; Macaskie and Dean 1989; Huang et al. 1990; Brady and Duncan 1994a; Brady et al. 1994b; Elahi et al. 2022). Naturally occurring bacteria that are capable of metal accumulation have been extensively studied since it is difficult to imagine that a single bacterium could be capable to remove all heavy metals from its polluted site (Clausen 2000). The objective of this study is to determine heavy metals resistance of bacteria, biochemical characteristics and growth studies were used to exploit these isolates for clean-up of tannery effluent waste water.

3.2 Materials and Methods

3.2.1 Isolation of Screening of Bacterial Isolates from Tannery Effluent

The sewage water samples were collected in and around Tanneries, Vaniyambadi, Vellore district, South India. The samples were collected in sterile plastic container and transport to laboratory for bacteriological analysis. The bacterial isolates were screened on Luria Bertani (LB) agar plates supplemented with 5 mg/l concentration of Cr, Cu, Ni, Pb and Zn metals by the standard pour plate method. Plates were incubated at 37 °C for 48 h and colonies differing in morphological characteristics were selected and used for further studies (Elahi et al. 2019, 2022).

3.2.2 Identification and Biochemical Characterization of Screened Bacterial Isolates

The shape and color of the colonies were examined under the microscope after Gram staining. Isolates were biochemically analyzed for the activities of catalase, starch hydrolysis and gelatin hydrolysis, mannitol test, motility, indole production and citrate utilization. The tests were used to identify the isolates according to Bergey's Manual of Systematic Bacteriology (Claus and Berkeley 1986).

3.2.3 Growth Studies of Screened Bacterial Isolates

Growth studies of sewage bacterial isolates was studied in 250 ml flasks containing 50 ml LB medium supplemented with 0.1 mM concentration lead. Flasks were inoculated with 0.5 ml of overnight culture and agitated on a rotary shaker at 37 °C. Growth was monitored as a function of biomass by measuring the absorbance at 620 nm using spectrophotometer and the optimal growth conditions with reference to pH and temperature were determined. The effect of pH on the growth of bacteria in the presence of lead was analyzed at five pH values 4.5, 5.5, 6.5, 7.5 and 8.5 and different temperatures 30, 37 and 45 °C was studied (Elahi et al. 2022).

3.2.4 Determination of MIC

Maximum resistance of the bacteria against increasing concentrations of lead on LB broth was evaluated until the strains unable to grow in the broth. The initial concentration used (100 ppm) was added from 100 mg/100 ml of stock solution. Concentration of metal up to 3000 mg/l was taken. The stock solution of Pb $(NO_3)_2$ (Ranbaxy) was prepared in distilled water and sterilized by autoclaving at 121 ºC, 15 psi for 15 min (Kalaimurugan et al. 2020).

3.3 Results and Discussion

3.3.1 Isolation of Screening of Bacterial Isolates from Tannery Effluent

The microbial colonies were isolated by pour plate method. Eleven bacterial isolates were purified using streak plate method**.** The bacterial isolates named based on the source from which it was isolated. The isolated bacterial colonies were screened with the metal concentration of 100 mg/l and the metal resistant isolates were isolated based on their cell density values measured at 620 nm by using UV–visible spectrophotometer (ELICO, India) (Kalaimurugan et al. 2020) (Table 3.1).

3.3.2 Identification and Biochemical Characterization of Screened Bacterial Isolates

From the gram-staining procedure it is found that the microbial strain was a gram-positive spherical bacterium that occurred in microscopic clusters formed yellow

Table 3.1 Screening of metal resistant bacterial isolates

S.No	Metals (5 mg/lit)	Days	Microbial strains										
			VLB*−1	VLB−2	VLB−3	VLB−4	VLB−5	VLB−6	VLB−7	VLB−8	VLB−9	VLB−10	VLB−11
1.	Cr	0	−	−	−	−	−	−	−	−	−	−	−
		1	+	+	+	+	+	++	+	+	+++	+	+
		2	++	+	+	+	++	+++	++	+	+++	++	+
2.	Cu	0	−	−	−	−	−	−	−	−	−	−	−
		1	+	+	+	+	+	+	+	+	+	+	+
		2	+	+	+	++	+	+	+	+	++	+	+
3.	Ni	0	−	−	−	−	−	−	−	−	−	−	
		1	+	+	+	+	+	++	+	+	+	+	+
		2	+	+	++	++	+	+++	+	++	++	++	+
4.	Pb	0	−	−	−	−	−	−	−	−	−	−	−
		1	+	+	+	+	+	+	+	+	++	+	+
		2	++	++	+	++	++	++	++	++	++++	++	+
5.	Zn	0	−	−	−	−	−	−	−	−	−	−	−
		1	+	+	+	+	+	+	+	+	+	+	+
		2	+	++	+	+	++	++	+	++	++	++	++

Note No growth; + poor growth; + + moderate; + + + growth
*VLB-Vaniyambadi Leather effluent Bacteria

Table 3.2 The morphological and biochemical characterization of bacterial isolates (VLB-9)

Sl. No	Characteristics	*Staphylococcus aureus* (VLB-9)
1.	Colony diameter	1–3 mm
2.	Colony color	Yellow
3.	Colony morphology	Slightly elevated, circular
4.	Gram staining	Positive
5.	Turbidity in broth	Uniform dispersed growth
6.	Motility	Non-motile
7.	Oxidase	Negative
8.	Catalase	Positive
9.	Mannitol sugar fermentation	Negative
10.	Coagulase	Positive
11.	Temperatures	
	30	+ + +
	37	+ +
	45	_
12.	pH	
	4.5	+
	5.5	+ +
	6.5	+ + +
	7.5	+ +
	8.5	_

Note No growth; + poor growth; + + moderate growth; + + + growth

colonies in nutrient medium. Further biochemical tests resulted positive for catalase, mannitol sugar fermentation and coagulase test confirmed that the isolated microbial strain VLB-9 was identified as *Staphylococcus aureus* (Elahi et al. 2022) (Table 3.2).

3.4 Growth Studies of *Saphylococcus Aureus*

3.4.1 Effect of Temperature

The measurements from the cultures incubated for 24 h were in good agreement according to bacterial resistance. Growth was monitored as a function of biomass by measuring the absorbance at 620 nm using spectrophotometer and the optimal growth conditions with reference to different temperature such as 30, 37 and 45 ℃. Figure 3.1 shows maximum removal of the metal was found to be at 30 ℃. Thus

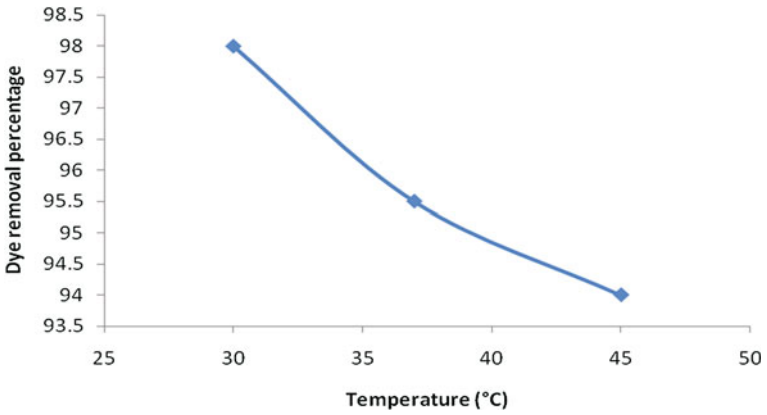

Fig. 3.1 Effect of temperature on biosorption of lead by *staphylococcus aureus*

the optimum temperature was found as 30 ºC (Raja et al. 2006; Kalaimurugan et al. 2020).

3.4.2 Effect of pH

The effect of pH on the growth of bacteria in the presence of lead was analyzed at five pH values such as 4.5, 5.5, 6.5, 7.5 and 8.5. Figure 3.2 shows maximum removal of the metal was found to be at 6.5 pH. Thus the optimum pH was found as 6.5 (Ahmed et al. 2005; Moodley et al. 2019).

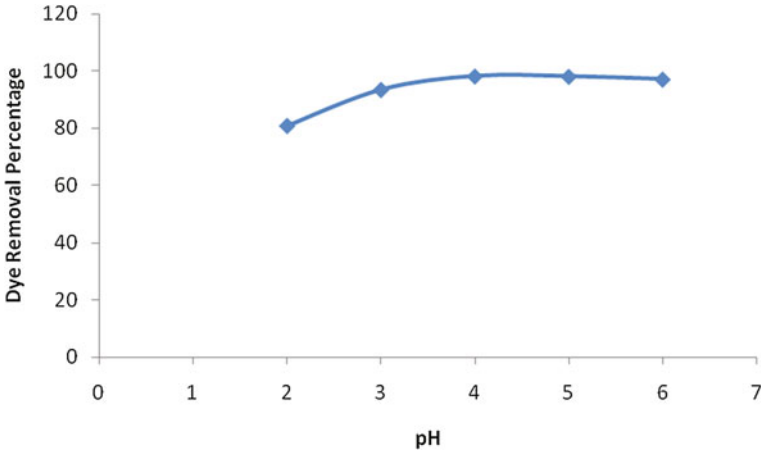

Fig. 3.2 Effect of pH on biosorption of lead by *staphylococcus aureus*

3.4.3 Determination of Minimum Inhibitory Concentration (MIC)

Staphylococcus aureus was highly resistant for various concentration of metal. The concentration such as 100, 500, 1000, 1500, 2000 and 2500 mg/l. Visible growth was observed in the broth containing up to 1000 mg/l concentration of lead as shown in Fig. 3.3 and Table 3.3. The growth of the isolated bacteria was inhibited from 1500 mg/l concentration of metal. Thus the minimum inhibitory concentration of the isolated bacteria was found as 1500 mg/l (Muthukumaran et al. 2014; Filali et al. 2004; Marzan et al. 2017).

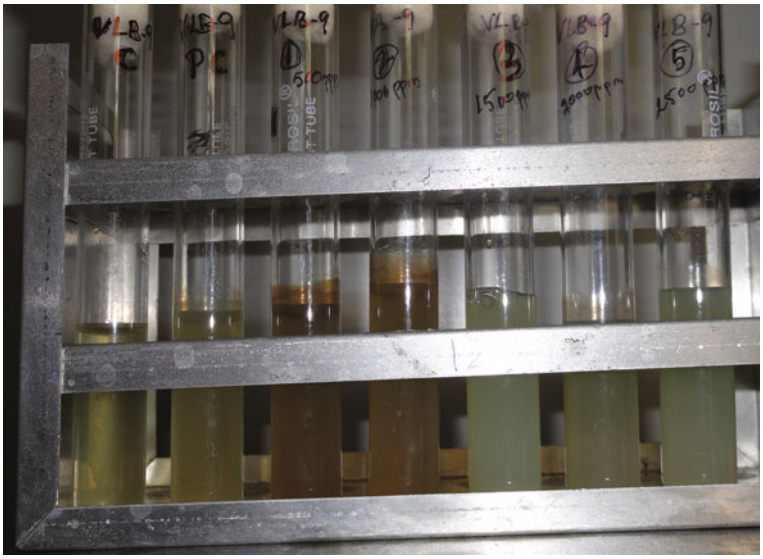

Fig. 3.3 Minimum inhibitory concentration of *staphylococcus aureus*

Table 3.3 Determination of minimum inhibitory concentration (MIC)

Sl. No	Metal ion concentration (ppm)	Growth of *Staphylococcus aureus*
1.	100	+ + +
2.	500	+ + +
3.	1000	+ +
4.	1500	–
5.	2000	–
6.	2500	–

Note No growth; + poor growth; + + moderate growth; + + + growth

3.5 Conclusion

The heavy metal resistant organism could be a potential agent for bioremediation of heavy metals pollution. *Staphylococcus* resistant to heavy metals and antibiotics. In the present study high degree of heavy metals resistance associated with multiple antibiotic resistances was detected in S*taphylococcus aureus.* S*taphylococcus aureus* exposed to high levels of heavy metals in their environment have adapted to this stress by developing various resistance mechanism. These mechanisms could be utilized for detoxification and removal of heavy metals from polluted environment. According to these results, the present study evaluates that S*taphylococcus aureus* were used to remediate heavy metal contaminated tannery effluent waste.

References

Ahmed N, Nawaz A, Badar (2005) Screening of copper tolerant bacterial species and their potential to remove copper from the environment. Bull Environ Contam Toxicol 74: 219−226

Brady D, Stoll AD, Starke L, Duncan JR (1994a) Bioaccumulation of metal cations by *Saccaromyces cerevisiae.* Appl Microbiol Biotechnol 41(1):149–154. https://doi.org/10.1007/BF00166098

Brady D, Stoll AD, Starke L, Duncan JR (1994b) Chemical and enzymatic extraction of heavy metal binding polymers from isolated cell walls of *Sccharomyces cerevisiae.* Biotechnol Bioeng 44(3):297–302. https://doi.org/10.1002/bit.260440307

Claus D, Berkeley RCW (1986) Genus *Pseudomonas.* In: Sneath PHA, Mair NS, Sharpe ME (eds) Bergey's manual of systematic bacteriology, vol 1, Williams & wilkins, Baltimore, pp. 140–219. ISBN 0–683–04108–8.

Clausen CA (2000) Isolating metal-tolerant bacteria capable of removing copper, chromium, and arsenic from treated wood. Waste Manag Res 18:264–268

Elahi A, Rehman A, Zajif Hussain SZ, Zulfiqar S, Shakoori AR (2022) Isolation and characterization of a highly effective bacterium *Bacillus cereus* b-525k for hexavalent chromium detoxification. Saudi J Biol Sci 29(4):2878–2885. https://doi.org/10.1016/j.sjbs.2022.01.027

Elahi A, Ajaz M, Rehman A, Vuilleumier S, Khan Z, Hussain SZ (2019) Isolation, characterization, and multiple heavy metal-resistant and hexavalent chromium-reducing Microbacterium testaceum B-HS2 from tannery effluent. J King Saud Univ Sci 31(4): 1437−1444. https://doi.org/10.1016/j.jksus.2019.02.007

Filali BK, Taoufik J, Zeroual Y, Dzairi FZ, Talbi M, Blaghen M (2004) Waste water bacterial isolates resistant to heavy metals and antibiotics. Curr Microbiol 41(3):151–156. https://doi.org/10.1007/s0028400

Huang C, Huang C, Morehart AL (1990) The removal of copper from dilute aqueous solutions by *Saccharomyces cerevisiae.* Water Res 24(4):433–439. https://doi.org/10.1016/0043-1354(90)90225-U

Kalaimurugan D, Balamuralikrishnan B, Durairaj K, Vasudhevan P, Shivakumar MS, Kaul T et al (2020) Isolation and characterization of heavy-metal-resistant bacteria and their applications in environmental bioremediation. Int J Environ Sci Technol 17(3):1455–1462. https://doi.org/10.1007/s13762-019-02563-5

Low KS, Lee CK, Liew SC (2000) Sorption of cadmium and lead from aqueous solution by spent grain. Process Biochem 36(1–2):59–64. https://doi.org/10.1016/S0032-9592(00)00177-1

Macaskie L, Dean ACR (1989) Microbial metabolism, desolubilisation and deposition of heavy metals: metal uptake by immobilised cells and application to the detoxification of liquid wastes. Adv Biotechnol Proc 12:159–172

Marzan LW, Hossain M, Mina SA, Akter Y, Chowdhury AMMA (2017) Isolation and biochemical characterization of heavy-metal resistant bacteria from tannery effluent in Chittagong city, Bangladesh: bioremediation viewpoint. Egypt J Aquat Res 43(1):65–74. https://doi.org/10.1016/j.ejar.2016.11.002

Moodley A, Kot W, Nälgård S, Jakociune D, Neve H, Hansen LH et al (2019) Isolation and characterization of bacteriophages active against methicillin-resistant staphylococcus pseudintermedius. Res Vet Sci 122:81–85. https://doi.org/10.1016/j.rvsc.2018.11.008

Muthukumaran P, Arvind J, Priya MN, Divya R, Indhumati E, Keerthika C (2014) Biosorption and screening of lead (Pb) tolerant indigenous Staphylococcus aureus isolated from tannery effluent contaminated site. Res Rev J Biotechnol 4(2).

Rai LC, Gaur JP, Kumar HD (1981) Phycology and heavy metal pollution. Biol Rev 56(2):99–151. https://doi.org/10.1111/j.1469-185X.1981.tb00345.x

Raja CE, Anbazhagan K, Selvam GS (2006) Isolation and characterization of a metal resistant *Pseudomonas aeruginosa* strain. World J Microbiol Biotechnol 22(6):577–585. https://doi.org/10.1007/s11274-005-9074-4

Chapter 4
Characterization of Chlorpyrifos Degrading *Pseudomonas* sp. Isolated from Contaminated Soil

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Abstract Chlorpyrifos is chemically known as O,O- diethyl-O-(3,5,6-trichloro-2-pyridyl) phosphorothioate belongs to organophosphorous pesticide. Usage of pesticide is still problematic and cause drastic changes in the ecosystem due to its moderate toxicity. The soil contaminated with chlorpyrifos was used to isolate CP degrading bacteria by standard pure culture technique. After isolation, the identified isolates were *P. aeruginosa* and *P. flourescens* and their pure cultures stored for further degradation studies. In nutrient broth and MSM medium, the growth of bacteria was observed by turbidometric measurements with different concentration of pesticides. The cultures of chlorpyrifos degrading bacteria were centrifuged for 20 min at 5000 rpm and the supernatants were collected. The collected supernatants subjected to analysis of UV-Vis spectrophotometer at 300 nm to find out the maximum absorption spectra. The control was used as a blank. *P. aeruginosa* and *P. flourescens* has the range of different values at different concentration of pesticides when absorbed at the maximum spectra of 300 nm. In *P. aeruginosa*, the chlorpyrifos residue was recorded in the 0.5c followed by 1.0c, 1.5c, 2.0c and 2.5c. The highest chlorpyrifos residue was obtained in 0.5c and 1.0c. In *P. flourescens*, the highest chlorpyrifos was recorded in the 0.5c, followed by 1.03c, 1.5c, 2.0c and 2.5c. The highest chlorpyrifos residue was obtained in 0.5c and 1.0c. After the degradation studies by UV-Vis spectrophotometer analysis, comparatively *P. aeruginosa* have shown the higher degradation of chlorpyrifos. The compound present in the treated sample after degradation of chlorpyrifos was separated and identified by GC-MS analysis. The chlorpyrifos was detected at Retention Time (RT) of 11.27 and the compound was identified as Thienol-3,5 Pyridine. This compound confirmed the degradation of chlorpyrifos by *Pseudomonas* sp.

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Abbreviations

CP	Chlorpyrifos
GC–MS-Gas	Chromatography-Mass Spectroscopy
MSM	Mineral Salt Medium
UV–Vis	Ultraviolet—Visible
Nm	Nanometer
RT	Retention Time
OD	Optical Density
µl	Microliter
ml	Milliliter
hrs	Hours
CFU	Colony Forming Unit
rpm	Rotation Per Minute
IMViC	Indole, Methylred, Voges Proskauer and Citrate Test
TSI	Triple Sugar Iron

4.1 Introduction

India is a country that relies overwhelmingly on agriculture; about 60–70% of the population work in this sector. Industries are rapidly occupying a significant percentage of arable land that is presently being cultivated. A substantial rise in agricultural output has been produced by the employment of modern technologies and agricultural inputs including fertiliser, hybrid seeds, and pesticides. The widespread usage of pesticides over time has led to issues brought on by their interactions with the environment's biological system. Based on their chemical composition, pesticides are synthesized in a wide range of forms, including organochlorine pesticides, organophosphorus pesticides, carbamate, pyrethroids, and biopesticides. Organophosphates are a significant component of herbicides, insecticides, pesticides and nerve gas. The ester derivatives of phosphoric acid are known as OPs, which undergo degradation quite quickly and are thought to be safest for use in agriculture. OPs, which are the ester forms of phosphoric acid, are normally regarded as acceptable for use in agriculture due to their relatively quick rates of degradation (Renita et al. 2021). Because of the prohibition of organochlorine pesticides like lindane and dichlorodiphenyltrichloroethane (DDT), the dependency of organophosphorus insecticides in agriculture has increased. Pesticides are poisonous agents that are purposefully introduced into the ecosystem to eradicate living organisms like insects

(insecticides), weeds (herbicides), rodents (rodenticides), fungi (fungicides), and others. Insecticides are generic poisons that affect more than just the "pests" they are intended to kill. Nearly 90% of pesticides never actually affect target species; rather, contaminates soil and water with an impact on unintended organisms (Vidali 2001). Organophosphate pesticides have an adverse effect on non-target plants and non-target animal species that inhabiting an extensive range of aquatic and terrestrial habitats (Blann et al. 2009).

Organophosphorous pesticides are a major and common category of pesticides now in use throughout, contributing for greater than 36% of the global market (Kanekar et al. 2004). Pesticide exposure not only has a lot of negative health consequences and connected to a number of significant human illnesses and diseases, ranging from respiratory issues to cancer. The signs of this pesticide exposure include nausea, blurred vision, breathing issues, sweating, headaches, slow heartbeats, numbness, and cramps in the abdomen (Vidya Lakshmi et al. 2009). In 2001, the United States Environmental Protection Agency (EPA) controlled the use of chlorpyrifos to specific crops and outlawed its residential usage in the country due to its toxicity (Eaton et al. 2008).

Chlorpyrifos is an agricultural insecticide used globally against various pests and insects. Based on the soil nature and texture, CP has a half-life varying between 10 and 120 days in the soil that undergoes degradation slowly in soil, arising a major environment and public health threats (Silva 2020). This chemical has broad spectrum action on the insect nervous system and cause mortality by inhibiting the important acetylcholine esterase enzyme. In humans, severe CP poisoning cases shown symptoms such as headache, nausea, dizziness, muscle twitching, weakness, increased sweating, and salivation (Ambreen and Yasmin 2021). It also shows immunotoxic effects on humans and animals. When these pesticides build up in agricultural fields, they permeate into the nearby water bodies and interrupt the metabolic processes of aquatic plants and animals. The persistent use of CP has generated numerous issues in environmental and biological systems. As a result, it is critical to remove chlorpyrifos (CP) from contaminated surroundings. CP detoxification is accomplished by physical, chemical, and biological techniques (Huang et al. 2021). A number of physicochemical techniques for removing chemicals from the environment, such as photodecomposition, chemical treatment, volatilization, and combustion are followed. These methods are ineffective for totally destroying CP and its metabolic degradation products (Natarajan et al. 2021).

Chlorpyrifos must be safely degraded by microorganisms in order to avoid leaving hazardous residues. Different types of bacteria can break down various pesticides, herbicides and insecticides (Munazza 2005). The technique of bioremediation involves employing microorganisms to transform hazardous or poisonous complex organic substances into smaller, non-toxic inorganic molecules (Pang et al. 2020). The persistence of most pesticides in soil is impacted by microbial activities. Toxic chemical removal from the environment through microbial biodegradation is a very appealing strategy. A simple, affordable, and eco-friendly method for removing CPF from soil and water is bioremediation (Rayu et al. 2017).

Microbial remediation effectively removes pollutants from the environment using bacteria, fungi, or algae. Many bacteria from the genera Pseudomonas, Alcaligenes, Bacillus, Xanthomonas, Enterobacter, and Ochrobactrum have been found and characterised as being capable of degrading CPF (Huang et al. 2021). According to reports, algae and fungi may also effectively break down TCP and chlorpyrifos. The major result of CPF's degradation in the soil is 3,5,6-TriChloro-2-Pyridinol (TCP) (Chen et al. 2012). Pseudomonas is one of them, a bacterial genus that is widely distributed in soil and is essential for the mineralization of organic matter. They can breakdown the majority of pesticides due to their metabolic adaptability (Sarkar et al. 2009).

Pseudomonas species can be used for bioremediation and some of its members are capable of metabolising chemical contaminants in the environment.

Pseudomonas is a diverse genus that includes several catabolic processes and enzymes involved in the breakdown of pesticides. The aim of this work was to identify and characterise CP-degrading microorganisms in order to assess their potential for degradation. In the present study, chlorpyrifos degrading *P. aeruginosa* and *P. flourescens* was isolated from chlorpyrifos contaminated soil. The growth of chlorpyrifos degrading *Pseudomonas* was assessed. The breakdown of chlorpyrifos by *Pseudomonas* was characterized by UV-Vis spectrophotometer. For further confirmation of chlorpyrifos degradation, the presence of breakdown product by GC-MS analysis was studies.

4.2 Materials and Methods

4.2.1 Collection of Soil Samples

The collection of soil samples was done randomly in the chlorpyrifos contaminated agricultural field, Erode District, Tamil Nadu, India. The selected agricultural field is regularly applied with agrochemicals for crop cultivation. The surface soil and debris layer were removed, at 10 cm depth soil samples were collected and transferred into a sterile container. The collected soil sample were transported to the laboratory and stored at 4 °C for further experiments (Singh et al. 2004).

4.2.2 Isolation of Chlorpyrifos Degrading Bacteria

From the collected soil sample, 1 g soil was weighed and serially diluted by the following standard microbiological plating methods. For the isolation of CP degrading bacteria, serially diluted samples were plated on the nutrient agar plate amended with 0.1% chlorpyrifos by spread plate technique. The inoculated plates were subjected to incubation at 37 °C for 24 to 48 h (Reetha and Kavi Karunya

2012). Then the samples were plated on the *Pseudomonas fluorescens* isolation agar plate by spread plate technique. After incubation duration for 24 h at 37°, bacterial colonies appeared. Further, the colonies were streaked into nutrient agar (NA) plates and sub-cultured sequentially to obtain pure colonies (Sharma et al. 2017).

4.2.3 Identification of CP Degrading Bacteria

The colonies were subjected to morphological and biochemical characterization by following Bergey's Manual of Determinative Bacteriology (Holt 1994). Colony characteristics used for identification include size, shape, color, margin, and elevation. Besides, the bacterial cells were stained and visualized under a compound microscope, supported by the Gram Staining Technique (Rayu et al. 2017). Subsequently, biochemical tests were performed, including Indole, Methyl-Red (MR), Voges Proskauer (VP), Citrate Utilization, Catalase, Oxidase, Urease, Lactose fermentation, and Hydrogen sulphide production.

4.2.4 Enrichment Technique and Isolation

Enrichment culture technique (Barathidasan et al. 2014) was followed to isolate potential CP degrading bacteria using Minimum Salt Medium (MSM). MSM composition as follows (in grams per liter of distilled water): Ammonium Nitrate ($(NH_4)_2NO_3$)—1 g; Hydrated Calcium Nitrate ($Ca(NO_3)_2{\cdot}2H_2O$)—0.04; Hydrated Magnesium Sulphate ($MgSO_4{\cdot}7H_2O$)—0.1 g; Potassium Chloride (KCl)—0.2 g; Hydrated Iron (ii) sulfate ($FeSO_4{\cdot}7H_2O$)—0.001, Dipotassium phosphate ($K_2HPO_4{\cdot}12H_2O$)—1.5; and Potassium dihydrogen phosphate (KH_2PO_4)—4.8. The pH of the above medium was adjusted to 7 using a Benchtop pH meter. The degradation efficiency of bacteria was compared in two different media such as MSM and Nutrient broth amended with CPD. In MSM, chlorpyrifos is the only carbon source for utilization of bacteria (Kumar 2011). The prepared Mineral Salt Medium (MSM) was sterilized in an autoclave at 121 °C for 15 min at 15 lbs (Sharma et al. 2016). The medium was allowed to cool. The different concentrations of chlorpyrifos were added to the tubes aseptically from 0.5 to 2.5%. About 0.1 ml bacterial culture was inoculated into the tubes. Then, the incubation of inoculated tubes was done at 37 °C for 2 days.

The prepared Nutrient Broth (NB) was sterilized in an autoclave at 121 °C for 15 min at 15 lbs. The medium was allowed to cool. The different concentrations of Chlorpyrifos pesticides were added to the tubes from 0.5 to 2.5%. About, 0.1 ml of bacterial culture was inoculated aseptically into the tubes. The incubation of tubes was done at 37 °C for 24–48 h.

4.2.5 UV Spectrophotometric Analysis

When the bacterial cultures were inoculated in the MSM medium lacking carbon source, the bacteria start utilizing alternative carbon source (CP) supplemented in MSM (Kumar 2011). CP degradation in the media was analysed by taking the Optical Density (OD) after centrifugation of the minimal media and nutrient media amended with chlorpyrifos at the absorbance maximum of 300 nm (Kumar et al. 2015).

4.2.6 GC-MS for Determination of Chlorpyrifos Intermediates

The identification of chlorpyrifos by efficient bacterial isolate was analyzed by gas chromatography-mass spectrometry (GC-MS) to identify degradation metabolites. GC-MS was set up with a split-splitless injector and attached to a triple quadruple mass spectrophotometer (Yin et al. 2021). The capillary column was used for the separation (Li et al. 2010). The carrier gas used at a constant flow rate of 1 ml/min was Helium gas (99.999%) and 3 μl injection volume was employed. The injector temperature was kept at 250 °C for 5 min in splitless mode. The oven temperature was set as follows:

- Initial temperature at 100 °C (hold for 2 min)
- 20 °C /min to 180 °C
- 10 °C/ min to 250 °C (hold for 2 min).

The total running time for GC-MS analysis was 15–20 min. Electron ionization and spectra were recorded at 70 eV. The GC-MS interface was placed at 250 °C and the ion source temperature was placed at 200 °C. The total ion current (TIC) chromatograms were recorded ranging from 40 to 500 m/z. The individual constituents of GC report were identified by comparing the Mass Spectra (MS) with the standard library of National Institute for Standard Technology mass spectra (NIST-MS).

4.3 Results and Discussion

The chlorpyrifos degrading and non-degrading bacterial colonies were appeared on the nutrient agar plates. The good numbers of chlorpyrifos degrading bacteria were obtained after 48 h of incubation. The bacterial colonies were obtained from the *Pseudomonas fluorescens* isolation agar plates. At 10^{-4} dilution, 153 CFU/ml was isolated. Isolation and exploration of indigenous microbial strains for *in-situ* detoxification is an environmentally friendly approach (Ortiz-Hern et al. 2010). Indigenous species are preferrable as they do not destructively affect the population and diversity of soil micro-flora (Farhan et al. 2021).

Table 4.1 Morphological characterization of CDB_1 and CDB_2 isolates

Isolates	Colony morphology	Pigmentation	Gram staining	Motility
CDB_1	Mucoid, elevated	Bluish green	Gram negative rods	Motile
CDB_2	Smooth convex glistening	Yellowish green	Gram negative rods	Motile

The isolates tolerated to grow on chlorpyrifos containing media were selected for screening. For pure culture, the colonies were picked up from the plates and sub-cultured by quadrant streak plate method. After overnight incubation, the culture was stored at 4 °C.

The isolated bacteria were subjected to morphological characterization by following standard staining procedures. The dominating colonies isolated were mucoid and elevated with appearing bluish green pigmentation. Other colonies were showing smooth, convex, glistening with yellowish pigmentation. The staining procedures confirmed the bacterial cells as Gram negative rod shaped. The motility test by hanging drop technique showed the isolates as motile bacteria. The results are represented in the Table 4.1.

The biochemical tests were performed for the identification of the organisms. The Indole test, Methyl Red (MR), Voges-Proskauer (VP) and citrate test showed the negative result for both CDB_1 and CDB_2 isolates. The catalase and oxidase test showed the positive result for both isolates. The TSI test showed negative result for CDB_1 and CDB_2.

Both the isolates were subjected to partial identification based on various biochemical tests with reference to the Bergey's Manual of Determinative Bacteriology. The isolates were identified as a member of *Pseudomonas* sp. and it was identified that CDB_1 as *Pseudomonas aeruginosa* and CDB_2 as *Pseudomonas fluorescens.* However, the results are like those which were obtained by concerning the identification of *Pseudomonas* sp. The results are represented in the Table 4.2.

After the incubation period, the nutrient and MSM medium at different concentration of pesticides, the turbidity was observed. The cultures were centrifuged at 5000 rpm for 20 min and the supernatant were collected. Spectral analysis was made using UV-Vis Spectrophotometer and the cell free extract was scanned in the range of 300 nm to find out the maximum absorption of spectra (Table 4.3). The control was used as a blank (Reetha and Kavi Karunya 2012; Kumar et al. 2015).

P. aeruginosa and *P. flourescens* had the range of different values at different concentration of pesticides when absorbed at the maximum spectra of 300 nm. The CP degrading *P. aeruginosa* had shown the maximum degradation when incorporated

Table 4.2 Biochemical characterization of CDB_1 and CDB_2 isolates

Isolates	Indole	Methyl red	Voges Proskauer	Citrate	Catalase	Oxidase	Gelatin	TSI		
								G	L	S
CDB_1	−	−	−	+	+	+	+	−	−	−
CDB_2	−	−	−	+	+	−	+	−	−	−

Table 4.3 UV-Vis spectrophotometer analysis of chlorpyrifos degrading *Pseudomonas*

Chlorpyrifos concentration	*P. aeruginosa*		*P. fluorescens*	
	Nutrient broth (OD at 300 nm)	MSM medium (OD at 300 nm)	Nutrient broth (OD at 300 nm)	MSM medium (OD at 300 nm)
Control	0.00	0.00	0.00	0.00
0.5	4.00	1.48	4.00	1.50
1.0	4.00	1.28	3.69	1.02
1.5	3.77	0.44	0.50	0.55
2.0	1.27	0.38	0.43	0.22
2.5	0.60	0.37	0.35	0.21

with 0.5c of chlorpyrifos after 2 days of incubation period. The results were showed in the Table 4.3. In case of *P. fluorescens*, the degradation of the chlorpyrifos pesticide was low comparing to *P.aeruginosa* under similar culture condition. For degradation of chlorpyrifos, MSM medium found suitable by both isolates when compared to nutrient broth. The change in the biodegradation rate depends on the concentration of the pesticides, substrate availability, incubation period and the ability of bacteria. The chlorpyrifos degradation by *P. aeruginosa* was further subjected to GC-MS analysis (Fig 4.1).

In the present study, GC-MS was used to identify degradation products of chlorpyrifos. The GC-MS is the most sensitive method to identify the degradation products of organochloropesticides (Farhan et al. 2021). After the degradation studies by UV-Vis Spectrophotometer analysis, comparatively the isolate I have given the higher degradation spectrum of chlorpyrifos. The compound present in the treated sample after degradation of chlorpyrifos was separated and identified by GC-MS analysis and it was showed in the Table 4.4 (Barathidasan et al. 2014).

The degradation of chlorpyrifos was confirmed by GC MS analysis. The chlorpyrifos was detected at Retention Time (RT) of 11.27 min. The reproducibility was confirmed using an injector (Kumar 2011). The cell free extracts of samples from the culture medium were subjected to GC MS analysis. This analysis was done to confirm the presence of degradative products of chlorpyrifos by microbial activity. The results of the present study represent that *P. aeruginosa* was known to degrade even high concentration of chlorpyrifos (2.5c). The active degradation of chlorpyrifos was obtained in 0.5c and 1.0c by *P. aeruginosa.* In *P. flourescens*, the highest chlorpyrifos degradation was recorded in the 0.5c, followed by 1.0c, 1.5c, 2.0c and 2.5c. The highest chlorpyrifos residue was obtained in 0.5c and 1.0c. The main potential CP degraders belonging to *Pseudomonas* sp. It has the potential to make use of CP when provided as sole carbon source (Amani et al. 2019).

By comparing both isolates, *Pseudomonas aeruginosa* have the higher degradation of chlorpyrifos (Manigandan and Nelson 2015). *Pseudomonas* sp. degrades many pesticides like Alachlor, Chlorprophan, DDT etc. and the residues found as Thienol 3,5 Pyridine, 3,4 Dihydroxy 1,6 bis (3-methoxy-phenyl)-hexa-2,4diene,

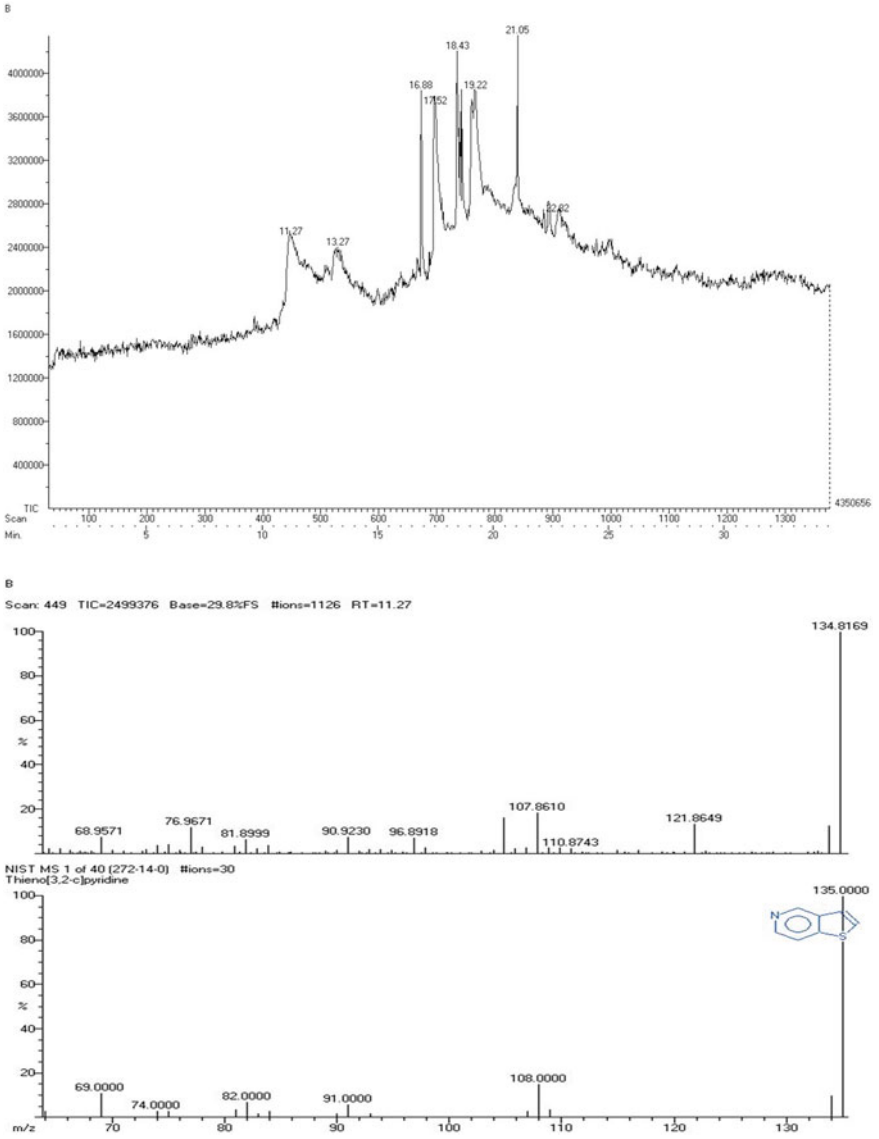
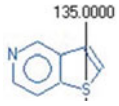
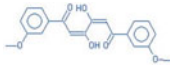
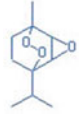


Fig. 4.1 Chromatogram showing Chlorpyrifos degradation by GC-MS Analysis

Oleic acid, n-hexadecanoic acid and Iso propyl stearate. The chlorpyrifos degradation was detected at Retention Time (RT) of 11.27 and the compound was identified as Thienol-3,5 Pyridine.

Although just a few species have been demonstrated to breakdown the compounds due to their complexity, bacteria and fungi are the primary transformers. Using microorganisms to immobilise or decompose contaminants is a common method

Table 4.4 GC-MS analysis of the treated sample

Compound name	Retention time	TIC	Structure
Thienol 3,5 pyridine	11.27	2,499,376	
3,4 dihydroxy-1,6-bis-[3-methoxy-phenyl]hexa-2,4-diene-1,6 diene	22.82	2,715,088	
n-hexadecanoic acid	17.52	3,702,304	
Isopropyl stearate	21.05	4,350,656	
Ascardiol epoxide	13.47	2,401,136	
Dodecanoic acid	16.88	3,841,936	
Oleic acid	18.43	4,204,384	

of removing them, and this process is known as bioremediation. The potential for these compounds to enter animals and subsequently human consumers of animal products makes biodegradation of OPs in the soil and water crucial (Chapman et al. 1986). Yadav et al. (2016) reported that bacteria contain catabolic genes that allow them to thrive in a variety of ecological niches at varying pH, temperature, and oxygen concentrations, which enables them to digest contaminants effectively.

From the study, it is concluded that CP undergoes degradation by many soil microorganisms. Bacterial degradations of CP are dependent on culture conditions, isolates and species of bacteria involved (Singh and Walker 2006). Pseudomonas sp. strains have been found to effectively breakdown the pesticide and its primary metabolite both in the absence and presence of external nutritional supplements, according to study on bacteria with the ability to degrade (Bose et al. 2021). Future research is proposed to investigate and develop the strains obtained in the current study for enhanced bioremediation of CP in soils. A bacterial consortium that uses symbiotic relationships among the strains to degrade CP must be developed. To improve our knowledge of the biodegradation process, additional research should focus on numerous biochemical factors, genes, and enzymes.

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References

Amani F, Student P, Akbar A, Sinegani S, Ebrahimi F, Nazarian S (2019) Biodegradation of chlorpyrifos and diazinon organophosphates by two bacteria isolated from contaminated agricultural soils. Biol J Microorg 7:27–39

Ambreen S, Yasmin S (2021) Novel degradation pathways for Chlorpyrifos and 3, 5, 6-Trichloro-2-pyridinol degradation by bacterial strain *Bacillus thuringiensis* MB497 isolated from agricultural fields of Mianwali. Pakistan Pestic Biochem Physiol 172:104750. https://doi.org/10.1016/j.pestbp.2020.104750

Barathidasan K, Reetha D, Milton DJ, Sriram N, Govindammal M (2014) Biodegradation of chlorpyrifos by co-culture of *Cellulomonas limi*. Afr J Microbiol 8:961–966. https://doi.org/10.5897/AJMR2013.6530

Blann KL, Anderson JL, Sands GR, Vondracek B (2009) Effects of agricultural drainage on aquatic ecosystems: a review. Crit Rev Env Sci Tech 39:909–1001. https://doi.org/10.1080/10643380801977966

Bose S, Senthil Kumar P, Dai-Viet NV (2021) A review on the microbial degradation of chlorpyrifos and its metabolite TCP. Chemosphere 283:131447. https://doi.org/10.1016/j.chemosphere.2021.131447

Chapman RA, Harris CR, Moy P, Henning K (1986) Biodegradation of pesticides in soil rapid degradation of isofenphos in a clay loam after a previous treatment. J Environ Sci Health B 21:269–276. https://doi.org/10.1080/03601238609372523

Chen S, Liu C, Peng C, Liu H, Hu M, Zhong G (2012) Biodegradation of chlorpyrifos and its hydrolysis product 3,5,6-trichloro-2-pyridinol by a new fungal strain *Cladosporium cladosporioides* Hu-01. PLoS ONE 7(10):e47205. https://doi.org/10.1371/journal.pone.0047205.g001

Eaton DL, Daroff RB, Autrup H, Bridges F, Buffler P, Costa LG, Coyle J, McKhan G, Mobley WC, Nadel L, Neubert D, Schulte-Hermann R, Spencer PS (2008) Review of toxicology of chlorpyrifos with an emphasis on human exposure and neurodevelopment. Crit Rev Toxicol 38:1–125. https://doi.org/10.1080/10408440802272158

Farhan M, Ahmad M, Kanwal A, Butt ZA, Khan QF, Raza SA, Qayyum H, Wahid A (2021) Biodegradation of chlorpyrifos using isolates from contaminated agricultural soil, its kinetic studies. Sci Rep 11:10320. https://doi.org/10.1038/s41598-021-88264-x

Holt JG (1994) Bergey's manual of determinative bacteriology, 9th edn. Lippincott Williams and Wilkins, Baltimore

Huang Y, Zhang W, Pang S, Chen J, Bhatt P, Mishra S, Chen S (2021) Insights into the microbial degradation and catalytic mechanisms of chlorpyrifos. Environ Res 194:110660. https://doi.org/10.1016/j.chemosphere.2021.130500

Kanekar PP, Bhadbhade B, Deshpande NM, Sarnaik SS (2004) Biodegradation of organophosphorus pesticides. Proc Ind Nat Sci Acad 70:57–70

Kumar S (2011) Bioremediation of chlorpyrifos by bacteria isolated from the cultivated soils. Int J Pharma Bio Sci 2:359–366

Kumar V, Niraj U, Virender K, Sitansh S (2015) A review on sample penetration and chromatographic determination of acephate and methamidophos in different sample. Arabian J Chem 8:624–331. https://doi.org/10.3390/biom10030433

Li X, Gan P, Peng R, Huang C, Yu H (2010) Determination of 23 organophosphorous pesticides in surface water using SPME followed by GC–MS. J Chromatographic Sci 48:183–187. https://doi.org/10.1093/chromsci/48.3.183

Manigandan G, Nelson R (2015) Isolation and molecular characterization of lamda Cyhalothrin pesticide degrading organisms. Adv Appl Sci Res 6:13–18

Munazza A (2005) Chlorpyrifos resistant bacteria from Pakistani soils: isolation, identification, resistance profile and growth kinetics: Pak. J Bot 37:381–388

Natarajan R, Banerjee K, Kumar PS, Somanna T, Tannani D, Arvind V et al (2021) Performance study on adsorptive removal of acetaminophen from wastewater using silica microspheres: kinetic and isotherm studies. Chemosphere 272:129896. https://doi.org/10.1016/j.chemosphere.2021.129896

Ortiz-Hernández ML, Sánchez-Salinas E, Olvera-Velona A, Folch-Mallol JL (2011) Pesticides in the environment: impacts and its biodegradation as a strategy for residues treatment. In: Stoytcheva M (ed) Pesticides—formulations, effects, fate. Londo, IntechOpen. https://www.intechopen.com/chapters/13026, https://doi.org/10.5772/13534

Pang S et al (2020) Insights into the microbial degradation and biochemical mechanisms of neonicotinoids. Front Microbiol 11:868. https://doi.org/10.3389/fmicb.2020.00868

Rayu S, Nielsen UN, Nazaries L, Singh BK (2017) Isolation and molecular characterization of novel chlorpyrifos and 3, 5, 6-trichloro-2-pyridinol-degrading bacteria from sugarcane farm soils. Front Microbiol 8:518. https://doi.org/10.3389/fmicb.2017.00518

Reetha D, Kavi Karunya S (2012) Efficiency of bacterial isolates in the degradation of malathion and parathion. Int J Pharm Biol Arch 3:659–665

Renita AA, Vardhan KH, Kumar PS, Ngueagni PT, Abilarasu A, Nath S, Kumari P, Saravanan R (2021) Effective removal of malachite green dye from aqueous solution in hybrid system utilizing agricultural waste as particle electrodes. Chemosphere 273:129634

Sarkar S, Seenivasan S, Prem Kumar R, Asir S (2009) Biodegradation of propargite by *Pseudomonas putida* isolated from tea rhizosphere. Hazard Mat 174:295–298. https://doi.org/10.17221/2184-pse

Sharma B, Sonika S, Aparna D, Sudipti A (2016) Spectrometric analysis of degradation if Chlorpyrifos pesticide by indigenous microorganisms isolated from affected soil. Int J Curr Microbial Appl Sci 5:742–749. https://doi.org/10.20546/ijcmas.2016.509.085

Sharma AK, Kasture A, Pandit J, Shirkot P (2017) Isolation, characterization and identification of chlorpyrifos tolerant Pseudomonas strain exhibiting extracellular organo-phosphorus hydrolase (OPH) activity from apple orchard soils of Himachal pradesh. Asian J Microbiol Biotechnol Env Sci 19(4):935–944

Silva MH (2020) Effects of low-dose chlorpyrifos on neurobehavior and potential mechanisms: a review of studies in rodents, zebrafish, and *Caenorhabditis elegans*. Birth Defects Res 112:445–479. https://doi.org/10.1002/bdr2.1661

Singh B, Walker A (2006) Microbial degradation of organophosphorus compounds. FEMS Microbiol Rev 30:428–471. https://doi.org/10.1111/j.1574-6976.2006.00018.x

Singh BK, Walker A, Morgan JA, Wright DJ (2004) Biodegradation of chlorpyrifos by Enterobacter strain B-14 and its use in bioremediation of contaminated soils. Appl Env Microbiol 70:4855–4863. https://doi.org/10.1128/AEM.70.8.4855-4863.2004

Vidali M (2001) Bioremediation: an overview. Pure Appl Chem 73:1163–1172. https://doi.org/10.1351/pac200173071163

Vidya Lakshmi C, Kumar M, Khanna S (2009) Biodegradation of chlorpyrifos in soil by enriched cultures. Curr Microbiol 58:35–38. https://doi.org/10.1007/s00284-008-9262-1

Yadav M, Shukla AK, Srivastva N, Upadhyay SN, Dubey SK (2016) Utilization of microbial community potential for removal of chlorpyrifos: a review. Crit Rev Biotechnol 36(4):727–742. https://doi.org/10.3109/07388551.2015.1015958

Yin X, Liu Y, Wen R, Wang Y, Chen Q, Kong B (2021) Characterization of selected Harbin red sausages on the basis of their flavour profiles using HS-SPME-GC/MS combined with electronic nose and electronic tongue. Meat Sci 172:108345. https://doi.org/10.1016/j.meatsci.2020.108345

Chapter 5
Studies on the Effects of Metribuzine on *Phormidium* and *Chroococcus* Species

S. Balakumar and N. Mahesh

Abstract The efficacy of metribuzine, a selective herbicide recommended for comprehensive weed control in rice, was investigated on *Phormidium* and *Chroococcus* species, common species of cyanobacteria obtained from the National Marine Cyanobacterial Facility (NFMC). A comparative study of the effects of metribuzine on both species of cyanobacteria measures their chlorophyll-a content and biochemical levels such as carbohydrate, protein, amino acid and lipid levels over 18 days of time- and dose-dependent exposure. was evaluated under laboratory conditions by Results showed that the herbicide exerted pleiotropic effects on both cyanobacterial species over the concentration range tested (2–10 μg). Chlorophyll a was more adversely affected by metribuzine at the highest concentration (10 μg) on day 18. On day 12, more than 50% inhibition of cyanobacterial growth was observed when 10 μg of metribuzine was applied. Carbohydrate, protein, amino acid, and lipid levels were reduced with herbicide, but by day 18, effects were noticeable at the highest concentrations for both cyanobacteria. Metribuzine concentrations in the 2 μg range also affected chlorophyll a and biochemical content in both cyanobacteria. *Phormidium* species were more affected by metribuzine than *Chroococcus* species. Since, *Phormidium and Chroococcus* species are abundant in paddy fields and can be used as inoculum for rice biofertilizer programs, protecting them from the potential residual effects of herbicides is critical to correctly managing local soil fertility.

Keywords *Phormidium* species · *Chroococcus species* · Metribuzin · Inhibitory action · Paddy fields

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5.1 Introduction

Although cyanobacteria are essentially microscopic, large colonies and mats are highly visible. They are a large, morphologically diverse group of photosynthetic prokaryotes that are involved in oxygenic photosynthesis and include unicellular to multicellular microorganisms found in nearly every habitat on earth (Carr and Whitton 1982; Vermass 2001). Cyanobacteria have a similar appearance to algae and have similar light, nutrient and carbon dioxide requirements. Even without light, some cyanobacteria can survive and grow using chemicals from the environment. They store carbon in different forms such as starches, oils and various sugars. Their main photosynthetic pigment is chlorophyll a (chla), and other photosynthetic pigments in cyanobacteria are various phycobiliproteins (phycoerythrin, phycocyanin, allophycocyanin) and carotenoids (beta-carotene, echinenone, myxoxanthophyll). Because of these later pigments and mucus, the color of cyanobacteria in nature can range from dirty yellow to various shades of blue-green, brown or black (Whitton and Potts 2000). Some species of the genus have small gas sacs inside their cells that float to the surface or sink to the bottom, depending on changing light and nutrient availability.

Cyanobacteria also live in a variety of terrestrial environments. Soil is one of the most potential habitats for algae, especially in moist or damp conditions. Cyanobacteria are one of the major constituents of nitrogen-fixing biomass in paddy fields. The agricultural importance of cyanobacteria in rice cultivation is related to their ability to fix atmospheric nitrogen on plants and soil (Whitton 2000; Silva and Silva 2007). Some genera of heterocysts are diazotrophic and can use atmospheric nitrogen as the sole nitrogen source, thus contributing to light-dependent nitrogen fixation in rice paddies worldwide, maintaining and maintaining soil fertility. It plays an important role in building. In addition to heterocyst nitrogen fixation, some non-heterocysts fix atmospheric nitrogen under microaerobic conditions. Some non-heterocyst forms often crusts in soil. Crust formation impedes soil erosion, increases rainwater retention, and reduces evaporative water loss (Thajuddin and Subramanian 2005).

The use of herbicide may have harmful effects on aquatic life. Agricultural runoff during rainfall often concentrates in lakes and ponds (Richards and Baker 1993). Cyanobacteria are one of the potential organisms that could benefit humanity in many ways. Cyanobacteria represent a vast potential resource in a variety of applications, including aquaculture, food, feed, fuel, fertilizer, pharmaceuticals, industry and environmental protection (Patterson 1996).

The aim of this study to investigate the adverse effect of herbicide metribuzine on *Phormidium* sp. and *Chroococcus* sp., found in the paddy field and the conservation of local soil fertility. Therefore, this study may help to improve the implementation of effective integrated rice pest management and likely to succeed for future use of cyanobacteria in rice fields.

5.2 Materials and Methods

5.2.1 Cultivation of Cyanobacteria

Filamentous and rounded diazotrophic cultures of the cyanobacteria, *Phormidium* sp. and *Chroococcus* sp., were obtained from NFMC. Purchased cultures were propagated in artificial synthetic nutrient III (ASN III) broth and placed in a rotary shaker at room temperature. The inoculated plates were regularly examined for cyanobacterial growth. Grown cultures were sub-cultured periodically and stored at 4 °C for future use.

5.2.2 Herbicide Stock Solution

Herbicide stock solution was prepared by dissolving 1 mg of herbicide in 100 ml of sterile ASN III medium to obtain a stock solution. Solutions of various concentrations were prepared from this herbicide stock solution as 2, 4, 6, 8, 10 μg/ml respectively.

5.2.3 Herbicide Efficacy

To test herbicide efficacy against the cyanobacterial species *Phormidium* sp and *Chroococcus* sp., were selected and treated with different herbicide concentrations. The required amount of herbicide was added to the liquid culture medium after sterilization. A volume of 0.5 ml of exponentially growing culture was then added equally to each different set of replicates (100 ml). Morphological changes supplemented with different herbicide concentrations were regularly observed. Chlorophyll a content and biochemical content were measured at individual day (6, 9, 12, 15, and 18) intervals. Experiments were performed in duplicate for 18 days under controlled conditions.

5.2.4 Determination of Chlorophyll a

The isolates were centrifuged at 5000 rpm for 10 min and the pellet was suspended in 4 mL of 80% methanol. The tubes were sealed to prevent solvent evaporation and incubated at 60 °C in the dark for 1 h in shaker. The contents were cooled and centrifuged at 5000 rpm for 5 min. The supernatant was colleceted and repeated again for more pigment extraction. The supernatant was made up to a known volume with 80% methanol. Absorbance was measured at 663 nm.

5.2.5 Carbohydrate Estimation

The cultures were centrifuged at 5000 rpm for 10 min and 100 mg of the pellet was placed in a test tube and hydrolyzed with 2 ml of concentrated H_2SO_4 at 100 °C for 30 min. 1 mL of 5% phenol and 5 mL of concentrated H_2SO_4 were added to 0.5 mL of hydrolyzate and mixed well. Color development was measured at 490 nm using a spectrophotometer. Glucose was used as standard.

5.2.6 Protein Estimation

The 100 mg of the pellet collected after centrifugation was suspemded with 10% TCA and centrifuged at 10,000 rpm for 10 min. The pellet was resuspended in 1N NaOH, boiled for 30 min, then re-centrifuged to remove light scattering material. Supernatant was made up to a known volume. To this 0.9 ml of distilled water and 5 ml of alkaline copper reagent were added to 0.1 ml of supernatant, left for 10 min, and finally 0.5 ml of Folin-Ciocalteu reagent was added. After 30 min, absorbance was read at 750 nm and protein levels were calculated using a standard chart made using bovine serum albumin.

5.2.7 Amino Acid Deduction

The 100 mg of the pellet after centrifugation was homogenized in 80% ethanol. The clear supernatant was adjusted to a known volume after centrifuged at 5000 rpm. 1 ml of this was pipetted into a test tube and diluted to 4 ml with distilled water. To this 1 ml of ninhydrin reagent was added and kept in a boiling water bath for 15 min. The tube was then cooled and 1 mL of 50% ethanol was added. The induced violet color was measured with a spectrophotometer at 540 nm.

A standard plot was made using a mixture of alanine, aspartic acid, tryptophan, proline, and lysine.

5.2.8 Lipid Estimation

The 100 mg of the pellet was homogenized and extracted by solvent (chloroform:methanol 2:1 (v/v)) and filtered by Whatman paper. The filtrate was vortexed with sodium sulfate to remove moisture. It was then collected in pre-weighed bottles and left overnight at room temperature in the dark. Total lipids were estimated by weighing the dried extract.

5.3 Results and Discussion

Successful rice farming is progressively reliant on the application of herbicides to remove unwanted weeds. However, heavy use of herbicides raises the level of chemical residues to levels that can inhibit the growth of native microbes such as cyanobacteria. Anything that affects these primary producers can affect the ecological balance of the entire paddy agroecosystem. Share the characteristics of This susceptibility of cyanobacteria to herbicides varies, but mainly depends on the type and type of herbicide. Evaluation of the toxic effect of metribuzine on cyanobacterial abundance and distribution shows progressive inhibition of cyanobacterial growth recorded compared to controls. These inhibitory effects could be attributed to the lethal effect of metribuzine on cyanobacterial growth and biochemical content. These results are consistent with those of Singh et al. (1983) reported that the thiocarbamate his benchocarb inhibited the growth of his Nostoc linckia. Similar results were also reported by Metting (2008), who found that the growth and dry weight of Anabaena oryzae and Auroscilla fertilisima were significantly reduced by the application of parathion. The herbicide metribuzine binds to soil components immediately after application, so cyanobacteria are constantly exposed to potential hazards from these herbicides (Table 5.1) (Gustavson et al. 2003).

Herbicides interfere with photosynthetic pigment synthesis by inhibiting perphyrin formation and biosynthetic steps in carotenoid synthesis, ultimately leading to chlorosis and precursor accumulation (Mohapatra et al. 1996). Such accumulation causes the necessary blockade of cell junctions that further impairs metabolic efficiency.

Cyanobacterial growth was measured in terms of chlorophyll 'a'. Chlorophyll a was recorded at 37.10 mg/mL, 32.57 mg/mL and 52.08 mg/mL, 44.26 mg/mL in the *Phormidium* and *Chroococcus* sp. controls on days 6 and 18, respectively. Concurrently, a loss of 5.45 and 34.60 mg/ml chlorophyll a content was observed in his *Phormidium* species treated with the lowest concentration (2 μg/ml) of metribuzine on days 6 and 18. At the highest concentration (10 μg/ml), a loss of chlorophyll a content of 24.03 and 51.62 mg/ml was observed in metribudine-treated formidium species on days 6 and 18 (Table 5.2; Fig. 5.1). In *Chroococcus* species treated with metribuzine, a loss of 5.61 and 30.

Chlorophyll a levels of 64 mg/ml were recorded at the lowest concentration (2 μg/ml) on days 6 and 18, respectively. At the highest concentration (10 μg/ml), a loss of chlorophyll a content of 25.12 and 43.52 mg/ml was recorded on days 6 and 18 (Table 5.2; Fig. 5.1). Similar observations were reported in.

Anabaena cylindrica, with the herbicides bentazone and molinat, which decreased chlorophyll a levels with increasing herbicide concentrations (Galhano et al. 2009). Yan et al. (1997) confirmed a decrease in growth rate of Anabaena sphaelica with increasing molinate concentration. Eladel et al. (1999) and Battah et al. (2001) also reported that thiobencarb induced a decrease in the specific growth rate of Anabaena he variabilis and significantly reduced its biomass yield. Kobbia et al. (2001) all tested concentrations (0.2–0.8 mg/l) photosynthetic inhibitors of simazine, triazine,

Table 5.1 Physico-chemical properties of metribuzin (Maria Diva et al. 1998)

Herbicide	Chemical family and chemical abstract service number	Chemical name	Chemical formula	Molecular weight ($g.mol^{-1}$)	Melting point (ºC)
Metribuzin	S-triazine CAS no: 21097–64-9	(4-amino-6-tert-butyl-4,5-dihydro-3-methylthio-1,2,4-triazine-5-one)	$C_8H_{14}N_4OS$	214.28	125.5–126.5

Table 5.2 The effect of metribuzin on chlorophyll-a in cyanobacteria

S.No	Concentration of Metribuzin (μg/ml)	Loss of chlorophyll-a in *Phormidium* sp., (mg/ml)					Loss of chlorophyll-a in *Chroococcus* sp., (mg/ml)				
		(Days)					(Days)				
		6	9	12	15	18	6	9	12	15	18
1	2	5.45	14.27	20.89	27.61	34.60	5.61	12.70	17.47	24.75	30.64
2	4	8.48	16.01	23.83	29.17	37.54	8.83	15.37	21.9	28.71	33.49
3	6	12.07	18.13	26.41	31.38	40.39	13.89	19.60	25.85	32.39	37.54
4	8	16.58	23.10	29.63	35.24	49.87	15.73	22.73	28.15	36.07	41.68
5	10	24.03	30.65	36.16	42.60	51.62	25.12	29.82	34.04	40.3	43.52

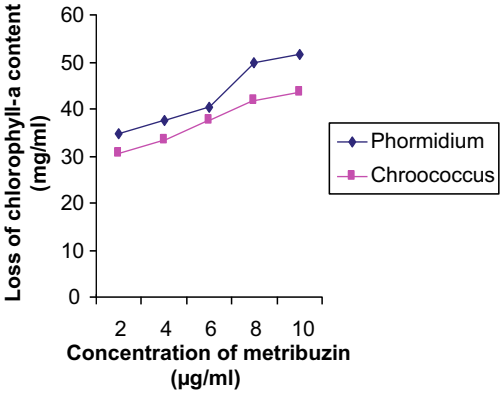

Fig. 5.1 The effect of metribuzin on chlorophyll-a content on 18th day

and reducing side PS II. Our results also contradict those of Gonzalez-Tome (1996), who studied Nostoc species. If double bentazone was used. Xia (2005) showed that thiobencarb had a negligible effect on chlorophyll-a synthesis in her Nostoc sphaeroides colonies.

Biochemical studies such as carbohydrate, protein, amino acid and lipid contents were analyzed in both control and herbicide-treated cyanobacterial species. At the highest concentration of herbicide (10 μg/ml), a significant decrease in the content of all biochemical components was observed on day 18 in both cyano-his bacteria. *Phormidium* species treated with the lowest concentration of metribuzine (2 μg/ml) on days 6 and 18 showed a loss of carbohydrate content at 0.8 and 12.8 μg/ml. At the highest concentration (10 μg/ml), a carbohydrate loss of 9.2 and 16.1 μg/ml was observed in metribudine-treated formidium species on days 6 and 18, respectively (Table 5.3; Fig. 5.2). *Chroococcus* sp. treated with the lowest concentration (2 μg/mL) of metribuzine had carbohydrate losses of 1.7 and 9.3 μg/mL on days 6 and 18, respectively.

At the highest concentration (10 μg/ml), a loss of carbohydrate content of 6.7 and 10.0 μg/ml was recorded on days 6 and 18, respectively (Table 5.3, Fig. 5.2).

Table 5.3 The effect of metribuzin on carbohydrate in cyanobacteria

S.No	Concentration of Metribuzin (μg/ml)	Loss of carbohydratre in *Phormidium* sp., (μg/ml)					Loss of carbohydrate in *Chroococcus* sp., (μg/ml)				
		(days)					(days)				
		6	9	12	15	18	6	9	12	15	18
1	2	0.8	4.9	7.0	9.8	12.8	1.7	2.6	6.1	8.0	9.3
2	4	4.9	5.8	7.6	9.9	13.5	2.3	3.6	7.1	8.1	9.4
3	6	6.2	7.0	9.1	12.3	15.0	2.6	5.1	7.2	8.6	9.5
4	8	6.5	7.8	9.9	12.4	15.2	5.7	6.4	7.7	8.9	9.9
5	10	9.2	9.6	12.2	14.0	16.1	6.7	7.3	8.6	9.1	10.0

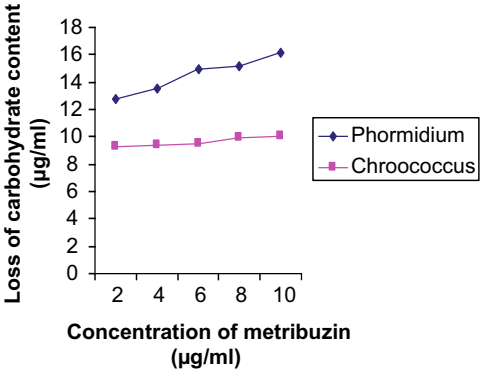

Fig. 5.2 The effect of metribuzin on carbohydrate content on 18th day

Concurrently, carbohydrate content losses of 10.6 μg/mL, 16.2 μg/mL and 7.6 μg/mL, 10.2 μg/mL were recorded in the *Phormidium* and *Chroococcus* sp. controls on days 6 and 7, respectively. Cobia et al. (2001) reported that simazine has the ability to suppress the carbohydrate content of Anabaena cylindrica. Garjano et al. (2009) reported that treatment with bentazone resulted in significant inhibition of carbohydrate synthesis. The loss of protein content was recorded as 9.4 μg/ml, 17.8 μg/ml, and 9.0 μg/mL and 11.4 μg/mL for *Phormidium* and *Chroococcus* sp. controls on days 6 and 18, respectively. were recorded at protein content losses of 1.7 and 15.9 μg/ml. At the highest concentration (10 μg/ml), a loss of protein content of 8.1 and 17.8 μg/ml was observed in metribudine-treated formidium spp. on days 6 and 18, respectively (Table 5.4; Fig. 5.3). *Chroococcus* species treated with the lowest concentration (2 μg/mL) of metribuzine showed a protein loss of 1.6 and 9.8 μg/mL on days 6 and 18, respectively. At the highest concentration (10 μg/ml), a protein loss of 7.1 and 11.4 μg/ml was recorded on days 6 and 18, respectively (Table 5.4; Fig. 5.3).

Yang et al. (1997) reported that molinate inhibited protein hydrolysis by inhibiting protease activity and resulted in total protein accumulation in cyanobacterial cells. Amino acid content losses of 3.0 and 11.1 μg/ml were recorded in *Phormidium*

Table 5.4 The effect of metribuzin on protein in cyanobacteria

S.No	Concentration of Metribuzin (μg/ml)	Loss of protein in *Phormidium* sp., (μg/ml)					Loss of protein in *Chroococcus* sp., (μg/ml)				
		6	9	12	15	18	6	9	12	15	18
1	2	1.7	4.8	9.8	13.7	15.9	1.6	3.1	4.6	7.9	9.8
2	4	3.5	5.8	10.4	13.8	16.6	4.9	5.8	8.7	10.0	10.9
3	6	5.5	7.3	11.5	14.5	16.8	5.3	7.0	9.1	10.1	11.2
4	8	6.7	8.6	12.1	14.9	17.6	6.0	7.6	9.2	10.3	11.3
5	10	8.1	9.4	12.6	15.6	17.8	7.1	7.8	9.6	10.5	11.4

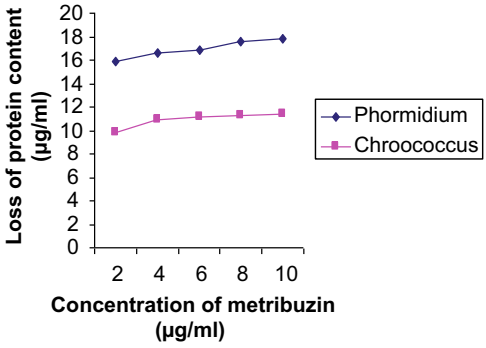

Fig. 5.3 The effect of metribuzin on protein content on 18th day

species treated on day 1. At the highest concentration (10 μg/ml), loss of amino acid content of 10.1 and 15.4 μg/ml was seen in metribudine-treated formidium species on days 6 and 18 (Table 5.5; Fig. 5.4). In his *Chroococcus* sp. treated with the lowest concentration (2 μg/ml) of metribuzine, a loss of amino acid content of 0.7 and 9.6 μg/ml was observed on days 6 and 18, respectively. At the highest concentration (10 μg/ml), 5, 4 and 11 amino acid content is lost. 4 μg/ml was recorded on days 6 and 18 (Table 5.5, Fig. 5.4). Concurrently, amino acid content losses of 10.8 μg/mL, 15.4 μg/mL and 7.2 μg/mL, 11.6 μg/mL were recorded in the *Phormidium* and *Chroococcus* sp. controls on days 6 and 18, respectively.

The *Phormidium* species treated on days 6 and 18 with the lowest concentration (2 μg/ml) of metribuzine showed a loss of lipid content at 0.11 and 1.07 mg/g. At the highest concentration (10 μg/ml), a lipid loss of 0.43 mg/g and 1.23 mg/g was seen in metribuzine-treated formidium species on days 6 and 18, respectively (Table 5.6; Fig. 5.5). No lipid loss in *Chroococcus* species treated with metribuzine 08 and 0.69 mg/g were recorded at the lowest concentration (2 μg/ml) on days 6 and 18, respectively. At the highest concentration (10 μg/ml), a loss of lipid content of 0.41 mg/g and 0.84 mg/g was recorded on days 6 and 18, respectively (Table 5.6, Fig. 5.5). At the same time, losses of amino acid content of 0.52 mg/g, 1.24 mg/g and 0.49 mg/g, 0.85 mg/g were recorded in *Phormidium* and Chrococcus sp. controls recorded on days 6 and 18, respectively rice field.

Table 5.5 The effect of metribuzin on amino acid in cyanobacteria

S.No	Concentration of Metribuzin (μg/ml)	Loss of amino acid in *Phormidium* sp., (μg/ml)					Loss of amino acid in *Chroococcus* sp., (μg/ml)				
		(days)					(days)				
		6	9	12	15	18	6	9	12	15	18
1	2	3.0	4.0	5.7	7.6	11.1	0.7	2.7	5.4	8.1	9.6
2	4	6.5	8.1	10.4	12.2	14.5	0.9	3.8	6.1	8.6	10.0
3	6	7.9	9.0	10.6	13.2	14.8	3.4	5.3	7.0	9.4	10.9
4	8	9.0	10.5	11.9	13.6	15.2	3.9	6.2	7.4	10.4	11.2
5	10	10.1	10.9	12.5	14.0	15.4	5.4	7.4	8.9	10.8	11.4

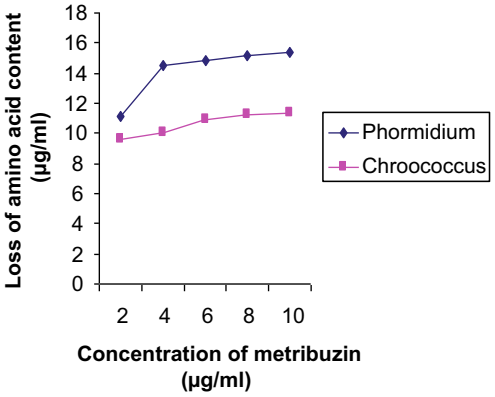

Fig. 5.4 The effect of metribuzin on amino acid content on 18th day

Table 5.6 The effect of metribuzin on lipid in cyanobacteria

S.No	Concentration of Metribuzin (μg/ml)	Loss of lipid in *Phormidium* sp., (mg/g)					Loss of lipid in *Chroococcus* sp., (mg/g)				
		(Days)					(Days)				
		6	9	12	15	18	6	9	12	15	18
1	2	0.11	0.32	0.41	0.68	1.07	0.08	0.23	0.35	0.53	0.69
2	4	0.22	0.44	0.50	0.75	1.16	0.19	0.33	0.45	0.59	0.77
3	6	0.30	0.55	0.63	0.89	1.23	0.27	0.39	0.54	0.69	0.83
4	8	0.39	0.62	0.68	0.921	1.235	0.35	0.48	0.59	0.74	0.842
5	10	0.43	0.67	0.70	0.925	1.239	0.41	0.53	0.64	0.76	0.848

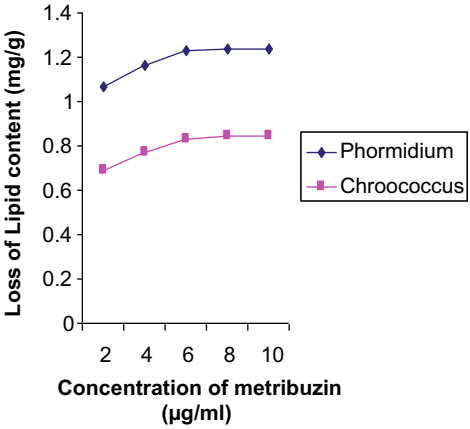

Fig. 5.5 The effect of metribuzin on lipid content on 18th day

A gradual decrease in chlorophyll-a and biochemical content was observed in *Phormidium* and *Chroococcus* species treated with different concentrations of metribuzine. Their levels were found to increase in controls compared to all concentrations of cyanobacterial species treated with metribuzine. This result confirms that chlorophyll a and biochemical levels were significantly reduced in cyanoHis bacterial species treated with metribuzine at his highest concentration (10 µg/ml) on day 18. The results also indicated that *Phormidium* was more sensitive to metribuzine than Chroococcus.

The data obtained in this study also demonstrated that metribuzine is capable of inhibiting the growth and biochemical components of *Phormidium* and *Chroococcus* species even at the lowest concentration (2 µg/ml). Similar observations were reported for haparosiphon with herbicide, where increasing herbicide concentrations decreased levels of chlorophyll, carbohydrates, proteins, amino acids, and lipids (Neveen et al. 2009). Similar results were also reported by his Shabana (1987), who found that the growth and dry weight of Anabaena oryzae and Aulosira fertilssima were significantly reduced by the application of atrazine. Our present results are in good agreement with those of Megharaj et al. match (1986) showed that herbicides exposed to the cyanobacteria Nostoc linckia, Synechococcus elongatus and elongatus, and his *Phormidium* tenue inhibited total carbohydrate levels even at the lowest concentrations. Other similar observations were made by many workers (Shabana 1985). Similarly, Machete-treated Anabaena dryorum showed the greatest reduction in carbohydrate, protein, and lipid content at all concentrations. Higher herbicide concentrations significantly decreased amino acid content (Kashyap and Pandey 1982). Gadkari (1988) reported that the photosynthetic herbicide metribuzine has the ability to inhibit the growth and nitrogenase activity of her Nostoc muscorum. *Phormidium* species have been shown to be sensitive to the herbicides 2,4-D, trifluralin, MCPA, and TCA (Cullimore and McCann 1977). Birmingham and Colman (1983) hypothesized that cyanobacteria may be more sensitive to herbicides than

eukaryotic algae. Also Kasai et al. (1993) concluded that, among the seven taxonomic groups, Volvocales and Cyanophyceae are the most sensitive to symmetry. Arvik et al. (1973) reported complete inhibition of cyanobacterial growth in liquid media in the presence of 1 ppm metribuzine.

A number of researchers have also reported the effects of herbicides on cyanobacteria tolerance, growth, and biochemical content (Venkataraman and Rajalakshmi 1971). Similar types of differential effects of different herbicides on the growth and biochemical content of different algae have been previously reported (DaSilva et al. 1975).

Herbicides are organic or inorganic chemicals used primarily to control pests. In high concentrations, these compounds are highly toxic to all plant systems. In addition to killing all target organisms when used in excess, they inhibit the growth of other non-target organisms (Cullimore and McCann 1977).

This study demonstrated the need to pay close attention to herbicide dosage and selection. Because a slightly higher dose of chemicals can kill beneficial microbes. From the foregoing discussion, it is clear that research on herbicide effects may affect the growth and biochemical composition of the cyanobacteria his *Phormidium* and his *Chroococcus* species.

5.4 Conclusion

Most studies of herbicide toxicity to cyanobacteria have been conducted with pure active ingredients. Currently we investigated the effect of marketable products used in the paddy field. The results obtained provided valuable information on the residual inhibitory effects of metribuzine herbicide formulations on the growth and biochemical content of cyanobacterial species. In terms of environmental protection measures, our results demonstrate the continuous and regular application of metribuzine in paddy fields. On the conflicting, we propose to ban the use of metribuzine in paddy fields because metribuzine has a strong inhibitory effect on soil microbiota, especially *Phormidium* and *Chroococcus* species. There is an urgent need to reduce herbicide contamination of the environment using safer, chemical-free pest control methods.

References

Arvik JH, Hyzak DL, Zimdahl RL (1973) Effect of metribuzin and two analogs on five species of algae. Weed Sci 21(3):173–175

Battah MG, Shabana EF, Kobbia IA, Eladel HM (2001) Differential effects of thiobencarb on growth and photosynthesis of *Anabaena variabilis* with changes in phosphate level. Ecotoxicol Environ Saf 49:235–239

Birmingham BC, Colman B (1983) Potential phytotoxicity of diquat accumulated by aquatic plants and sediments. Wat Air Soil Pollut 19:123–131

Carr NG, Whitton BA (1982) The biology of cyanobacteria. Water Air Soil Pollut 19:211–222

Cullimore DR, McCann AE (1977) Influence of four herbicides on the algal flora of a prairie soil. Plant Soil 46:499–510
DaSilva EJ, Hetiksson LE, Henriksson E (1975) Effect of pesticides on blue-green algae and nitrogen fixation. Arch Environ Contam Toxicol 3:193–204
Eladel HM, Henley WJ, Kobbia IA (1999) Effect of thiobencarb on growth and photosynthesis of the soil algae *Protosiphon botrycides* (Chlorophyta). J Appl Phycol 10:547–554
Gadkari D (1988) Assessment of the effects of the photosynthesis-inhibiting herbicides diuron, DCMU, metamitron and metribuzin on growth and nitrogenase activity of *Nostoc muscorum* and a new cyanobacterial isolate strain G4. Biol Fertil Soils 6(l):50–54
Galhano V, Peixoto F, Gomes-Laranjo J, Fernandez-Valiente E (2009) Differential effects of bentazon and molinate on *Anabaena cylindrica* an autochthonous cyanobacterium of portuguese rice field agro-ecosystems. Water Air Soil Pollut 197:211–222
Gonzalez-Tome R (1996) Estudio de diversas tecnicas agronomicas en relacion con las cianobacterias fijadoras de N_2 en el cultivo del arroz. Ph.D dissertation. Facultad de Ciencias Biologicas, Universidad de Valencia, Valencia (in Spanish).
Gustavson K, Mohlenberg F, Schliiter L (2003) Effects of exposure duration of herbicides on natural stream periphyton communities and recovery. Arch Environ Contam Toxicol 45:48–58
Kasai F, Takamura N, Hatakeyama S (1993) Effects of simetryne on growth of various freshwater algal taxa. Environ Pollut 79:77–83
Kashyap AK, Pandey KD (1982) Inhibitory effects of rice-field herbicide machete on *Anabaena doliolum* and protection by nitrogen sources. Z Pflanzenphysiol 107(4):339–346
Kobbia IA, Battah MG, Shabana EF, Eladel HM (2001) Chlorophyll-a fluorescence and photosynthetic activity as tools for the evaluation of simazine toxicity to protosiphon botryoides and *Anabaena variabilis*. Ecotoxicol Environ Saf 49:101–105
Maria Diva L, Sebastiae claudino DS, Maria Olimpia OR (1998) Mechanism of metribuzin herbicide sorption by humic acid samples from peat and vermi compost. Analytica Chimica Acta 368:155–164
Megharaj M, Venkateswarlu K, Rao AS (1986) Growth response of four species of soil algae to monocrotophos and quinalphos. Environ Pollut Ser A Ecol Biol 42(l):15–22
Metting B (2008) The systematics and ecology of soil algae. Bot Rev 47:195–312
Mohapatra PK, Schubert H, Schiewer U (1996) Effect of dimethoate on transthylakoid pH gradient of intact *Synechocystis* sp. Bull Environ Contami Toxicol 57(5):722–728
Muhammad MJ, Muhammad INB, Hasnain W, Ahsan A, Muhammad AN, Al-Doss AA (2022) Efficacy of metribuzin doses on physiological, growth, and yield characteristics of wheat and its associated weeds, front. Plant Sci. https://doi.org/10.3389/fpls.2022.866793
Neveen AR, Nadia M, Shafey EI (2009) Harmful effects of endosulfan treatment on cyanobacterial distribution and some macromolecules of soybean plant. Afr J Biotechnol 8:6277–6281
Patterson GML (1996) Biotechnological applications of cyanobacteria. J Sci Ind Res 55:669–684
Richards RP, Baker DB (1993) Pesticide concentration patterns in agricultural drainage networks in the Lake Erie basin. Environ Toxicol Chem 12:13–26
Shabana EF (1985) Use of algal batch assays to assess the toxicity of atrazine to some selected blue-green algae: 1. Influence of atrazine on the growth, pigmentation and carbohydrate contents of *Aulosira fertilissima*, *Anabaena oryzae*, *Nostoc muscorum* and *Tolypothrix tenuis*. Egypt J Physiol 12(l):67–76
Shabana EF (1987) Use of batch assays to assess the toxicity of atrazine to some selected cyanobacteria. II. Effect of atrazine on heterocyst frequency, nitrogen and phosphorus metabolism of four heterocystous cyanobacteria. J Basic Microbial 27(4):215–223
Silva PG, Silva HJ (2007) Effect of mineral nutrients on cell growth and self-flocculation of a biofertilizer. Bioresour Technol 98:607–611
Singh RK, Singh BD, Singh HN (1983) Inhibition of photosystem II of nitrogen-fixing blue green algae *Nostoc linckia* by the rice field herbicide benthiocarb. Z Allg Microbiol 23(7):435–441
Thajuddin N, Subramanian G (2005) Cyanobacterial biodiversity and potential applications in biotechnology. Curr Sci 89:47–57

Venkataraman GS, Rajalakshmi B (1971) Tolerance of blue-green algae to pesticides. Curr Sci 40:143–144

Vermass WFJ (2001) Photosynthesis and respiration in cyanobacteria. Nature encyclopedia of life sciences. Nature Publishing Group, London

Whitton BA, Potts M (2000) Introduction to the cyanobacteria. In: Whitton BA, Potts M (eds) The ecology of cyanobacteria. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 1–11

Whitton BA (2000). Soils and rice fields. The ecology of cyanobacteria. pp 233–255

Xia J (2005) Response of growth, photosynthesis and photoinhibition of the edible cyanobacterium *Nostoc sphaeroides* colonies to thiobencarb herbicide. Chemosphere 59:561–566

Yan GA, Yan X, Wu W (1997) Effects of the herbicide molinate on mixotrophic growth, photosynthetic pigments and protein content of *Anabaena sphaerica* under different light conditions. Ecotoxicol Environ Saf 38:144–149

Chapter 6
Emerging Methodologies for the Molecular Analysis of Soil Microbiota from Polluted Soil Sites

Ridhuvarshini, Pavethra, Sophia Reena, and Sivaranjani

Abstract The soil microbiome performs a wide range of crucial functions; however, we have a limited understanding of its biodiversity. Extracting microbes from polluted sites could reveal potential microbes that could be used to mitigate pollution better than conventional microbes. Soil DNA may be extracted directly, amplified using polymerase chain reaction, and profiled to reveal more about the soil microbiome's taxonomy and function than ever before. Current procedures frequently combine DNA sequencing with other methods like denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE), single-strand conformation polymorphism (SSCP), terminal restriction fragment length polymorphism (TRFLP), amplified rDNA restriction analysis (ARDRA), amplified ribosomal intergenic spacer analysis (ARISA), and cloning. The advantages and disadvantages of these methods are discussed, and new developments that have relevance as an appliance shedding light on the soil microbial ecology are also included. Soil diversity cannot be assessed using just one approach; therefore, picking the right one and using newly discovered information can significantly improve our understanding of soil microbes for their specific applications in mitigating.

Keywords Soil microbiome · Bacterial ecology · Polluted soil · Emerging methodologies · Microbial profiling · Direct DNA sequencing

6.1 Introduction

The diversity of the soil microbiome is little understood, even though the soil microbiome performs a wide variety of essential tasks. Direct DNA extraction, amplification by polymerase chain reaction, and profiling of soil microbes have made hitherto undisclosed information on the taxonomy and function of the soil microbiome accessible. It is common practise to combine DNA sequencing with other

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techniques, including DGGE, TGGE, SSCP, TRFLP, cloning, and amplification of rDNA restriction and ribosomal intergenic spacer regions. Innovations that have significance as an appliance shining a light on the soil microbial ecology are also mentioned, along with the pros and cons of these approaches. We can learn much more about soil bacteria if we use the correct method for assessing soil diversity and incorporate newly found information.

Additional advancements are provided by more recent techniques like microarrays and high-throughput sequencing. In this review, various molecular methods used to study soil microbiota are reviewed, along with the pros and cons of each method.

6.2 Bioinformatic Analysis

The initial computational method was based on the sequencing platform, with distinct software modules envisioned for early analysis of diverse systems. However, the programs need to be linked to establish automated "channels" to identify the possible genes and taxonomic groups illustrated by the sequencing data and their relative frequency of occurrence. When one genome is sequenced in short reads, it creates overlapping expansions that allow the "assemblage" of shorter reads into longer ones. When the genomic sequence is presented as a "scaffold," the job becomes much less complicated. Extended sequences can be matched by searching the NCBI databases. Arranging the various sequences of the initiator in the best possible way is critical for further study. PHYLIP, the PHYLogeny Inference Package, can silently infer phylogenetic trees and sequence family relationships. Genome annotation is assigning biological meaning to DNA sequences and identifying genes. There is a need for software that can build de-novo genomes from short-reads (i.e., less than 50 b), with significant errors projected with extended-reads of 100 b; nevertheless, verifying restricted sequences is challenging. Therefore, it is highly improbable that microbial genomes from diverse and mixed populations can be reconstructed using high-throughput, short-read-length techniques. A mix of tactics catering to readers with varying attention spans (short, medium, and long) would be required. With the expanding breadth of metagenomic research and public databases comes the requirement for high-performance computing and automated applications. Independent and autonomous organizations are developing alternative public pathways to examine metagenomic data. Disparities in metabolic profiles among a few biomes have been uncovered using the mg-RAST server (Meyer et al. 2008; Dinsdale et al. 2008). The sequence was analyzed using BLASTX in addition to ribosomal (GREENGENES, RDP-II), chloroplast, and mitochondrial databases (via ACLAIME). MEGAN is a one-of-a-kind method that rapidly assesses the biodiversity of metagenomic samples using visual outputs, allowing researchers to compare and contrast many data sets, such as functional evaluations and metadata (Huson et al. 2007) (Fig. 6.1).

Understanding the subject's research hot spots and potential future research trajectories can be done using statistics and examining the literature's subject keywords (Shi et al. 1999). This study employed VOS viewer version 1.6.16 (van Eck and

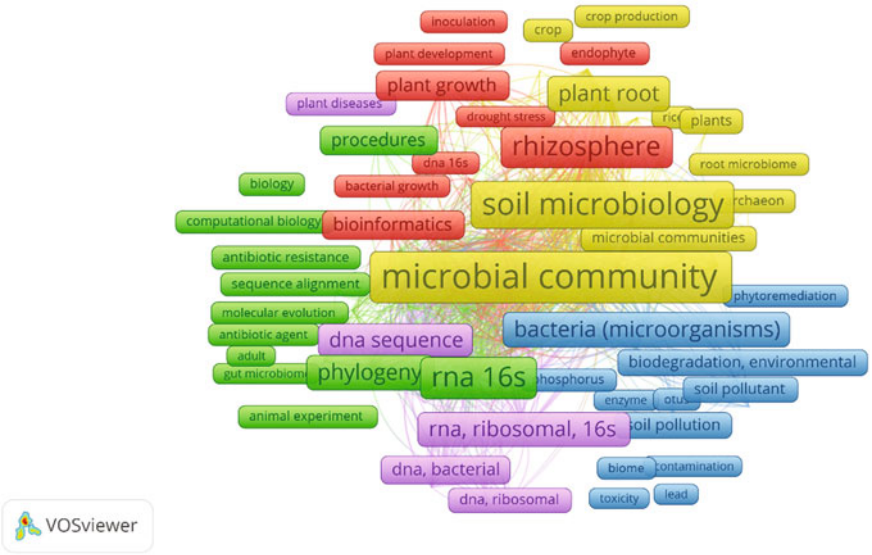

Fig. 6.1 VOSviewer mapping of keywords

Waltman 2010) to assess descriptive data and consider recent research and historical trends. The Scopus database output for the keyword "TITLE-ABS-KEY (Soil microbiota AND molecular analysis)" is also included for co-occurrence analysis in the VOS viewer. The nodes linked with the soil microbial community, soil bacteria, plant-microbe interactions, rDNA, and molecular analysis are deduced from the yellow, blue, red, violet, and green color clusters. The size of the nodes (frames) reveals how frequently they are used, and the separation between the frames and connecting lines reveals how interconnected and linked they are. All nodes are most connected to the cluster of adsorption keywords, the core content, and the most key phrase for all keywords. Figure 6.2 displays the global collaborations identified by the document search. The blue color of the map represents global research collaboration. The pink border that divides the states demonstrates the authors' level of involvement. It is incredible how the countries that have published the most articles on the soil microbiome have worked together. A generic function summary of bibliometrix analysis done by Bibliometrix R-Tool (Aria and Cuccurullo 2017) for the Scopus database search of a given keyword is provided in Table 6.1.

6.3 Community Profiling Techniques and Limitations

Community fingerprinting techniques have been widely employed in microbiota-ecology research, greatly enhancing our understanding of the variety of soil microbiota. For example, denaturing gradient gel electrophoresis (DGGE), temperature

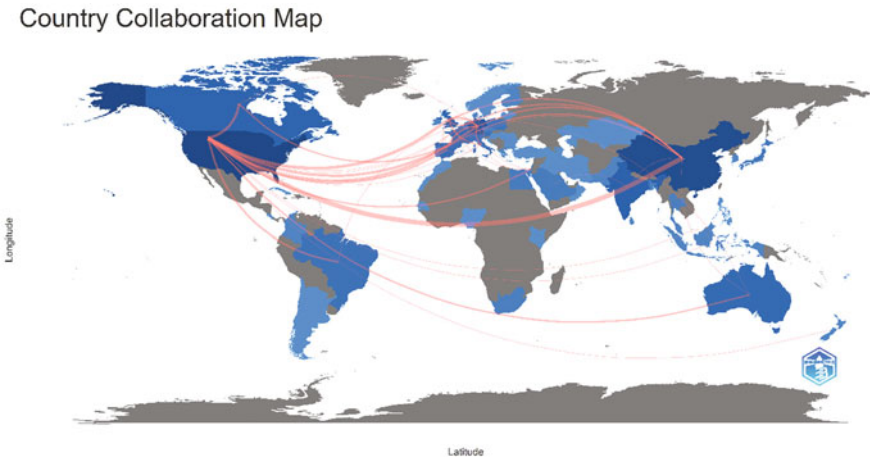

Fig. 6.2 Country collaboration map of research of the title

Table 6.1 Scopus database keyword search and paper hits

Description	Results
Main information about the data	
Timespan	2008:2022
Sources (Journals, Books, etc.)	139
Documents	277
Annual growth rate (%)	30.15
DOCUMENT AVERAGE AGE	3.6
Average citations per doc	52.39
References	20,472
Document contents	
Keywords plus (ID)	3319
Author's keywords (DE)	920
Authors	
Authors	1595
Authors of single-authored docs	6
Authors collaboration	
Single-authored docs	6
Co-authors per doc	6.49
International co-authorships (%)	35.02

(continued)

Table 6.1 (continued)

Description	Results
Document types	
Article	222
Book	2
Book chapter	8
Editorial	1
Erratum	1
Note	1
Review	41
Short survey	1

gradient gel electrophoresis (TGGE), single-strand conformation polymorphism (SSCP), terminal restriction fragment length polymorphism (T-RFLP), amplified rDNA restriction analysis (ARDRA). In addition, amplified tRNA-serine-leucine-nucleotide polymorphism (ART) is a method for identifying (van Elsas 2000).

While each community profiling approach is distinct, many share the same DNA features used in the separating process. Both denaturant gradient gel electrophoresis (DGGE) and thermal gradient gel electrophoresis (TGGE) use the melting behavior of DNA to separate fragments of the same size but different sequences; this is because DNA with different sequences (i.e., different G + C contents) melts at different locations in a polyacrylamide gel (TGGE). Because of the potential for many bands and an exaggerated estimation of diversity, the use of degenerate primers is limited in DGGE/TGGE. Since the advent of DGGE, these methods have become the most widely used community fingerprinting techniques in bacterial ecology, making their advantages and disadvantages clear to anybody interested in studying soil microbial communities. Soil fungal communities may be analyzed rapidly and effectively with DGGE/TGGE, making it a helpful tool for studying changes in community composition (Anderson et al. 2003a). The techniques' benefits include the ability to study and compare several samples on a single gel and the speed and simplicity of such comparisons. Specialized software programs have substantially improved the study of community fingerprints by permitting comparison of the position and relative intensity of distinct bands within gels. This paves the way for further statistical analysis of the data and an appreciation of the ecological conclusions drawn from it. Accurate comparison, however, is highly dependent on suitable internal standards and the prior establishment of gel resolution and quality. The need to compare multiple gels owing to a large number of samples emphasizes the significance of this. One of the fundamental limitations of DGGE is its lack of repeatability across gels, even if the gel-making process may be improved by using, for example, the same flow rate of denaturant solutions and the same equipment for each gel (Fromin et al. 2002).

The methods used to create such profiles of a whole community are not without their flaws. Compared to more oversized products, smaller DNA fragments (500 bp)

provide better resolution across bands in a profile, limiting the taxonomic information that can be gleaned by sequencing excised bands (Landeweert et al. 2003). More importantly, even the most sensitive staining techniques sometimes fail to identify all the variability that occurs in a sample, especially for the less prominent members of the group. It has also been shown that a single band on a gel may contain many sequence types (Schmalenberger and Tebbe 2003). T-RFLP uses automated DNA sequencing technology to provide significantly better throughput than gel-based community profiling approaches (Marsh 1999). T-RFLP varies from conventional RFLP in that it employs fluorescently tagged PCR primers (forward, reverse, or both primers) before restriction digestion and size measurement of fluorescently labeled terminal restriction fragments using a DNA sequencer. T-RFLP may simplify the community profile without compromising the variety found by identifying the last segment of each 18S rDNA or ITS sequence in a sample. T-RFLP is an improvement on the ARDRA method of community profiling. There are methods like ARISA, which are similar, but they assess whole amplicons instead of simply the terminal fragments that result after restriction digestion (Leckie et al. 2004). Using an automated DNA sequencer for ARISA and T-RFLP increases throughput and improves accuracy in sizing the generated fragments by adding an internal standard in each sample and maintaining stable operating parameters. Although T-RFLP has been used to analyze the diversity of soil fungi and to detect ECM fungi in soil samples (Lord et al. 2002; Dickie et al. 2002), a robust T-RFLP database is necessary for identifying individual fungi species. The lack of ease with which sequence information may be extracted from T-RFLP peaks makes identifying previously unknown species in a sample a formidable task. Occasionally, artificial identification has been made using virtual restriction digests based on public database sequences; however, this is risky because it presumes that only one species or operational taxonomic unit (OTU) can have a peak of that size in a sample and that the database sequence is correctly identified and of high quality.

Cloning PCR amplicons from environmental DNA has also been used to evaluate soil fungal diversity (Jumpponen 2003). However, clones may be screened using RFLP to categorize clones into OTUs prior to DNA sequencing, reducing the number of clones required for sequencing and making it difficult to determine how many clones must be analyzed to sample the diversity contained in a single sample effectively. The development of collectors or species abundance curves shows that the number of 18S rDNA clones that need to be analyzed in agricultural soil is far smaller than the number of clones that would have been required for a library of bacterial 16S rDNA to have covered the diversity microbiota (Anderson et al. 2003). These researchers found that more ITS clones than 18S rDNA clones from the same sample needed to be screened to ensure coverage of the fungal diversity in the sample. Without analyzing many clones, it is difficult to discriminate between familiar and unusual sequence types in a sample.

DNA sequencing and phylogenetic analysis are commonly used in tandem with community profiling methods for the taxonomic identification of species present in a sample; however, it is crucial to remember that environmental DNA samples may also contain chimera DNA sequences. The techniques mentioned above provide an

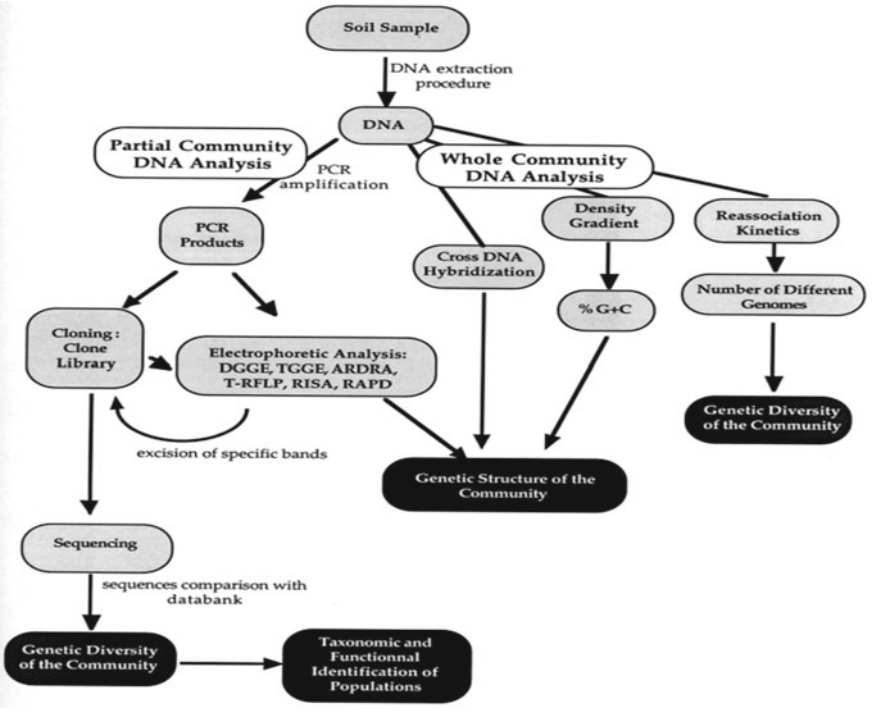

Fig. 6.3 Schematic representation of the different molecular approaches

array of adequate resources for assessing soil fungus populations. Although each has certain technical limitations that prevent it from being fully utilized, its widespread application in bacterial ecology over the past decade has led to steady development and refinement. Each community profiling method varies in its taxonomic resolution due to factors such as the PCR primers chosen for the first PCR amplification of the community DNA and the lack of extensive sequencing information in public databases. Fungus ecologists have been working on loosening these constraints for years, and their efforts will likely bear fruit soon (Figs. 6.3 and 6.4).

6.4 Current Developments in Molecular Ecology of Soil Fungi Profiling

The spatial and temporal dynamics of resident fungus, as well as the diversity of fungi in plant roots, the rhizosphere, and/or bulk soil, have been considerably improved by recent investigations of soil fungal ecology employing PCR amplification from total extracted DNA.

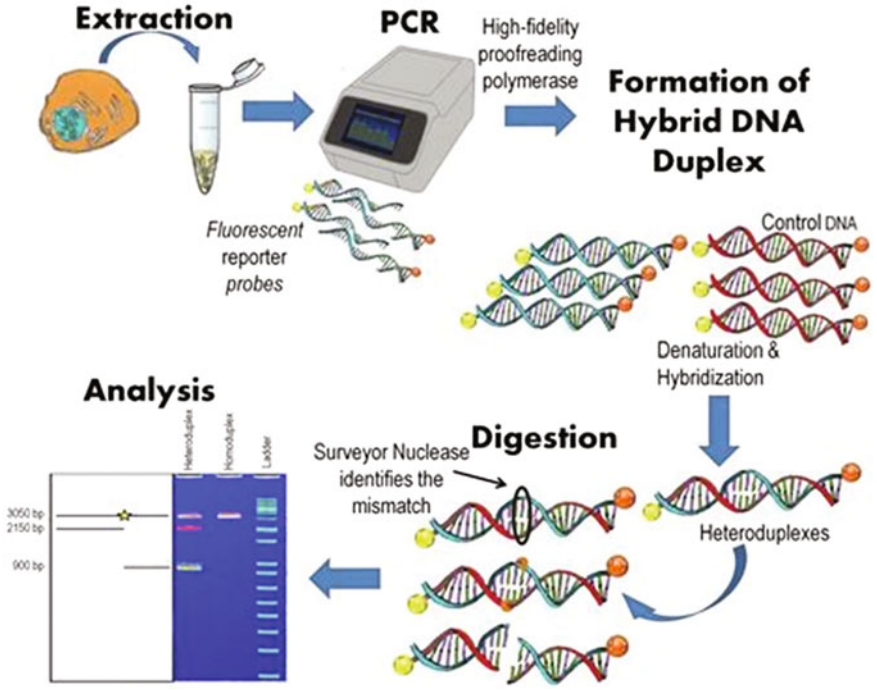

Fig. 6.4 Extraction and analysis of genetic information of soil microbes

Support for the long-held belief that growing fungi from the soil only allows for a limited perspective of variety was provided by the cloning and sequencing of 18S and ITS rDNA PCR products from total soil DNA (Hunt et al. 2004). Using 18S rDNA amplification in conjunction with TGGE and DGGE in both microcosms and field soil, we show that fungal diversity is lower in the rhizosphere compared to that in bulk soil, and that there is an influence of plant age. The bare soils around glacier ice edges and terminal moraines contain unique fungal communities, as shown by an analysis of 18S rDNA clones. Soil community composition has been shown to shift in response to various treatments, including petrochemical pollution, nitrogen addition, controlled vegetation burning, elevated atmospheric CO_2 concentration, and the addition of compost or manure, as determined by studies employing 18S or ITS rDNA amplification coupled with cloning, DGGE, SSCP, or T-RFLP analysis. The DGGE-detected communities lacked the taxonomic diversity of the T-RFLP-detected communities. However, because several DNA extraction methods and primers were employed, it is unclear to what extent these differences reflect variations in the relative resolution of the approaches.

However, the taxonomic richness of the DGGE profiles was similar to that of an isolated fungal assemblage from the same host, despite sequencing differences. While Phoma and Microdochium were predicted to be major players in the A. arenaria root fungal community based on isolated assemblages, they were not found using DGGE.

Additional research using 18S rDNA PCR and DGGE on A. arenaria endophytes demonstrated that the taxonomic structure of AM fungal assemblages in roots and that inferred from spore diversity are unrelated. It has been common practise to directly amplify AM fungus from whole root DNA for quite some time. Testing for AM infection in roots using competitive PCR has been shown to be doable. While it has been around for some time, it has just lately been put to use to resolve pressing ecological concerns. Helgason and coworkers (1998) used partial 18S rDNA PCR from total root DNA and cloning to compare AM fungal diversity in the roots of monoculture arable crop plant hosts and forest plant species. It was believed that the prevalence of AM fungus, which can infect a wide range of host plants, was not due to monoculture planting but rather an effect of agronomic management approaches on diversity. Different AM communities have been found in the roots of several plant species, however, according to a different study that used the same techniques in combination with T-RFLP (Vandenkoornhuyse et al. 2003). More evidence that floristic diversity may have a substantial effect on endophyte diversity was provided by Johnson and colleagues (2004), who compared the AM fungal assemblages in the roots of Plantago lanceolata bait plants in microcosms grown in monoculture and mixed plant communities. They were able to do so by employing a PCR and T-RFLP technique based on an 18S rDNA subset.

6.5 Popular Approaches

Community profiling techniques have recently made significant strides in soil mycology. These methods are already being used in labs all over the world, and it is expected that our knowledge of the composition and evolution of soil fungal communities will expand dramatically in the near future as a result. But researchers shouldn't just choose any old set of primers or community profiling technique to assess fungal diversity; they should weigh the pros and cons carefully in light of their goals, the fungi of interest, and the data they want to analyse. In addition, we have emphasised in this review the limits of the methodologies that must be taken into account in the context of individual studies and their respective hypotheses. Some of these restrictions apply across all fields of ecology, whereas others have been addressed in soil bacterial community investigations, leading to improved methods. Diversity analysis based on taxonomic richness and relative abundance, along with community dynamics, is far more difficult than analysing, say, the taxonomic richness of soil fungus. This is because fungi are mycelial in structure, and spores are likely to coexist in any given sample. The technique of stable isotope probing (SIP), which classifies organisms according to their nucleic acids' abilities to use certain stable isotope-marked substrates, has been successfully applied to the separation of soil bacteria into their respective functional groups (Morris et al. 2002). In particular, its benefits to soil fungus have just lately been recognised. These scientists present a convincing case for using this method to investigate microbial DNA in soil. Our findings show that SIP may be used to examine fungal RNA, which integrates 13C quicker

than DNA. This is in contrast to bacterial DNA, which incorporates carbon isotopes more slowly. In a practical setting, analysing fungal RNA obtained from soil enables the detection and identification of metabolically active community members rather than dormant individuals. Consequently, this is a major development in our understanding of fungus and the ecosystems in which they exist. Fungal DNA and RNA levels in soil have also been quantified using real-time PCR (Lueders et al. 2004). Opportunities for further development of metagenomics techniques and microarray technology to study the composition and activity of microbial communities seem promising. These methodologies, when used in tandem with community profiling techniques, allow us to get a more nuanced understanding of fungal communities and the roles they play in the ecological processes of soil.

6.6 Isotopic Labeling

This can be done with the use of circumstantial evidence, such as when scientists try to link a change in the abundance of a certain group or set of genes with the event in question. By using isotopes, substrates may be individually verified for membership in a certain category (Dumont and Murrell 2005). Micro-autoradiography (MAR) requires the introduction of radiolabeled substrates or nucleotides, such as 3H-labelled thymidine, into the soil. While FISH applied to soil may allow for the identification of live cells in their natural setting, it suffers from the same limitations as other direct imaging techniques (Wagner et al. 2006; Rogers et al. 2007). In order to better identify certain subpopulations, it is encouraged to label soil-extractable PLFAs or nucleic acids. When methane oxidizers in soil are exposed to 13CH4, stable isotope probing (SIP) is most often observed in the interaction of 13C with PLFAs or DNA (Bull et al. 2000; Radajewski et al. 2000). Growing cells take up 13C from certain substrates in their DNA and rRNA. In order to separate the 13C-enriched fraction from the unlabeled fraction of isolated soil nucleic acids, density gradient centrifugation can be used. Methods for SIP are outlined by Kreuzer-Martin (2007); these techniques are useful for examining separate groups that make use of specific substrates like methane, and they may also give more broad information on the members of actively forming soil communities. Many soil communities, for instance, have been studied using various 13C substrates in conjunction with DNA and RNA-SIP (Manefield et al. 2002; Lueders et al. 2004; Whiteley et al. 2006).

6.7 BrdU Labeling

The thymidine nucleotide analogue 5-bedroom-20-deoxyuridine (BrdU) can be used alone or in conjunction with an exogenous substrate to mark dividing soil cells. Using any of the current community DNA analysis techniques, DNA from the less active minority may be compared to DNA from cells containing BrdU. In soils from several

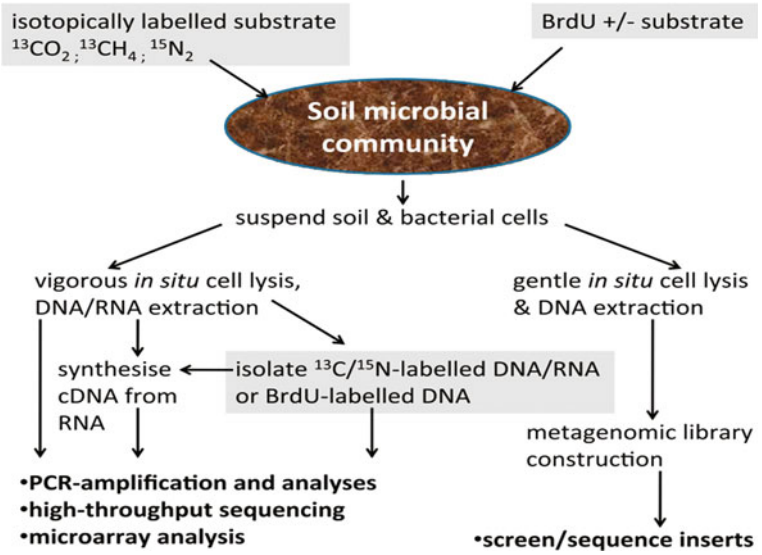

Fig. 6.5 Flow chart showing DNA and RNA extraction from soil. The soil microbial community will include bacteria, archaea, fungi, protists, and microfauna

sites that were incubated at either 4 or 25 °C, we found that BrdU was useful for assessing both the total and growing communities (Figs. 6.5 and 6.6). The data reveal that, in contrast to the profiles generated from actively reproducing bacteria that absorbed BrdU, whole-genome DNA profiles do not cluster according to soil type or incubation temperature. Other possible uses for BrdU include identifying members of disease-resistant plant-related active microbial communities in soil and identifying bacteria connected with arbuscular mycorrhizal fungus hyphae (Artursson and Jansson 2003). Researchers from many institutions collaborated on the project (Hjort et al. 2007).

6.8 The Potential of Microarrays in Soil

Microarrays have been constructed in recent years using sequence data from publically available bacterial genomes deposited in databases like GenBank at the NCBI. Recent literature on the use of microarrays in soil microbial ecology (Sessitsch et al. 2006). The PhyloChip, designed by Gary Andersen and his colleagues at the Lawrence Berkeley National Lab in the United States, is a phylogenetic microarray that uses databases of 16S rRNA gene sequences. With a total of 500,000 probes, this high-density array has the potential to identify w9000 unique species or taxa. To reflect the ever-evolving nature of DNA databases, these microarrays are routinely

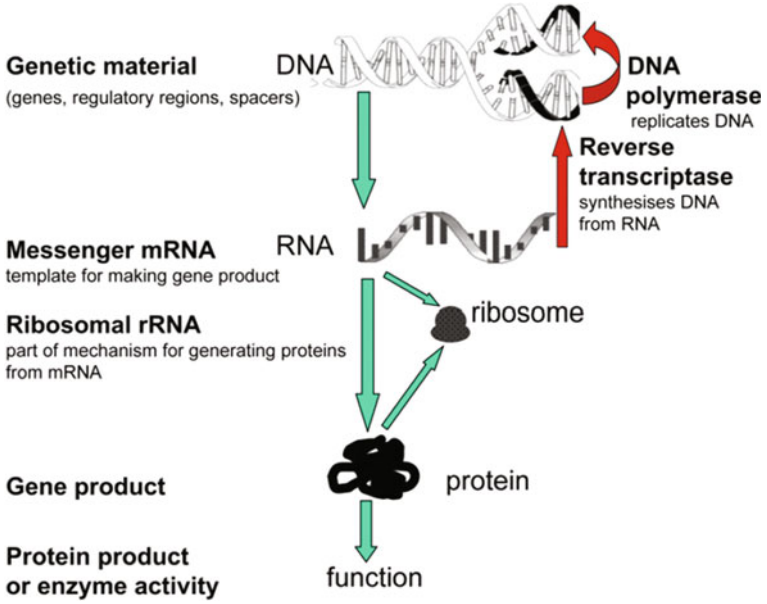

Fig. 6.6 The relationship between DNA, RNA forms, protein functions, and pathways

upgraded to include the most recent data. Some researchers believe that these techniques, as opposed to traditional cloning/sequencing procedures, may show a greater diversity of bacterial phyla and species in soil (DeSantis et al. 2007). In addition to showcasing the richness of ecosystems, these arrays can provide insight into how various taxa are represented within a given treatment, such as rhizosphere vs. bulk soil (DeAngelis et al. 2009). When it comes to identifying the people who make a community tick, rRNA may soon supplant genomic DNA as the gold standard. While it can provide some insight into the composition of known microbial communities, the array does not include all of the functional genes involved for the major activities in soil. The GeoChip, created by Jizhong Zhou at the University of Oklahoma in the United States, is based on functional gene sequences, whereas phylochips are based on non-functional gene sequences. By employing GeoChip 2, for instance, researchers have shown that the number and variety of genes involved in organic C degradation in soils is on the rise. GeoChip 2 has the potential to uncover over 10,000 genes in over 150 different functional areas. To wit: (Zhang et al. 2007). Functional genes in several species may be identified using probes, yielding information on biodiversity. The already outstanding identification rate of 47,000 genes across 292 gene families utilising the new and improved GeoChip 3 is expected to rise as additional sequencing data becomes available. The RNA that is collected from soil can provide information about which genes were active right before sampling, which is something that DNA cannot do. Microarrays, on the other hand, rely on already known DNA sequence information, therefore they are unable to identify new groups

that may become critical to particular functions. Because so little is known about the taxonomic diversity and functional gene sequences of soil fungi, microarrays for soil fungal communities are also now unavailable.

6.9 High-Throughput Sequencing

Traditional cloning and Sanger sequencing take a long time, thus only a small number of samples may be processed at once. Automatic diagnostic techniques (sometimes known as "lab-on-a-chip" technology) are now under development. Utilizing these techniques, researchers may now analyse hundreds of samples to study microbial functional diversity. Thanks to recent advancements in massively parallel high-throughput pyrosequencing techniques, hundreds of thousands of sequences may be processed in parallel. At now, the "GS FLX Titanium Series" machine can sequence one million fragments with an average read length of 400 bp; however, this will increase to 800 bp in the near future. Information on the most recent models may usually be found on company websites, and other commercial high-throughput sequencing equipment offer a more extensive variety of shorter sequences. In comparison to the Applied Biosystems Inc. SOLiD3 system, which promises to sequence 10–15 Gb every 6–7 day run with reads of up to 50b, the Illumina Inc. Genome AnalyserIIx, as of this writing, offers to sequence 20–25 Gb per 9–10 day run. With the fast development of new technologies, new approaches like nanopore sequencing are being created (Mardis 2008). In a 2009 study (Stoddart et al. However, weighing the benefits and drawbacks of each approach is outside the scope of this study and is being done on several online forums. In order to alter the DNA that has been collected from soil, PCR amplification with gene-specific primers can be performed. The relative abundance of bacterial and archaeal 16S rRNA genes (the mean from four soils was w140,000 bacterial; w9000 archaeal sequences) and the total taxonomic diversity of these species in soil were determined using universal 16S rRNA gene primers, for example (Roesch et al. 2007). This approach has the limitation that it assumes all prokaryotes have 16S rRNA gene sequences comparable to the primers used in the PCR amplification stage. However, many bacteria and archaea are able to be cultured despite deviating from the standard primer sequence for this gene. But the technology will develop into a more useful tool as more and more critical pieces may be scheduled with each iteration. Recent advances in high-throughput sequencing have rendered direct and unbiased sampling unproductive because of their inability to unearth uncommon but ecologically significant categories without prior selection. In the future, only the bioinformatic processing of sequence data is anticipated to limit the potential expansion of sequencing, which is now useful and will become much more so as more advanced methods are developed (Pop and Salzberg 2008).

6.10 Conclusion

There are obstacles to exploring the microbiota due to the large number and variety of soil microbiota and the varied nature of the soil, irrespective of the site being polluted or otherwise. Results from small samples that may be used for the study have made traditional protocols unappealing; nevertheless, cutting-edge methods increase the number of soil samples included. Understanding the fundamental mechanisms of each method for investigating microbiota diversity and activity is crucial for mitigating the demerits and capitalizing on the strengths of the various approaches.

References

Anderson IC, Campbell CD, Prosser JI (2003) Diversity of fungi in organic soils under a moorland–Scots pine (Pinus sylvestris L.) gradient. Environ Microbiol 5(11):1121–1132. https://doi.org/10.1046/j.1462-2920.2003.00522.x

Aria M, Cuccurullo C (2017) Bibliometrix: an R-tool for comprehensive science mapping analysis. J Informetr 11(4):959–975. https://doi.org/10.1016/j.joi.2017.08.007

Artursson V, Jansson JK (2003) Use of bromodeoxyuridine immunocapture to identify active bacteria associated with arbuscular mycorrhizal hyphae. Appl Environ Microbiol 69(10):6208–6215. https://doi.org/10.1128/AEM.69.10.6208-6215.2003

Bull ID, Parekh NR, Hall GH, Ineson P, Evershed RP (2000) Detection and classification of atmospheric methane oxidizing bacteria in soil. Nature 405(6783):175–178. https://doi.org/10.1038/35012061

DeAngelis KM, Brodie EL, DeSantis TZ, Andersen GL, Lindow SE, Firestone MK (2009) Selective progressive response of soil microbial community to wild oat roots. ISME J 3(2):168–178. https://doi.org/10.1038/ismej.2008.103

DeSantis TZ, Brodie EL, Moberg JP, Zubieta IX, Piceno YM, Andersen GL (2007) High-density universal 16S rRNA microarray analysis reveals broader diversity than typical clone library when sampling the environment. Microb Ecol 53(3):371–383. https://doi.org/10.1007/s00248-006-9134-9

Dickie IA, Xu B, Koide RT (2002) Vertical niche differentiation of ectomycorrhizal hyphae in soil as shown by T-RFLP analysis. New Phytol 156(3):527–535. https://doi.org/10.1046/j.1469-8137.2002.00535.x

Dinsdale EA, Edwards RA, Hall D, Angly F, Breitbart M, Brulc JM et al (2008) Functional metagenomic profiling of nine biomes. Nature 452(7187):629–632. https://doi.org/10.1038/nature06810

Dumont MG, Murrell JC (2005) Stable isotope probing—linking microbial identity to function. Nat Rev Microbiol 3(6):499–504. https://doi.org/10.1038/nrmicro1162

Fromin N, Hamelin J, Tarnawski S, Roesti D, Jourdain-Miserez K, Forestier N et al (2002) Statistical analysis of denaturing gel electrophoresis (DGE) fingerprinting patterns. Environ Microbiol 4(11):634–643. https://doi.org/10.1046/j.1462-2920.2002.00358.x

Hjort K, Lembke A, Speksnijder A, Smalla K, Jansson JK (2007) Community structure of actively growing bacterial populations in plant pathogen suppressive soil. Microb Ecol 53(3):399–413. https://doi.org/10.1007/s00248-006-9120-2

Hunt J, Boddy L, Randerson PF, Rogers HJ (2004) An evaluation of 18S rDNA approaches for the study of fungal diversity in grassland soils. Microb Ecol 47(4):385–395. https://doi.org/10.1007/s00248-003-2018-3

Huson DH, Auch AF, Qi J, Schuster SC (2007) MEGAN analysis of metagenomic data. Genome Res 17(3):377–386. https://doi.org/10.1101/gr.5969107

Johnson D, Vandenkoornhuyse PJ, Leake JR, Gilbert L, Booth RE, Grime JP et al (2004) Plant communities affect arbuscular mycorrhizal fungal diversity and community composition in grassland microcosms. New Phytol 161(2):503–515. https://doi.org/10.1046/j.1469-8137.2003.00938.x

Kreuzer-Martin HW (2007) Stable isotope probing: linking functional activity to specific members of microbial communities. Soil Sci Soc Am J 71(2):611–619. https://doi.org/10.2136/sssaj2006.0093

Landeweert R, Leeflang P, Kuyper TW, Hoffland E, Rosling A, Wernars K, Smit E (2003) Molecular identification of ectomycorrhizal mycelium in soil horizons. Appl Environ Microbiol 69(1):327–333. https://doi.org/10.1128/AEM.69.1.327-333.2003

Leckie SE, Prescott CE, Grayston SJ, Neufeld JD, Mohn WW (2004) Characterization of humus microbial communities in adjacent forest types that differ in nitrogen availability. Microb Ecol 48(1):29–40. https://doi.org/10.1007/s00248-003-1020-0

Lord NS, Kaplan CW, Shank P, Kitts CL, Elrod SL (2002) Assessment of fungal diversity using terminal restriction fragment (TRF) pattern analysis: comparison of 18S and ITS ribosomal regions. FEMS Microbiol Ecol 42(3):327–337. https://doi.org/10.1111/j.1574-6941.2002.tb01022.x

Lueders T, Wagner B, Claus P, Friedrich MW (2004) Stable isotope probing of rRNA and DNA reveals a dynamic methylotroph community and trophic interactions with fungi and protozoa in oxic rice field soil. Environ Microbiol 6(1):60–72. https://doi.org/10.1046/j.1462-2920.2003.00535.x

Manefield M, Whiteley AS, Ostle N, Ineson P, Bailey MJ (2002) Technical considerations for RNA-based stable isotope probing: an approach to associating microbial diversity with microbial community function. Rapid Commun Mass Spectrom 16(23):2179–2183. https://doi.org/10.1002/rcm.782

Marsh TL (1999) Terminal restriction fragment length polymorphism (T-RFLP): an emerging method for characterizing diversity among homologous populations of amplification products. Curr Opin Microbiol 2(3):323–327. https://doi.org/10.1016/S1369-5274(99)80056-3

Meyer F, Paarmann D, D'Souza M, Olson R, Glass EM, Kubal M et al (2008) The metagenomics RAST server–a public resource for the automatic phylogenetic and functional analysis of metagenomes. BMC Bioinform 9(1):1–8

Morris SA, Radajewski S, Willison TW, Murrell JC (2002) Identification of the functionally active methanotroph population in a peat soil microcosm by stable-isotope probing. Appl Environ Microbiol 68(3):1446–1453. https://doi.org/10.1128/AEM.68.3.1446-1453.2002

Pop M, Salzberg SL (2008) Bioinformatics challenges of new sequencing technology. Trends Genet 24(3):142–149. https://doi.org/10.1016/j.tig.2007.12.006

Radajewski S, Ineson P, Parekh NR, Murrell JC (2000) Stable-isotope probing as a tool in microbial ecology. Nature 403(6770):646–649. https://doi.org/10.1038/35001054

Roesch LFW, Fulthorpe RR, Riva A, Casella G, Hadwin AKM, Kent AD et al (2007) Pyrosequencing of 16S rRNA reveals contrasts in microbial diversity of four soils. ISME J 1(4):283–290. https://doi.org/10.1038/ismej.2007.53

Rogers SW, Moorman TB, Ong SK (2007) Fluorescent in situ hybridization and micro-autoradiography applied to ecophysiology in soil. Soil Sci Soc Am J 71(2):620–631. https://doi.org/10.2136/sssaj2006.0105

Schmalenberger A, Tebbe CC (2003) Bacterial diversity in maize rhizospheres: conclusions on the use of genetic profiles based on PCR-amplified partial small subunit rRNA genes in ecological studies. Mol Ecol 12(1):251–262. https://doi.org/10.1046/j.1365-294x.2003.01716.x

Sessitsch A, Hackl E, Wenzl P, Kilian A, Kostic T, Stralis-Pavese N et al (2006) Diagnostic microbial microarrays in soil ecology. New Phytol 171(4):719–735. https://doi.org/10.1111/j.1469-8137.2006.01824.x

Shi D, Xie C, Wang J, Xiong L (2021) Changes in the structures and directions of heavy metal-contaminated soil remediation research from 1999 to 2020: a bibliometric and scientometric study. Int J Environ Res Public Health 18(14). https://doi.org/10.3390/IJERPH18147358

van Eck NJ, Waltman L (2010) Software survey: VOSviewer, a computer program for bibliometric mapping. Scientometrics 84(2):523–538. https://doi.org/10.1007/s11192-009-0146-3

van Elsas JD, Duarte GF, Keijzer-Wolters A, Smit E (2000) Analysis of the dynamics of fungal communities in soil via fungal-specific PCR of soil DNA followed by denaturing gradient gel electrophoresis. J Microbiol Methods 43(2):133–151. https://doi.org/10.1016/s0167-7012(00)00212-8

Vandenkoornhuyse P, Ridgway KP, Watson IJ, Fitter AH, Young JPW (2003) Co-existing grass species have distinctive arbuscular mycorrhizal communities. Mol Ecol 12(11):3085–3095. https://doi.org/10.1046/j.1365-294x.2003.01967.x

Wagner M, Nielsen PH, Loy A, Nielsen JL, Daims H (2006) Linking microbial community structure with function: fluorescence in situ hybridization-microautoradiography and isotope arrays. Curr Opin Biotechnol 17(1):83–91. https://doi.org/10.1016/j.copbio.2005.12.006

Whiteley AS, Manefield M, Lueders T (2006) Unlocking the 'microbial black box'using RNA-based stable isotope probing technologies. Curr Opin Biotechnol 17(1):67–71. https://doi.org/10.1016/j.copbio.2005.11.002

Zhang Y, Zhang X, Liu X, Xiao Y, Qu L, Wu L, Zhou J (2007) Microarray-based analysis of changes in diversity of microbial genes involved in organic carbon decomposition following land use/cover changes. FEMS Microbiol Lett 266(2):144–151. https://doi.org/10.1111/j.1574-6968.2006.00511.x

Chapter 7
Biodecoloration of Synthetic Reactive Red and Reactive Black Dyes by Using *Aspergillus niger* and *Pleurotus ostreatus*

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Abstract The present study is alternative for removal of synthetic dyes particularly reactive red 120 and reactive black 5 dyes by *Aspergillus niger* and *Pleurotus ostreatus* by shake flask system for 7 days. The efficiency of decoloration of reactive red and reactive black, at room temperature (28 ± 2 °C), by *Aspergillus niger* are 78% and 66% respectively**.** Similarly, *Pleurotus ostreatus* shows 64 and 77% of decoloration for reactive red 120 and reactive black 5 respectively. The optimization of the decolorization efficiency was then carried out for the fungal colonies. The various physical parameters such as pH (3, 4, 5 and 6), temperature (30, 37 and 45 °C) and C:N ratio (1:2, 1:1, 2:1) were optimized to obtain maximum decolorization of the commercial reactive dyes by *Aspergillus niger* and *Pleurotus ostreatus.*

Keywords Reactive red 120 · Reactive black 5 · *Aspergillus niger* and *Pleurotus ostreatus* · Decolourisation

7.1 Introduction

Textile dye effluents pose environmental hazards because of color and toxicity. All dyes used in the textile industry are designed to resist fading upon exposure to sweat, light, water, many chemicals including oxidizing agents and microbial attack. In literature, there have been reported many fungal strains that are able to degrade dyes by their capacity to produce a range of enzymes (extra cellular enzymes like laccase, phenol oxidase, aryl oxidases, peroxidase, etc.). To name a few, fungal strains used in dye degradation are white rot fungus, *Phanerochaete chrysosporium*, *Geotrichum candidum*, *Trametes versicolor*, *Bjerkandera adusta*, various species of *Penicillium*, *Pleurotus ostreatus*, *Pycnoporus cinnabarinus*, *Pyricularia oryzae*,

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Aspergillus niger, etc. which can decolorize and/or biosorb diverse dyes (Martorell et al. 2017; Kaushik and Malik 2009a).

Evaluation of indigenous soil fungi for decolourization of textile dyes, Microbial decolourization of textile dyes through isolates obtained from contaminated sites (Raju et al. 2008; Kaushik and Malik 2009b) and decolorisation of reactive red 11 and 152 azo dyes under aerobic conditions reported by Kodam and Gawai (2006). Colour removal of textile dyes by culture extracts obtained from White- rot fungi was reported by Mehmet et al. (2009).

Large literature exists regarding the potential of these fungi to oxidize phenolic, non-phenolic, soluble and non-soluble dyes (Heinfling et al. 1998). In particular laccase from *Pleurotus ostreatus*, *Schizophyllum commune*, *Sclerotium rolfsii* and *Neurospora crassa*, seemed to increase up to 25% the degree of decolorization of individual commercial triarylmethane, anthraquinonic, and indigoid textile dyes using enzyme preparations (Siripong et al. 2009). This study focuses on the biological decolourization of textile effluents through microbial isolates obtained from contaminated sites. The fungal colonies were isolated from the effluent samples and the soil from the contaminated sites (Feng et al. 2014).

7.2 Materials and Methods

The dye used in this study was reactive red 120 and reactive red 5 dye obtained from Department of Textile Technology, Kumaraguru College of Technology, Coimbatore. The effluent was collected from a textile unit located in Erode, Tirupur and Salem district, Tamil Nadu, India.

7.2.1 Sample Collection

The dye effluent samples and soil sample from the dye contaminated sites were collected from various textile industries located in Perundurai, Tirupur and Salem district, Tamil Nadu. The isolation of fungal colonies from these samples was then carried out.

7.2.2 Isolation of Fungal Colonies from the Collected Effluent and Samples

The effluent dye samples were serially diluted and fungal colonies are isolated by using Potato Dextrose Agar (Hi-Media) plates by pour plate techniques and plates were incubated at room temperature (28 ± 2 °C) for 5 days (Ahmed et al. 2018).

7.2.3 Decolourization Studies

The isolated bacterial and fungal colonies were then studied for their ability to decolorize the effluent dyes. This assay was measured in the terms of reduction percentage using a spectrophotometer (Dos Santos et al. 2007).

Dye removal (%) was calculated as

$$\text{Dye removal (\%)} = \frac{\text{Initial absorbance} - \text{Final absorbance}}{\text{Initial absorbance}} \times 100$$

The dye removal % was calculated after 48 h and the fungal colonies with high decolorization efficiency were selected.

7.2.4 Decolorization Studies on the Isolated Fungal Colonies by Using Minimal Media

The decolorization studies were carried out for the isolated fungal colonies on a specialized media called Bushnell & Haas medium of the following composition (g/L): NH_4NO_3, 0.5; $MgSO_4 \cdot 7H_2O$, 0.1; K_2HPO_4, 0.5; NaCl, 1 g/L; glucose, 10; yeast extract, 2% and pH 6. Similarly, two synthetic dyes namely Reactive red 120 and reactive black 5. These dyes were prepared at a concentration of 50 mg/100 mL and the decolorization assay mixture consists of 10% of dye. The samples were withdrawn after 7 days and were centrifuged at 10,000 rpm for 15 min. The Absorbance of the supernatant was measured by using UV-visible spectrophotometer (ELICO, India).

7.2.5 Optimization of Decolorization of the Synthetic Dyes by Fungal Strains

The optimization of the decolorization efficiency was then carried out for the fungal colonies. The effect of pH (3, 4, 5 and 6), Temperature (30, 37 and 45 °C), C:N ratios (1:2, 1:1, 2:1) were studied and these parameters are optimized to obtain maximum decolorization of the commercial dyes using the fungal colonies.

7.3 Results and Discussion

7.3.1 Isolation of Fungal Colonies from the Effluent and Soil Samples

Totally about 13 different effluent and soil samples were collected from Perundurai, Tirupur and Salem district, Tamil Nadu. About 8 different fungal colonies were obtained from the samples. Out of 8 different fungal strains two are identified as *Aspergillus niger* and *Pleurotus ostreatus.*

7.3.2 Decolorization of the Synthetic Dyes Using the Selected Fungal Cultures

The screened fungal strains were then tested for their ability to decolorize the synthetic dyes. Two synthetic dyes namely reactive red and reactive black were used for these studies. The synthetic dye removal % of the screened by two fungal strains (Table 7.1).

7.3.3 Optimization of the Decolorization Efficiency for the Selected Fungal Strains

The optimization of the decolorization efficiency was then carried out for the screened fungal strains such as *Aspergillus niger* and *Pleurotus ostreatus.* The effect of various physical parameters such as pH, Temperature, C:N ratio and shaking conditions were studied. The optimum conditions for the decolorization of the synthetic dyes was determined (Kameche et al. 2022).

Table 7.1 Synthetic dye removal % of the fungal cultures—after 7 days

Culture name	Dye removal % Reactive red 120 (520 nm)	Dye removal % Reactive black 5 (574 nm)
Aspergillus niger	78	66
Pleurotus ostreatus	64	77

Table 7.2 Effect of temperature on the decolorization efficiency—after 7 days

Culture name	% Dye removal Reactive red 120 (520 nm)	% Dye removal Reactive black 5 (574 nm)
Incubation temperature: 30 °C		
Aspergillus niger	78	40
Pleurotus ostreatus	64	77
Incubation temperature: 37 °C		
Aspergillus niger	51	35
Pleurotus ostreatus	34	55
Incubation temperature: 45 °C		
Aspergillus niger	16	22
Pleurotus ostreatus	21	17

7.3.4 Effect of Temperature on the Decolorization Efficiency

The effect of temperature on the decolorization of the commercial dyes was analyzed at three different incubation temperatures 30, 37 and 45 °C. The decolorization % was calculated for the samples incubated at different temperatures and the following table gives the decolorization % after 7 days for the two fungal strains (Agrawal and Verma 2019) (Table 7.2).

The maximum dye removal was found to occur at the incubation temperature of 30 °C. Thus, the optimum temperature for decolorization for the three synthetic dyes by the fungal strains was found to be 30 °C (Ali and El-Mohamedy 2012).

7.3.5 Effect of pH on the Decolorization Efficiency

The effect of pH on the decolorization of synthetic dyes by the fungal strains was analyzed. The decolorization media was prepared at different pH 3, 4, 5 and 6 and the assay mixture decolorization % was calculated. The following table gives the synthetic dye removal % of the four fungal strains at different pH (Sosa-Martínez et al. 2020) (Table 7.3).

The maximum dye removal was found to occur at pH 6. Thus, the optimum pH for the decolorization of the synthetic dye by the fungal strains was determined to be 5.

Table 7.3 Effect of pH on the decolorization efficiency—after 7 days

Culture name	% Dye removal Reactive red 120 (520 nm)	%Dye removal Reactive black 5 (574 nm)
pH 3		
Aspergillus niger	17	15
Pleurotus ostreatus	22	10
pH 4		
Aspergillus niger	36	45
Pleurotus ostreatus	31	48
pH 5		
Aspergillus niger	78	61
Pleurotus ostreatus	64	77
pH 6		
Aspergillus niger	56	45
Pleurotus ostreatus	38	48

7.3.6 Effect of C:N Ratio on the Decolorization Efficiency

The effect of the C:N ratio on the decolorization efficiency was determined by using the decolorization media with different Carbon:Nitrogen source ratios such as 1:2, 1:1, 2:1. The following table gives the dye removal % of the fungal strains at different C:N ratios (Table 7.4).

The maximum dye removal % occurred at the C:N ratio of 1:2. Thus, the optimum C:N ratio for synthetic dye decolorization was determined to be 1:2 for the fungal strains (Mahmoud et al. 2017).

Table 7.4 Effect of C:N ratio on the decolorization efficiency—after 7 days

Culture name	% Dye removal Reactive red 120 (520 nm)	% Dye removal Reactive black 5 (574 nm)
1:1		
Aspergillus niger	11	14
Pleurotus ostreatus	12	22
2:1		
Aspergillus niger	29	52
Pleurotus ostreatus	36	21
1:2		
Aspergillus niger	56	85
Pleurotus ostreatus	48	51

7.4 Conclusion

Environmental pollution caused by the release of a wide range of compounds as a consequence of industrial progress has now assumed serious proportions. Textile dyes are one of the most prevalent chemicals in use today. With increasing usage of the wide variety of dyes in the industries pollution from the effluents has become increasingly alarming. Microbial decolorization and degradation is and environmentally friendly and cost—effective process. The fungal strains were isolated from the effluent and soil samples from the contaminated sites. These strains were subjected to initial screening through the decolorization studies on effluent dyes. The screened strains were then tested for their ability to decolorize three synthetic dyes Reactive Red 120 and Reactive Black 5 then final screening was done based on these studies. After final screening, 2 fungal strains were selected. The fungal strains were identified as *Aspergillus niger* and *Pleurotus ostreatus.* The various physical parameters such as temperature, pH, C:N ratio were optimized for the decolorization of the these two synthetic reactive dyes by *Aspergillus niger* and *Pleurotus ostreatus.*

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References

Agrawal K, Verma P (2019) Biodegradation of synthetic dye alizarin cyanine green by yellow laccase producing strain Stropharia sp. ITCC-8422. Biocatal Agric Biotechnol 21:101291. https://doi.org/10.1016/j.bcab.2019.101291

Ahmed PM, Pajot HF, de Figueroa LIC, Gusils CH (2018) Sustainable bioremediation of sugarcane vinasse using autochthonous macrofungi. J Environ Chem Eng 6:5177–5185. https://doi.org/10.1016/j.jece.2018.08.007

Ali NF, El-Mohamedy RSR (2012) Microbial decolourization of textile wastewater. J Saudi Chem Soc 16:117–123. https://doi.org/10.1016/j.jscs.2010.11.005

Dos Santos AB, Cervantes FJ, van Lier JB (2007) Review paper on current technologies for decolourisation of textile wastewaters: perspectives for anaerobic biotechnology. Bioresour Technol 98:2369–2385. https://doi.org/10.1016/j.biortech.2006.11.013

Feng C, Fang-yan C, Yu-Bin T (2014) Isolation, identification of a halotolerant acid red B degrading strain and its decolorization performance. APCBEE Proc 9:131–139. https://doi.org/10.1016/j.apcbee.2014.01.024

Heinfling A, Martínez MJ, Martínez AT, Bergbauer M, Szewzyk U (1998) Purification and characterization of peroxidases from the dye-decolorizing fungus *Bjerkandera adusta.* FEMS Microbiol Lett 165:43–50. https://doi.org/10.1111/j.1574-6968.1998.tb13125.x

Kameche K, Amrani S, Mouzaoui S, Aït-Amar H (2022) Biodegradation of diazo dye Evans blue by four strains of Streptomyces isolated from soils of Algeria. Biocatal Agric Biotechnol 46:102529. https://doi.org/10.1016/j.bcab.2022.102529

Kaushik P, Malik A (2009a) Fungal dye decolourization: recent advances and future potential. Environ Inter 35(127–141):127. https://doi.org/10.1016/j.envint.2008.05.010

Kaushik P, Malik A (2009b) Microbial decolourization of textile dyes through isolates obtained from contaminated sites. J Sci Ind Res 68:325–331

Kodam KM, Gawai KR (2006) Decolorisation of reactive red 11 and 152 azo dyes under aerobic conditions. Indian J Biotechnol 5:422–424

Mahmoud MS, Mostafa MK, Mohamed SA, Sobhy NA, Nasr M (2017) Bioremediation of red azo dye from aqueous solutions by *Aspergillus niger* strain isolated from textile wastewater. J Environ Chem Eng 5:547–554. https://doi.org/10.1016/j.jece.2016.12.030

Martorell MM, Pajot HF, Ahmed PM, de Figueroa LIC (2017) Biodecoloration of Reactive Black 5 by the methylotrophic yeast *Candida boidinii* MM 4035. J Environ Sci (China) 53:78–87. https://doi.org/10.1016/j.jes.2016.01.033

Mehmet A, Unyayar MA, Erkurt EA, Arkci NB, Bilen E, Ozyurt M (2009) Colour removal of textile dyes by culture extracts obtained from White rot fungi. Afr J Microbiol Res 3(10):585–589

Raju NS, Venkataramana GV, Girish ST, Raghavendra P, Shivashankar (2008) Isolation and evaluation of indigenous soil fungi for decolourization of textile dyes. J Appl Sci 7(2):298–300

Siripong P, Oraphin B, Sanro T, Duanpor P (2009) Screening of fungi from natural sources in Thailand for degradation of polychlorinated hydrocarbons. Am Eurasian J Agric Environ Sci 5:466–472

Sosa-Martínez JD, Balagurusamy N, Montañez J, Peralta RA, Moreira RFPM, Bracht A et al (2020) Synthetic dyes biodegradation by fungal ligninolytic enzymes: process optimization, metabolites evaluation and toxicity assessment. J Hazard Mater 400:123254. https://doi.org/10.1016/j.jhazmat.2020.123254

Chapter 8
Feasibility and Performance Evaluation of *Mentha Aquatica* in Treating Domestic Wastewater

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Abstract For life to exist, water is essential. Several conventional methods and combinatorial approaches are in practice to clean wastewater, which needs to be maintained. Additionally, wasted water must be treated and used for various purposes. Domestic wastewater is defined as water used for activities such as dish washing, laundry, and house cleaning. Different kind of ways to treat household wastewater. It can be purified using various techniques, including physical, chemical, biological, and natural processes. Physical methods require expert or qualified personnel for maintenance and control, along with the best equipment for use. Each tactic uses various techniques. The chemicals used in chemical wastewater treatment methods can cause harm to people. However, biological treatment requires both aerobic and anaerobic systems. *Mentha aquatica* was used in this study as a natural source for treating domestic wastewater. The chemical oxygen demand (COD), biological oxygen demand (BOD), and turbidity measurements were used to evaluate the water quality. It was noted that the chemical oxygen demand and biological oxygen demand readings had significantly decreased on days 11 and 21 of treatment. Thus, the observed data confirm *M. aquatica* as a potential replacement for current wastewater treatment methods.

Keywords Wastewater purification · Aquatic plants · Natural methods · BOD and COD reduction · Removal efficiency

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8.1 Introduction

Germs and hazardous contaminants found in sewage are capable of causing serious harm to the ecosystems and living beings through infections, severe illnesses and sometimes lethal (Muthukumaran et al., 2022, 2021a, b; Mahesh et al., 2022a, b). A high concentration of pollutants in wastewater decreases the water's quality and its nutrient compositions and assists in the spread of the disease (Priyadharshini et al., 2022; Anandaraj et al., 2018; Kanmani et al., 2017). An estimated 70% of the country's pure water sources are said to be tainted with bacteria. Additionally, India's water quality scores 120 of 122 countries, which is quite low. Due to microbiologically polluted water, several diseases, including cholera, diarrhoea, dysentery, hepatitis A, typhoid, and polio, can spread. This contamination causes significant changes in variables including pH, biological oxygen demand (BOD), chemical oxygen demand (COD), and the number of heavy metals in the water. Heavy metals are released into the environment from the gamut of process industries like food and pharmaceuticals, cosmetics, leather, and other chemical processing industries (Mahesh et al., 2022b; Manikandan et al., 2022). Water contamination can result in fatal complications from the release of industrial chemicals, poor maintenance, oil spills, sewage overflow, and other causes (Muthukumaran et al., 2021a, b). The contaminated water is highlighted as the cause of the increased rate of ailments. The WHO estimates that water contamination causes more than 4 lakh diarrhea-related deaths each year. Before this contaminated water from diverse sources may be used for domestic tasks like drinking, irrigation, farming, gardening, and toilet flushing, it must first be treated.

To clean up contaminated wastewater, numerous physical, chemical, biological, and natural techniques are available (Magar and Ansari, 2017; Gupta at al., 2012). This research aims to develop a natural approach for cleaning contaminated household water that can be applied to many tasks, including cleaning, gardening, and other things. Natural purifying agents, such as plants, are thought of as environmentally friendly ways to purify water because they unintentionally filter water (Foroughi et al., 2010; Dahija et al., 2019). Plants from both the land and the sea can be utilised as filters. Aquatic plants including hyacinth, azolla, duckweed, cattail, lotus (Abd Rasid et al., 2019) and poplar are frequently employed for wetland water remediation because of their high accumulation of heavy metals, high tolerance, or rapid growth and high biomass output (Musa and Idrus, 2021; Mustafa and Hayder, 2021). This filtration process has no negative side effects because it is a natural process. The use of water hyacinth for wastewater treatment in California, Florida, Massachusetts, and Texas are examples of this (Téllez et al., 2008; Lateef et al., 2013). Chemical methods involve adding purifying chemicals to the water. Chemical procedures include the removal of heavy metals, chelation, adsorption, and chlorination (Kumar et al., 2019; Raskin et al., 1997). Physical procedures, including filtration, distillation, and sedimentation, are also available (Dayana and Suresh Babu, 2021; Leiknes, 2009).

Depending on the industrial, agricultural, and municipal wastewater releasing operations, the contaminants found in the wastewater may vary. Pollutants are categorized into three major classes as listed inorganic, organic, and biological contaminants. Heavy metals that are toxic and carcinogenic are the most prevalent inorganic water pollutants (Balakumar et al., 2022; Lakherwal, 2014). Other compounds that can be quite harmful include nitrates, sulfates, phosphates, fluorides, chlorides, and oxalates. Fungicides, insecticides, and herbicides are the sources of the hazardous organic pollutants. Other polynuclear hydrocarbons (PAHs) that can contaminate materials include phenols, polychlorinated biphenyls, halogenated aromatic hydrocarbons, formaldehyde, poly-brominated biphenyls, biphenyls, detergents, oils, and greases. Regular hydrocarbons, alcohols, aldehydes, ketones, proteins, lignin, drugs, and other compounds are also present in the wastewater. Various bacteria that flourish in sewage may be the root of many diseases. Some of the potentially harmful microorganisms include bacteria, fungi, algae, plankton, viruses, and other worms. These pollutants in the water remain as suspended, solvated, or colloidal particles (Sudarsan et al., 2015). Aquatic plants that filter water already exist can eliminate these contaminants. According to a study (Avelar et al., 2014) evaluating the performance of the species Mentha aquatica in constructed wetlands of horizontal subsurface flow with regard to the removal of coliforms bacteria in an effluent from the primary treatment of sewage, the results are inferred to show positive results. As a result, the treatment of domestic waste water can be considered.

8.2 Materials and Method

8.2.1 Sample Collection

A *Mentha aquatica* plant sample was obtained from a nearby nursery and corroborated at the Central Council for Research in Siddha. The residential wastewater that was obtained after washing clothes and dishes was collected in a glass tank that measured 5 feet by 5 feet.

8.2.2 Wastewater Analysis

The amounts of pH, BOD, COD, turbidity, chlorine, nitrate (NO_3^-), and sulfate were measured in the collected residential wastewater sample. A pH meter is used to measure the pH, and a LAQUA twin nitrate meter is used to measure the nitrate (Saleh et al., 2020; Priya et al., 2012).

8.2.3 Determination of COD

The amount of oxygen, measured in milligrams, needed to stabilize or oxidize the oxidizable compounds contained in one litre of effluent under certain circumstances is known as the chemical oxygen demand of an effluent. A 2.5 mL tube was filled with 2.5 mL of the sample, 1.5 mL of potassium dichromate (0.25 N), a spatula of mercuric sulfate ($HgSO_4$), and 3.5 mL of COD acid. The tube was then heated to 150 °C in a COD reactor for two hours. After cooling, the sample was titrated with an indicator and standard ferrous ammonium sulfate (0.1 N). The last point is reddish brown in colour. The same procedure was used to create a blank sample using 2.5 mL of distilled water (Islam et al. 2014).

8.2.4 Determination of BOD

Prepare the deionized water-based 2000 mg/L BOD standard solution. WTW company provided BOD standard powder. The medium for the serial BOD concentrations was the solution. Apply the following equation to the designated BOD medium while adding the BOD solution at concentrations ranging from 0.0 to 1000 mg/L. Inoculation with natural or modified sludge is carried out after the addition of 164 mL of baseline medium and 50 mg/L of standard solution (C1) to 2,000 mg/L of C2 V2 in a BOD container (Inoculums). The OxiTop S12 digital electronic detectors are used to recover every bottle, and they are then placed in a rack shaker in an incubator for five days at 20 °C (Devi and Dahiya, 2008).

8.2.5 Turbidity Determination

Try to wait until the samples are at room temperature before analysing them if the turbidity is less than 40 units. Mix the sample thoroughly for even distribution of the solids. Pour the substance into the turbid meter tube once all the air bubbles have disappeared. The turbidity can be calculated using either the instrument scale or the proper calibration curve.

8.2.6 Phosphate Determination

Before adding it to the cell, the reaction mixture was boiled and cooled. The supporting electrolyte was made up of saturated ammonium acetate and acetic acid (5 mL each), was added to the combination of 10 mL. As opposed to a standard calomel electrode, the applied voltage was changed to +0.8 V during the interval.

The galvanometer spot was set to zero and the sensitivity control was set to an appropriate level. Up to 0.1 mL of the equivalence point's distance, the titrant thiomalic acid solution was added from the burette in 0.5 mL increments, and then for the remaining 2 mL in 0.5 mL increments. The gas stream was circulated through the solution for about a minute (more diluted solutions may take up to three minutes), then it was passed over the surface, and finally the galvanometer deflection was read to help with the precipitation of the copper ions after each addition (current). The volume of the solution has been changed in the current readings to account for the addition of the reagent. An amperometric titration curve was constructed using the obtained result, and the equivalency point was then determined by reading off the curve (Gaballah et al., 2019; Salem, 1996; Nivetha et al., 2016).

8.2.7 Chlorine Determination

A quick and incredibly accurate method for measuring free chlorine has been developed using differential pulse voltammetry and an Au working electrode. As per the literature, this standardized protocol is highly promising for monitoring the level of remaining free chlorine in drinking water. The electrochemical experiments were carried out in a separate compartment cell. Pt wire, an Ag/AgCl (saturated KCl) disc, and a 1.6 mm diameter Au disc were used as the working, reference, and counter electrodes, respectively. The Au working electrode's surface was pre-treated by being polished with an aluminium oxide suspension, followed by a meticulous sonication in water and a rinsing with Milli-Q water. A solution of 0.1 mol/dm^3 potassium chloride served as the supporting electrolyte. The DPV measurements were performed using the BD-101 electrochemical analyzer with a C-1B cell stand. For the DPV measurements, the pulse amplitude was set at 25 mV, and the scan rate was 20 mV/s. Potassium chloride was added to a 20 cm^3 water sample until the final concentration was 0.01 mol dm^{-3}. For all measurements, the thermostat was set at a temperature of around 25 °C (Ahmed 2011; Mangione and Carlyle, 2012).

8.3 Results and Discussion

For samples derived from home wastewater, levels of BOD, COD, turbidity, pH, chloride, phosphate, and nitrate were monitored. These factors were assessed over the course of 21 days, and the findings were tallied. To evaluate the effectiveness of the water mint for treating wastewater, the acquired findings were compared. Three different sets of values are noted. Before adding the water mint plant to the samples, the initial set of values for the aforementioned parameters was measured on day 0. Using the tests conducted on Day 11, the second set of data was determined. All the previously indicated parameters have clearly dropped. Additionally, a greater

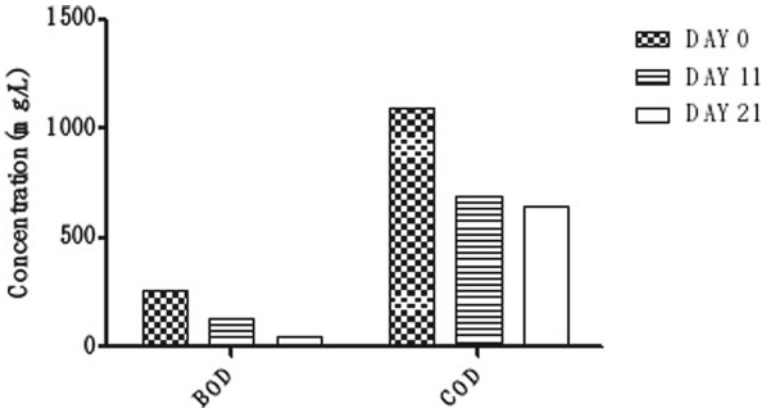

Fig. 8.1 BOD and COD values of domestic wastewater treated with *Mentha aquatica*

reduction was noted on the 21st day. The results and observations are discussed in this section.

8.3.1 BOD and COD

From day 0 to day 21, there was a gradual decrease in the capacity of water mint to remove BOD and COD. The BOD range initially is 263 mg/L, lowers to 136 mg/L on day 11, and finally drops to 49 mg/L on day 21 because of the plant's great capacity to absorb toxins from the residential wastewater. On day 0, the COD levels were roughly 1100, 697 mg/L on day 11, and 647 mg/L on day 21. The effectiveness of BOD removal on the eleventh day was 48.288%, exceeding the 44.4% figure of BOD removal recorded for Lotus. (Jyoti 2017). The BOD elimination efficiency was almost 64% after 21 days, which was somewhat closer to the value of 66.6% recorded for Lotus. Similar to this, after 11 days, COD elimination efficiency was 36.64%, and after 21 days, it was 7%. After 20 days, Lotus' COD elimination efficiency was reported to be 32%. This leads us to the conclusion that the first 11 days are when the COD for water mint is actively reduced (Fig. 8.1).

8.3.2 Turbidity

The domestic wastewater was turbid on day 0, which indicates that heavy metal pollution has caused the water to be significantly contaminated and seem to be a greyish black in colour. On day 0, a turbidity measurement of 319 NTU was taken. The turbidity gradually dropped to 36.8 NTU by the observation period's eleventh day, and by the twenty-first day it had totally vanished to 4.7 NTU. On days 11 and

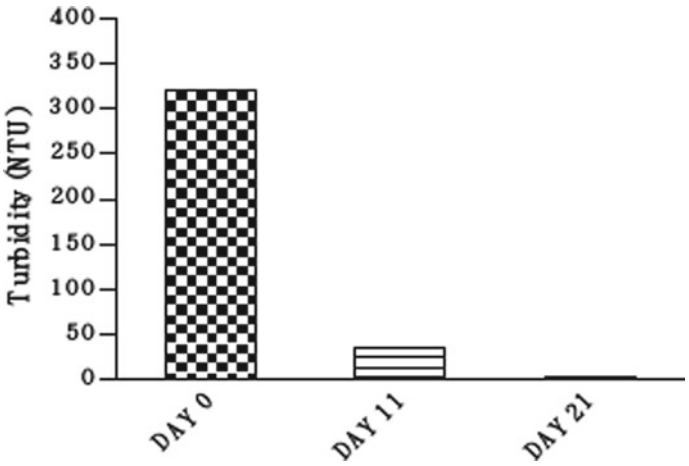

Fig. 8.2 Turbidity values of domestic wastewater treated with *Mentha aquatica*

21, respectively, the efficiencies of turbidity reduction were 88.46 and 87.23%. This was consistent with Lotus claimed TDS removal efficiencies, which were 87% and 86% after 10 days and 20 days, respectively (Jyoti 2017) (Fig. 8.2).

8.3.3 pH Value

From day 0 to day 21, the pH efficiency is shown in Fig. 8.3 to have gradually decreased. Due to the plant's exceptional ability to absorb contaminants from household wastewater, the pH range initially ranged from 8.34 to 7.66 on day 11 and 7.31 on day 21. At 25 °C, the pH of the home wastewater was determined. This consistent pH drop is consistent with the pH changes observed in water caltrop, which was employed to treat sewage from local governments (Kumar and Chopra 2018).

8.3.4 Removal Efficacy of Chlorides, Phosphorus and Nitrates

The chloride as Cl-starting value was around 1250 mg/L on day 0, and it swung to a range of 1799 mg/L on day 11, before decreasing to 1550 mg/L on day 21. The value changed because of the substance's water solubility and lack of biodegradability. The pollutants steadily decreased as the amount of chloride was in the wastewater (Fig. 8.4). Water mint was found to have 13.84% efficacy in removing chloride after 21 days, but Lotus, Water Caltrops, and Hydrilla have not yet been reported on.

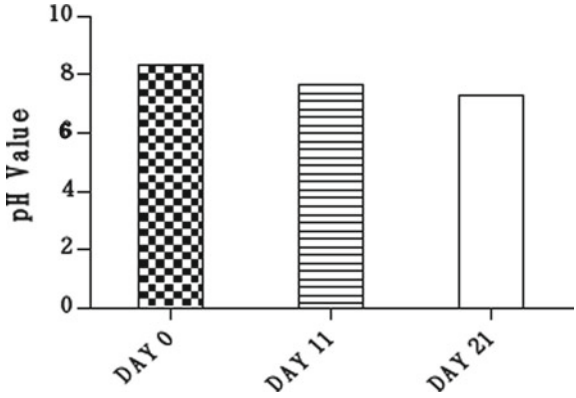

Fig. 8.3 pH value of domestic wastewater treated with *Mentha aquatica*

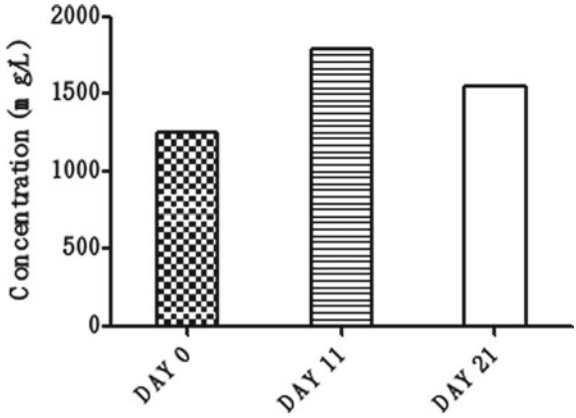

Fig. 8.4 Chloride concentration of domestic wastewater treated with *Mentha aquatica*

The overall phosphorous concentration of the detergents affects pH. On day 1, the total phosphorus level was roughly 0.32 mg/L, and by day 11, it varies between 0.17 and 0.17 mg/L. This serves as an example of the full phosphorous removal process. Phosphorus falls to 0.13 mg/L on the last day of observation. At the end of 11 days, the total phosphorous removal efficiency was 46.88%, and 23.53% at the end of 21 days. Lotus and Hydrilla had total phosphorous removal efficiencies of 66% and 65%, respectively, after 10.5 days (Kanabkaew and Puetpaiboon, 2004) (Fig. 8.5).

On the eleventh day of observation, Fig. 8.6 depicts the fluctuation in nitrate removal from home wastewater. On day eleven of observation, the value rose to 31 mg/L from an initial value of about 29 mg/L. The result had dropped to 27 mg/L on the twenty-first day of monitoring, notwithstanding a fluctuation on the eleventh day, which was seen. This variation in the nitrate concentration can be ascribed to the water mint's ability to remove ammonia from home wastewater, which necessitates the conversion of NH_3 to NO_3^- by active nitrification. Due to the de-nitrification process, we later see a decrease in the concentration of nitrate. Similar results were reported by Selvarani et al. (2015) for Duckweed.

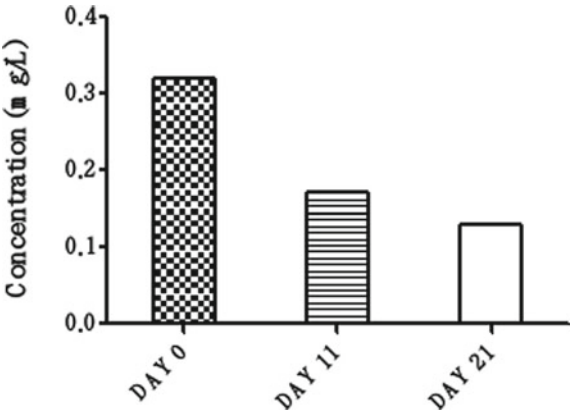

Fig. 8.5 Total phosphate concentration of domestic wastewater treated with *Mentha aquatica*

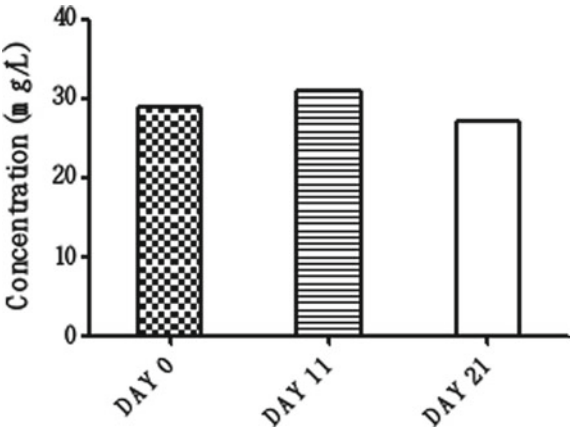

Fig. 8.6 Nitrate concentration of domestic wastewater treated with *Mentha aquatica*

Table 8.1 clearly shows that all parameters have significantly decreased after receiving treatment with *Mentha aquatica* for 21 days.

Table 8.1 Cumulative result of parameters of *Mentha aquatica* treated domestic wastewater

Parameters	Units	Day 0	Day 11	Day 21
pH value	–	8.34	7.66	7.31
Turbidity	NTU	319	36.8	4.7
Chlorides as Cl^-	mg/L	1250	1799	1550
BOD	mg/L	263	136	49
COD	mg/L	1100	697	647
Total phosphorus	mg/L	0.32	0.17	0.13
Nitrate as NO_3^-	mg/L	29	31	27

8.4 Conclusion

The study thus reveals the advantage of using water mint to clean domestic wastewater. The findings showed a sharp reduction in effectiveness from day 0 to day 21 for the criteria of BOD, COD, turbidity, and phosphorus. The BOD and COD levels fell from 263 to 49 and 1100 to 647 mg/L, respectively. Turbidity was reduced by 4.7 NTU, from 319 NTU. The pH readings ranged between 8.34 and 7.31. The effectiveness of the other criteria might also be impacted by environmental variables like nitrate and chloride. The value of the chlorides ranged from 1250 to 1550 mg/L, and the value of the nitrate dropped from 29 to 27 mg/L. The analyses cited above show that water mint is quite good at filtering out pollutants from home wastewater. Using water mint to filter water is convenient and eco-friendly.

References

Abd Rasid NS, Naim MN, Man HC, Bakar NA et al (2019) Evaluation of surface water treated with lotus plant; *Nelumbo nucifera*. J Environ Chem Eng 7(3):103048. https://doi.org/10.1016/j.jece.2019.103048

Ahmed AS (2011) Measurement of biological oxygen demand (BOD) in sewage wastewater using modified inoculums. J Agric Sci Technol A (1):244–248. https://doi.org/10.13140/RG.2.2.11764.60803

Anandaraj B, Eswaramoorthi S, Rajesh TP (2018) Chromium(VI) adsorption by *Codium tomentosum*: evidence for adsorption by porous media from sigmoidal dose-response curve. Int J Environ Sci Techno 15(12):2595–2606. https://doi.org/10.1007/s13762-017-1488-7

Avelar FF, de Matos AT, de Matos MP et al (2014) Coliform bacteria removal from sewage in constructed wetlands planted with *Mentha aquatica*. Environ Technol 35(16):2095–2103. https://doi.org/10.1080/09593330.2014.893025

Balakumar S, Mahesh N, Kamaraj M et al (2022) Outlook on bismuth-based photocatalysts for environmental applications: a specific emphasis on Z-scheme mechanisms. Chemosphere 303(1):135052. https://doi.org/10.1016/j.chemosphere.2022.135052

Dahija S, Bešta-Gajević R, Jerković-Mujkić A, et al (2019) Utilization of *Mentha aquatica* L. for removal of fecal pathogens and heavy metals from water of Bosna River, Bosnia and Herzegovina. Int J Phytoremediation 21(8):807–815. https://doi.org/10.1080/15226514.2019.1566883

Dayana PS, Suresh Babu P (2021) Advances in biopolymer composites and biomaterials for the removal of emerging contaminants. Phys Sci Rev. https://doi.org/10.1515/PSR-2021-0056

Devi R, Dahiya RP (2008) COD and BOD removal from domestic wastewater generated in decentralised sectors. Bioresour Technol 99(2):344–349. https://doi.org/10.1016/j.biortech.2006.12.017

Foroughi M, Najafi P, Toghiani A et al (2010) Analysis of pollution removal from wastewater by *Ceratophyllum demersum*. Afr J Biotechnol 9(14):2125–2128

Gaballah MS, Ismail K, Beltagy A et al (2019) Wastewater treatment potential of water lettuce (*Pistia stratiotes*) with modified engineering design. J Water Chem Technol 41(3):197–205. https://doi.org/10.3103/S1063455X1903010X

Gupta VK, Ali I, Saleh TA et al (2012) Chemical treatment technologies for waste-water recycling—an overview. RSC Adv 2(16):6380–6388. https://doi.org/10.1039/C2RA20340E

Islam BI, Musa AE, Ibrahim EH et al (2014) Evaluation and characterization of tannery wastewater. J For Prod Ind 3(3):141–150

Jyoti D (2017) Comparative study of the role of *Nelumbo nucifera* (sacred lotus) in phytoremediation for sewage untreated water from Khan River and Annapurna lake water at Indore, Madhya Pradesh. 3(5). https://doi.org/10.22623/IJAPSA.2017.3043.PQEHN

Kanabkaew T, Puetpaiboon U (2004) Aquatic plants for domestic wastewater treatment: lotus (*Nelumbo nucifera*) and Hydrilla (*Hydrilla verticillata*) systems. Aquatic 26(5):750

Kanmani P, Aravind J, Kamaraj M et al (2017) Environmental applications of chitosan and cellulosic biopolymers: a comprehensive outlook. Bioresour Technol 242:295–303. https://doi.org/10.1016/j.biortech.2017.03.119

Kumar S, Narayanasamy S, Venkatesh RP (2019) Removal of Cr (VI) from synthetic solutions using water caltrop shell as a low-cost biosorbent. Sep Sci Technol 54(17):2783–2799. https://doi.org/10.1080/01496395.2018.1560333

Kumar V, Chopra, AK (2018) Phytoremediation potential of water caltrop (*Trapa natans* L.) using municipal wastewater of the activated sludge process-based municipal wastewater treatment plant. Environ Technol 39(1):12–23

Lakherwal D (2014) Adsorption of heavy metals: a review. Int J Environ Sci Dev 4(1):41–48

Lateef SK, Soh BZ, Kimura K (2013) Direct membrane filtration of municipal wastewater with chemically enhanced backwash for recovery of organic matter. Bioresour Technol 150:149–155. https://doi.org/10.1016/j.biortech.2013.09.111

Leiknes T (2009) The effect of coupling coagulation and flocculation with membrane filtration in water treatment: a review. Res J Environ Sci 21(1):8–12. https://doi.org/10.1016/S1001-0742(09)60003-6

Magar R, Ansari M (2017) Waste water treatment using water hyacinth

Mahesh N, Balakumar S, Shyamalagowri S, et al (2022a) Carbon-based adsorbents as proficient tools for the removal of heavy metal from aqueous solution: a state of art—review emphasizing recent progress and prospects. Environ Res 213. https://doi.org/10.1016/j.envres.2022.113723

Mahesh N, Balakumar S, Danya U et al (2022b) A review on mitigation of emerging contaminants in aqueous environment using bio machines as sustainable tools: progress and limitations. J Water Process Eng 47:102712. https://doi.org/10.1016/j.jwpe.2022.102712

Mangione R, Carlyle D (2012) Chlorine: properties, applications, and health effects. Nova Science Publishers, New York

Manikandan A, Suresh Babu P, Shyamala Gowri S, et al (2022) Emerging role of microalgae in heavy metal bioremediation. J Basic Microbiol 62(3–4). https://doi.org/10.1002/jobm.202100363

Mishra S, Srivastava S, Tripathi RD, et al (2006) Lead detoxification by coon tail (*Ceratophyllum demersum* L.) involves induction of phytochelatins and antioxidant system in response to its accumulation. Chemosphere 65(6):1027–1039. https://doi.org/10.1016/j.chemosphere.2006.03.033

Musa MA, Idrus S (2021) Physical and biological treatment technologies of slaughterhouse wastewater: a review. Sustainability 13(9):4656. https://doi.org/10.3390/su13094656

Mustafa HM, Hayder G (2021) Recent studies on applications of aquatic weed plants in phytoremediation of wastewater: a review article. Ain Shams Eng J 12(1):355–365. https://doi.org/10.1016/j.asej.2020.05.009

Muthukumaran P, Suresh Babu P, Kamaraj M (2021a) Microplastics menace: the new emerging lurking environmental issue, a review on sampling and quantification in aquatic environments. Int J Environ Sci Techno. https://doi.org/10.1007/s13762-021-03591-w

Muthukumaran P, Suresh Babu P, Karthikeyan S (2021b) Tailored natural polymers: a useful eco-friendly sustainable tool for the mitigation of emerging pollutants—a review. Int J Environ Sci Techno 18:2491–2510. https://doi.org/10.1007/s13762-020-03048-6

Muthukumaran P, Suresh Babu P, Shyamalagowri S et al (2022) Polymeric biomolecules based nanomaterials: production strategies and pollutant mitigation as an emerging tool for environmental application. Chemosphere 307(4):136008. https://doi.org/10.1016/j.chemosphere.2022.136008

Muthukumaran P, Suresh Babu P, Shyamala Gowri S, et al Nanotechnological approaches as a promising way for heavy metal mitigation in an aqueous system. J Basic Microbio. https://doi.org/10.1002/jobm.202100365

Nivetha C, Subraja S, Sowmya R, Induja NM (2016) Water lettuce for removal of nitrogen and phosphate from sewage. IOSR J Mech Civ Eng 13(2):104–107

Priya A, Avishek K, Pathak G (2012) Assessing the potentials of Lemna minor in the treatment of domestic wastewater at pilot scale. Environ Monit Assess 184(7):4301–4307. https://doi.org/10.1007/s10661-011-2265-6

Priyadharshini DS, Kiruthiga R, Udayabhaskar R et al (2022) Graphene oxide-based nanomaterials for the treatment of pollutants in aquatic environment: recent trends and perspectives—a review. Environ Pollut 306(1):119377. https://doi.org/10.1016/j.envpol.2022.119377

Priyadharshini DS, Suresh Babu P, Manikandan S, et al Phycoremediation of wastewater for pollutant removal: a green approach to environmental protection and long-term remediation. Environ Pollut 290:117989. https://doi.org/10.1016/j.envpol.2021.117989

Raskin I, Smith RD, Salt DE (1997) Phytoremediation of metals: using plants to remove pollutants from the environment. Curr Opin Biotechnol 8(2):221–226. https://doi.org/10.1016/S0958-1669(97)80106-1

Saleh IA, Zouari N, Al-Ghouti MA (2020) Removal of pesticides from water and wastewater: chemical, physical and biological treatment approaches. Environ Technol Innov 101026. https://doi.org/10.1016/j.eti.2020.101026

Salem FB (1996) Determination of phosphate in water samples. Crit Rev Anal Chem 15(3):225–236. https://doi.org/10.1515/REVAC.1996.15.3.225

Selvarani AJ, Padmavathy P, Srinivasan A et al (2015) Performance of duckweed (Lemna minor) on different types of wastewater treatment. Int J Fish Aquat 2(4):208–212

Sudarsan JS, Roy RL, Baskar G et al (2015) Domestic wastewater treatment performance using constructed wetland Sustain. Water Resour Manag 1(2):89–96. https://doi.org/10.1007/s40899-015-0008-5

Téllez TR, López EM, Granado GL et al (2008) The water hyacinth, *Eichhornia crassipes*: an invasive plant in the Guadiana River Basin (Spain). Aquat Invasions 3(1):42–53. https://doi.org/10.3391/ai.2008.3.1.8

Chapter 9
Screening of Bacterial Isolates from Coal Mining Region in Chhattisgarh

Rajni Kumari, K. Harshan, Prashanth Rajan, Anand Prem Rajan, and Thomas Theodore

Abstract Microorganisms that can thrive under intense conditions have generated considerable interest in recent days on account of their exceptional physiology, nature, and essential industrial applications. This could be because of the special sequences that they possess in their DNA which can produce proteins with unrivalled biocatalytic properties. The present study aims at determining the microbial diversity of mine soil collected from Indian Coal Mines, South Eastern Coal Field Ltd (SECL), Korba district, Chhattisgarh. The soil samples were evaluated based on their color, texture and structure. Bacteria were isolated using iron salts-purified agar (ISP) media by serial dilution method and spread-plate method. The bacterial isolates were of yellow and brown colours. Microscopic observation revealed that the isolates were Gram-negative, rod-shaped bacteria. Bacteria were subjected to glucose test, lactose test, fructose test, triple sugar iron agar test, mannitol motility test, catalase test, oxidase test, citrate test, hydrogen sulphide test, phenylalanine deaminase test, indole test, methyl red test, and Voges–Proskauer test. Molecular assay of 16S rRNA sequencing revealed the isolate to be *Enterobacter cloacae.*

Keywords Microbial diversity · Coal-mine bacteria · *Enterobacter cloacae*

Misc Extremophilic Enterobacter cloacea screened and reported for the first time in the coal mines of Chhattisgarh

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9.1 Introduction

Potentially poisonous elements (PTEs) from diverse anthropogenic sources have found a home in soil. Since the beginning of time, it has served as a reservoir for a sizable number of PTEs due to the unlawful and negligent dumping of household, agricultural, mining, and industrial (Palansooriya et al. 2020; Zhou and Wang 2019). Coal harbors bacteria of multiple genera. Bacteria are found ubiquitously in the environment: at great heights, in deepest sea sediments, at extremes of heat and cold, and in highly contaminated sites. These bacteria found in coal mines are extremely rugged in nature, hence may prove to have numerous industrial applications. They also perform coal bio–beneficiation (Mukherjee et al. 2018) along with a role in many other biogeochemical processes. Coal mine soil has numerous strains of *Methanogen* sp. and *Thiobacillus* sp. (Brock 1978). The first bacterium *Thiobacillus ferrooxidans* was isolated from acidic bioleaching environments, (Colmer and Hinkle 1947), (Donati and Sand 2007). It is difficult to describe the exact role and significance of each organism in these active populations, as the intimate connection between sulphide biohydrometallurgy and microbial physiology is not completely understood (Belly and Brock 1974).

Overburden of coal mine spoil signifies disturbed habitat for the soil organisms due to its low pH and high internal temperature (Juwarkar et al. 2014). The quantity of Gram-negative bacteria from coal mine spoils of different geographical regions has also been reported (Marsh and Norris 1983). It was discovered that the Gram-negative bacteria were resistant to acidic environments as well as Fe, Cd, and Cr. They were recovered from acid mine drainage (AMD) of rat-hole coal mines (Ka-ot and Joshi 2022).

Heavy metal resistant bacteria were reported in Bokaro coalfield, Jharkhand soil samples as *Acinetobacter gyllenbergii* (KM029963; NK-7), *Enterobacter ludwigii* (KM029957; NK-1), *Enterobacter ludwigii* (KM029959; NK-3), *Enterobacter ludwigii* (KM029960; NK-4), *Enterobacter cloacae* (KM029962; NK-6), *Enterobacter cloacae* (KM029964; NK-8), *Klebsiella pneumonia* (KM029958; NK-2), *Klebsiella oxytoca* (KM029961; NK-5) (Gandhi et al. 2015).

In Singareni Collieries, Andhra Pradesh BDRC1 (*Bacillus* sp.) (KJ643909.1), BDRC2 (*Bacillus* sp.) (KJ643910.1), and BDRC3 *Bacillus* sp. (KJ643911.1) were the bacterial species that dominated the soil (Machulla et al. 2005). The bacteria found in mining areas are good for bioremediation of aquatic areas by accumulating metals in their cells via a process known as biosorption (Mishra and Ma 2005).

The largest area of acid sulfate soil materials in Finland which caused large-scale fish mortality in 2006 due to acid run-off. *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* were isolated from acid sulfate soil materials (Arkesteyn 1980).

"Potential acid sulfate soil materials" are metal sulphide, containing anoxic residues found various coastal areas of Australia and the Baltic (Rickard and Luther 2007).

Bacterial molecular studies are done by molecular sequencing. Isolates from Australian potential acid sulphate soil (PASS) and acid sulphate soil (ASS) had 16S rRNA gene sequences that showed similarity to the iron-oxidizing bacteria *Alicyclobacillus* species and *Thiomonas* species. The isolates from both PASS and ASS plates during molecular phylogenetic studies resulted in 40 operational taxonomic units (OTUs) (Ohba and Owa 2005).

Acidithiobacillus thiooxidans was first discovered in 1947, in an acid mine drainage in Japan. Nevertheless, experience from low-temperature sulphide mineral environments and acid mine drainage (AMD) has shown a significantly simpler mixed population in soil. A single acidophile capable of low-temperature growth on Fe^{2+} and/or inorganic sulphur compounds (ISCs) has been reported (Baker-Austin et al. 2007). The common bacteria *Acidithiobacillus ferrooxidans* (*A. ferrooxidans*) and *Acidiphilium multivorum* (*A. multivorum*), which mediate Fe^{2+} oxidation and Fe^{3+} reduction under an acidic environment, respectively, are found throughout acid mine drainage (Jin et al. 2020; Wang et al. 2020). The molecular evolution of boreal PASS and ASS isolates were reported from the Risofladan experimental field, Finland (Kupka et al. 2009).

Coal sources in India are mostly dispersed along different valleys such as Pench-Kanhan valley, Damodar valley, Wardha-Godavari valley, Sone-Mahanadi valley, etc. The greater part of the coal stores is situated in the south-eastern region of India bound between 24° North latitude and 78° East longitude in West Bengal, Chhattisgarh, Southern Uttar Pradesh, Eastern Madhya Pradesh, Odisha, and Jharkhand. Altogether, there are 57 coal mines present in these areas. The entire Gondwana coalfields range up to 64,000°km^2. Overall, 194 billion tons of coal reserves are predicted including non-coking coal reserves of approximately 165 billion tons (Anand and Prasad 2013).

Coal contains four different forms of sulphur, namely, organic sulphur-containing compounds of calcium and barium, pyritic sulphur (FeS_2), elemental sulphur, and sulphates of iron. Among these, organic and pyritic sulphur is the most commonly known. Acidic mine water is produced by bacteria *Thiobacillus thiooxidans* and *Thiobacillus ferrooxidans* that are present in coal deposits (Natarajan 1978). Chemoautotrophs such as *Thiobacillus ferrooxidans* oxidizes ferrous to ferric iron and forms sulphuric acid resulting in acid mine drainage (AMD):

$$4FeS_2 + 15O_2 + 2H_2O \xrightarrow{\text{Microbial Action}} 2Fe_2(SO_4)_3 + 2H_2SO_4$$

Microbial action is also affected by the coal types (lignite, bituminous, subbituminous, and anthracite) along with the matrix and contents of the coal matrix (Natarajan 1978).

Different mesophilic organisms, thermophiles and fungi were found to exist in coal-mining waste dumps (Tichý et al. 1998). These species have been found to play an active role in microbial desulphurization. Various strains of heterotrophs such as *Acinetobacter*, *Beijerinckia* sp., *Pseudomonas* sp., *Rhizobium* sp. execute organic sulphur oxidation.

Our in-depth literature survey has brought to light that the bacteria of Chhattisgarh coalfields are still unexplored. No industrially useful bacteria have ever been reported so far from these virgin soils, which prompted us to design and carry out this present research work.

9.2 Materials and Methods

9.2.1 Study Area

South Eastern Coalfields Limited (SECL), Korba, Chhattisgarh is a major coal-producing company of India. The company has its head office at Bilaspur. It has a total of 92 mining sites. There are 70 underground mines and 22 open-cast mines.

9.2.2 Sample Location Area

See Fig. 9.1.

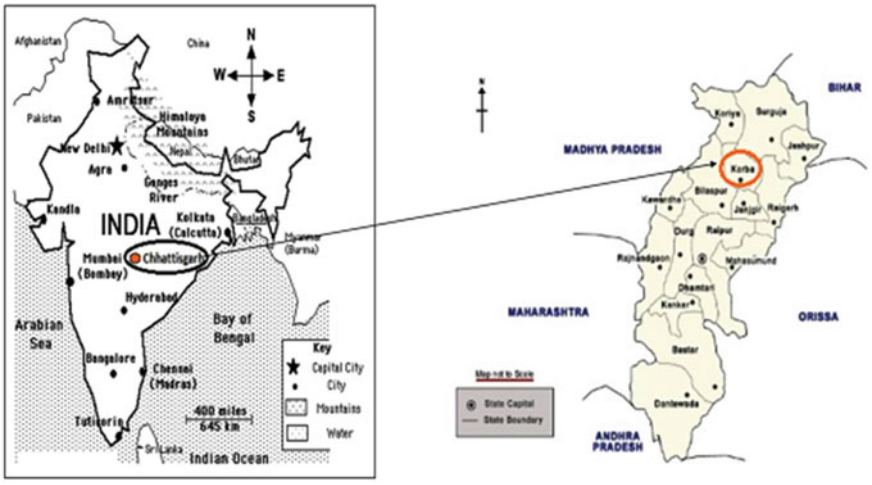


Fig. 9.1 Map of India and Korba district in Chhattisgarh, the location of South Eastern Coalfields Limited

Fig. 9.2 Sample collection area from a coal mine of South Eastern Coal Field Limited (SECL), Korba District, Chhattisgarh

9.2.3 Sampling Site

The mining soil samples were collected at 50 feet depth (22.35° N; 82.68° E) from South Eastern Coal Field Limited (SECL), Korba District, Chhattisgarh. The soil samples were collected in sterile ziplock bags and brought to our laboratory. The samples were examined for their physicochemical characteristics such as colour, texture, density, porosity, and pH (Fig. 9.2).

9.2.4 Growth Media and Culture Conditions

9.2.4.1 Iron Salt Purified Media (ISP)

Bacterial isolation from soil samples was carried out using Iron salts-purified agar (ISP) media.

ISP media was prepared using three sterilized solutions:

Solution A: Ferrous sulphate ($FeSO_4 \cdot 7H_2O$—33.4 g/L) was added to 300 mL of distilled water and the pH was adjusted to 2.5 using 6 M sulphuric acid (H_2SO_4). The solution was filter sterilized.

Solution B: Ammonium sulphate $(NH_4)_2SO_4$—6.0 g/L), potassium chloride (KCl—0.2 g/L), magnesium sulphate ($MgSO_4 \cdot 7H_2O$—1.0 g/L), calcium nitrate ($Ca(NO_3)_2$—0.02 g/L) were dissolved in 550 mL double-distilled water. The pH of the solution was adjusted to 3.0 and autoclaved.

Solution C: Purified agar was soaked in 150 mL of double-distilled water and autoclaved. Solution B was mixed with solution C with gentle mixing and then solution A was added to this mixture. The mixture was then poured into Petri plates and allowed to solidify.

9.2.4.2 Colony Forming Units (CFU)

5.0 g of soil samples were taken in Erlenmeyer flasks containing 50 mL of deionized double-distilled water. The plates were incubated at 28 °C in the dark for 48 h. The colony forming units (CFU) was calculated using the formula:

$$\text{CFU} = \frac{\text{Number of colonies}}{\text{Volume of solution taken}} \times \text{Dilution factor}$$

9.2.4.3 Bacterial Isolation

Soil samples mixed in ISP media were shaken rigorously and then subjected to serial dilution followed by spread plating. In order to isolate pure culture, isolated bacteria were grown in ISP media (Manning 1975). Cultures were streaked directly on ISP media to obtain isolated colonies. Individual colonies were cultured in broth at 30°C in Erlenmeyer flasks on a rotary shaker at 150 rpm.

9.2.4.4 Microscopic Examination

Gram staining was done and observed under 10× and 100× magnification.

9.2.4.5 Biochemical Characteristics of the Bacteria

The biochemical characteristics of the bacteria were studied. The extra-cellular enzymatic activities were performed by gelatin hydrolysis and starch hydrolysis. The intracellular enzyme activities were determined by the urease test, catalase reaction, indole production test, methyl red test, Voges-Proskauer test, citrate utilization test, and triple sugar iron test. Different carbon sources were used to study the effect of carbon source on the growth of bacteria.

9.2.4.6 Primers

From the purified genomic DNA, the 16S rRNA gene was amplified using polymerase chain reaction (PCR) with universal primers 27F [5′—AGA GTT TGA TCC TGG CTC AG—3′] and 1492R [5′—ACG GCT ACC TTG TTA CGA CTT—3′] (Mageswari et al. 2012).

9.2.4.7 16S rRNA Sequencing

The PCR reaction mixture of a total volume of 20.0 μL, containing 6.0 μL of nuclease-free water, 1.0 μL of forward primer, 1.0 μL of reverse primer, 2.0 μL of DNA, 10.0 μL of PCR master mix was prepared in a 200 μL PCR tube. The amplification was carried out in a thermal cycler and consisted of 30 cycles. It was performed under the following conditions: initial denaturation at 94 °C for 1 min, denaturation at 94 °C for 1 min, annealing at 49 °C for 1 min, extension at 72 °C for 1 min, final extension at 72 °C for 10 min. The PCR product (10.0 μL) was analyzed using 1.5% agarose gel stained with 5% 10.0 mg/mL ethidium bromide.

9.2.4.8 Phylogenetic Analysis

The analysis of the evolutionary pattern of genes was achieved by the construction of the phylogenetic tree. MEGA v5.2.2 (Molecular Evolutionary Genetics Analysis version 5.2.2) software was used for the construction of the phylogenetic tree. This software has the advantages of collection of databases, alignment of large sequences, and phylogenetic tree construction. This information can be used for evolutionary analysis in basic biology, biomedicine, and other evolution studies. The alignment of DNA sequences was used to perform the trace file editor option. MEGA 5.2.2 has derived models for the evaluation of nucleotide and amino acid substitutions. The trees are constructed using neighbor joining, maximum likelihood, maximum parsimony with bootstrap, and branch length. It computes distances by pair-wise method within and between groups. The maximum likelihood method is used for referring to evolutionary trees to select the best-fit substitution models for amino acid as well as nucleotide sequences to represent the evolutionary rate (Tamura et al. 2011).

9.3 Results and Discussion

The soil samples were brown to yellow in color with a pH of 7.2. The normal temperature during collection was 37 °C.

9.3.1 Colony Forming Units (CFU)

Very few colonies were obtained in the test samples. CFU was found to be 3.0×10^{-4} CFU/mL for 48 h.

Table 9.1 Colony morphology of the isolated bacteria from the coal mines of South Eastern Coal Fields Limited (SECL), Korba District, Chhattisgarh

	Bacteria	Colony morphology characterization		Selective media	Bacterial shape	Elevation	Size (mm)
		Colour	Shape				
1	Isolate 1	Yellow	Circular with irregular margin	ISP media	Rod	Convex	1
2	Isolate 2	Grey	Circular with irregular margin	ISP media	Rod	Flat	1
3	Isolate 3	White	Circular with regular margin	ISP media	Coccus	Pulvinate	2

9.3.2 Bacterial Isolation

Three distinct bacterial strains were obtained from the test samples. The colony morphology characterization of the isolates is provided in Table 9.1. From the obtained isolates, the pale-yellow colony forming bacteria was found to be the most dominant. Hence, pale-yellow colonies were chosen for identification and characterization due to their abundance in initial plating (Jamal et al. 2016).

9.3.3 Colony Morphology

Colony morphology is a significant feature for the isolated bacteria. Margins of the colony were irregular but circular with slight elevation for isolate 1. The pale yellow pigmentation may be due to the oxidation of ferrous ions in the ISP media.

9.3.4 Microscopic Examination

The isolates were found to be Gram-negative rods around 2.6 μm long and 1.0 μm wide. The cells were motile and possessed flagella.

Table 9.2 Biochemical characteristics of the isolated bacteria from coal mine soil

Biochemical test	Result
Glucose test	Positive
Lactose test	Negative
Triple sugar iron agar	Positive
Mannitol motility test	Positive
Catalase test	Positive
Oxidase test	Negative
Citrate test	Positive
Hydrogen sulphide test	Negative
Phenylalanine deaminase test	Negative
Indole test	Negative
Methyl red test	Negative
Voges-Proskauer test	Positive

9.3.5 Biochemical Assay

The biochemical characterization was performed for Isolate-1 as a vital part in the characterization of the isolated bacteria. Table 9.2 consolidates the result of reactions of isolate-1 during various biochemical tests.

The results of biochemical tests show that isolate-1 may be from the *Enterobacteriaceae* family.

9.3.6 16S rRNA Bacterial Sequencing

Molecular analysis was done to confirm the bacterial species. The assembled sequence is given in Fig. 9.3.

The obtained sequence was found to be:

AAACAGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAACGGTA
ACAGGAAGCAGCTTGCTGCTTCGCTGACGAGTGGCGGACGGGTGAGTAATGTCTG
GGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAA
CGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCGGATGTGCCCAG
ATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGG

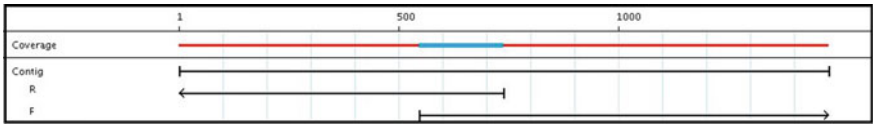


Fig. 9.3 The assembled sequence for isolate-1

TCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGG
AGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGC
GTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGTGTT
GAGGTTAATAACCTCAGCAATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCC
GTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGC
GTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGGCTCAACC
TGGGAACTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAGAGGGGGGTAGAATTC
CAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGG
CCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGAT
TAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTG
AGGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGC
AAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGG
TTTAATTCGATGCAACGCGAAGAACCTTACCTACTCTTGACATCCAGAGAACTTAGC
AGAGATGCTTTGGTGCCTTCGGGAACTCTGAGACAGGTGCTGCATGGCTGTCGTCA
GCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTT
GTTGCCAGCGGTCCGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGG
AAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGC
TACAATGGCGCATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAA
GTGCGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCT
AGTAATCGTGGATCAGAATGCCACGGTGAATACGTTCCCGGGCCTTGTACACACCG
CCCGTCACACCATGGGGAGTGGGTTGCAAAAGAAGTAGGTAGCTTAACCTTCGGG
AGGGCGCTTACCACTTTGTGATTCATGACTGGGGTGAGC

The analysis of the sequence fragments was done by using Blast algorithm at NCBI (www.ncbi.nlm.nih.gov/nuccore/KC990822.1). The complete 16S rRNA gene sequence of the isolated *Enterobacter cloacae* strain DRA1 genomic sequence has been deposited under the accession number KJ947344.

9.3.7 Phylogenetic Analysis

The phylogenetic analysis result of *Enterobacter cloacae* genes is presented in Fig. 9.4. The phylogenetic tree of 4 bacterial genes showed an independent evolution of *Enterobacter cloacae* compared to *Citrobacter murliniae*. *Citrobacter murliniae* is resistant to metal ions (Boechat et al. 2017). In the bootstrap test, the percentage of replicating trees for 4 bacterial sequences is represented next to the branches. The genes of *Enterobacter* showed homology with that of *Citrobacter*, *Leclercia,* and *Salmonella* bacteria. The results show that the evolution of Isolate-1, i.e., *Enterobacter cloacae* including these 3 bacteria originated from the same ancestor as they are present in one clad (Fig. 9.5).

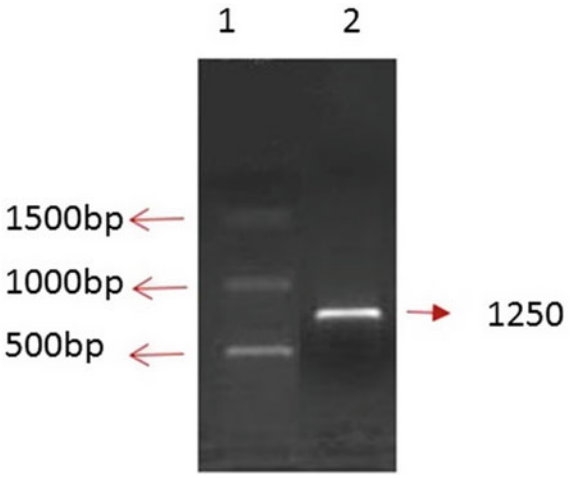


Fig. 9.4 16S rRNA sequencing of the isolated bacterial colony (expected size 1250 bp) Lane 1. 1 kb DNA ladder, Lane 2. Amplified DNA product

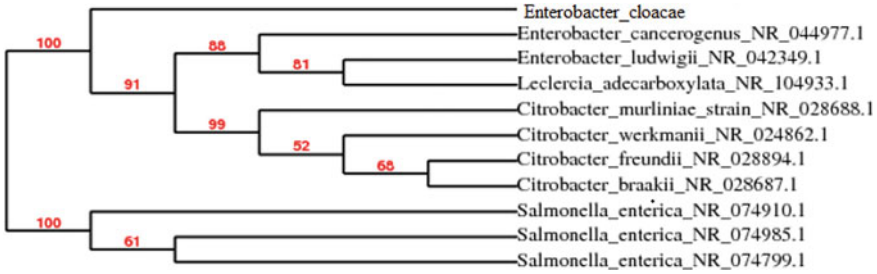
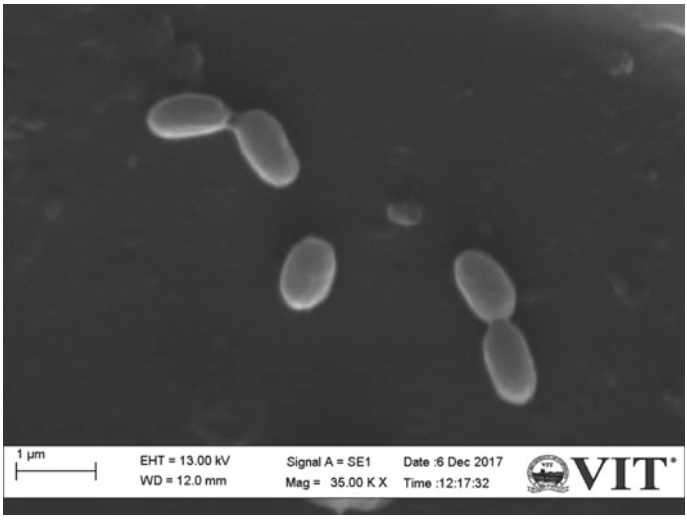


Fig. 9.5 The phylogenetic tree for *Enterobacter cloacae* gene in coal mine bacteria

The optimal temperature of culture for Isolate-1, i.e., *E. cloacae* was observed to be 30–37 °C and the optimal pH was observed over a wide range (pH 5–9). The bacteria could suitably be utilized for bioremediation at optimized temperature and pH conditions (Panda and Sarkar 2012).

Enterobacter strains grown in ISP medium has been reported to show faint growth, which demonstrated that they might not be strict chemolithotrophs, but rather they could still use this media even without any hydrocarbon source. This stress-tolerance ability may be credited to their abundance in the coal mine (Arnold et al. 2004).

9.3.8 Scanning Electron Microscopy (SEM)



The Scanning electron micrograph shows that the isolate-1, namely, *Enterobacter cloacae* was 1 µm in length.

9.4 Conclusion

In conclusion, *Enterobacter cloacae* was found to be the most abundant bacteria out of the three isolates obtained from the mining soils of South Eastern Coal Field Limited, Korba District, Chhattisgarh. This bacterium was isolated and grown on ISP media which exhibited pale yellow colonies. Biochemical studies and molecular studies confirmed it to be *Enterobacter cloacae*. As this strain is isolated from a harsh mining environment, it mimics the chemolithotrophs. It has a vast industrial potential for bioremediation, e-waste treatment, metal leaching, and bioleaching applications. Further studies need to be carried out on the growth kinetics of the isolate and its industrial applications.

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Conflict of Interest On behalf of all the authors, the corresponding author states that there is no conflict of interest.

References

Anand MR, Prasad DN (2013) Recent trends in production and import of coal in India. https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Anand+MR%2C+Prasad+DN+%282013%29+Recent+trends+in+production+and+import+of+coal+in+India.+Springer+11%3A+1-14&btnG=

Arkesteyn GJMW (1980) Pyrite oxidation in acid sulphate soils: the role of microorganisms. Plant Soil 54(1):119–134. https://doi.org/10.1007/BF02182004

Arnold JW, Boothe DH, Suzuki O, Bailey GW (2004) Multiple imaging techniques demonstrate the manipulation of surfaces to reduce bacterial contamination and corrosion. J Microsc 216(3):215–221. https://doi.org/10.1111/J.0022-2720.2004.01415.X

Baker-Austin C, Dopson M, Wexler M, Sawers RG, Stemmler A, Rosen BP, Bond PL (2007) Extreme arsenic resistance by the acidophilic archaeon "Ferroplasma acidarmanus" Fer1. Extremophiles 11(3):425–434. https://doi.org/10.1007/S00792-006-0052-Z

Belly RT, Brock TD (1974) Ecology of iron oxidizing bacteria in pyritic materials associated with coal. J Bacteriol 117(2):726–732. https://doi.org/10.1128/JB.117.2.726-732.1974

Boechat CL, Giovanella P, Amorim MB, de Sá ELS, de Oliveira Camargo FA (2017) Metal-resistant rhizobacteria isolates improve Mucuna deeringiana phytoextraction capacity in multi-metal contaminated soils from a gold mining area. Environ Sci Pollut Res 24(3):3063–3073. https://doi.org/10.1007/S11356-016-8103-1

Brock TD (1978) The habitats, pp 12–38. https://doi.org/10.1007/978-1-4612-6284-8_2

Colmer AR, Hinkle ME (1947) The role of microorganisms in acid mine drainage: a preliminary report. Science 106(2751):253–256. https://doi.org/10.1126/SCIENCE.106.2751.253

Donati E, Sand W (2007) Microbial processing of metal sulfides. https://doi.org/10.1007/1-4020-5589-7.pdf

Gandhi VP, Priya A, Priya S, Daiya V, Kesari J, Prakash K, Jha K, Kumar K, Kumar N (2015) Isolation and molecular characterization of bacteria to heavy metals isolated from soil samples in Bokaro Coal Mines, India. Pollution 1(3), 287–295. https://jpoll.ut.ac.ir/article_53929.html%0Ahttp:/jpoll.ut.ac.ir/article_53931.html

Jamal Q, Ahmed I, Rehman SU, Abbas S, Kim KY, Anees M (2016) Isolation and characterization of bacteria from coal mines of Dara Adam Khel, Pakistan. Geomicrobiol J 33(1):1–9. https://doi.org/10.1080/01490451.2014.964886

Jin D, Wang X, Liu L, Liang J, Zhou L (2020) A novel approach for treating acid mine drainage through forming schwertmannite driven by a mixed culture of Acidiphilium multivorum and Acidithiobacillus. Elsevier. https://www.sciencedirect.com/science/article/pii/S0304389420310979

Juwarkar AA, Misra RR, Sharma JK (2014) Recent trends in bioremediation, pp 81–100. https://doi.org/10.1007/978-3-642-41837-2_5

Ka-ot AL, Joshi SR (2022) Application of acid and heavy metal resistant bacteria from rat-hole coal mines in bioremediation strategy. J Basic Microbiol 62(3–4):480–488. https://doi.org/10.1002/JOBM.202100241

Kupka D, Liljeqvist M, Nurmi P, et al (2009) Oxidation of elemental sulfur, tetrathionate and ferrous iron by the psychrotolerant Acidithiobacillus strain SS3. Elsevier. https://www.sciencedirect.com/science/article/pii/S0923250809001594

Machulla G, Bruns MA, Scow KM (2005) Microbial properties of mine spoil materials in the initial stages of soil development. Soil Sci Soc Am J 69(4):1069–1077. https://doi.org/10.2136/SSSAJ2004.0271

Mageswari A, et al (2012) Optimization and immobilization of amylase obtained from halotolerant bacteria isolated from solar salterns. Elsevier. https://www.sciencedirect.com/science/article/pii/S1687157X12000388

Manning HL (1975) New medium for isolating iron-oxidizing and heterotrophic acidophilic bacteria from acid mine drainage. Appl Microbiol 30(6):1010–1016. https://doi.org/10.1128/AM.30.6.1010-1016.1975

Marsh R, Norris PR (1983) The isolation of some thermophilic, autotrophic, iron-and sulphur-oxidizing bacteria. Academic.Oup.Com. https://academic.oup.com/femsle/article-abstract/17/1-3/311/548097

Mishra R, Ma ZY (2005) Friction stir welding and processing. Elsevier. https://www.sciencedirect.com/science/article/pii/S0927796X05000768

Mukherjee D, Selvi VA, Ganguly J, Ram LC, Masto RE (2018) Exploratory study of archaebacteria and their habitat in underground, opencast coal mines and coal mine fire areas of Dhanbad. J Geol Soc India 91(5):575–582. https://doi.org/10.1007/S12594-018-0907-9

Natarajan KA (1978) Desulphurisation of coals with Thiobacillus ferrooxidans. Repository.Ias.Ac.In. http://repository.ias.ac.in/72404/

Ohba H, Owa N (2005) Vertical distribution of physico-chemical properties and number of sulfur-oxidizing bacteria in the buried layer of soil profiles with marine-reduced sulfur compounds. Soil Sci Plant Nutr 51(3):379–388. https://doi.org/10.1111/J.1747-0765.2005.TB00043.X

Palansooriya KN, Shaheen SM, Chen SS, Tsang DCW, Hashimoto Y, Hou D, Bolan NS, Rinklebe J, Ok YS (2020) Soil amendments for immobilization of potentially toxic elements in contaminated soils: a critical review. Environ Int 134:105046. https://doi.org/10.1016/J.ENVINT.2019.105046

Panda J, Sarkar P (2012) Isolation and identification of chromium-resistant bacteria: test application for prevention of chromium toxicity in plant. J Environ Sci Health - Part A Toxic/Hazard Subst Environ Eng 47(2):237–244. https://doi.org/10.1080/10934529.2012.640895

Rickard D, Luther GW (2007) Chemistry of iron sulfides. Chem Rev 107(2):514–562. https://doi.org/10.1021/CR0503658

Tamura K, Peterson D, et al (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Academic.Oup.Com. https://academic.oup.com/mbe/article-abstract/28/10/2731/973375

Tichý R, Lens P, Grotenhuis JTC, Bos P (1998) Solid-state reduced sulfur compounds: environmental aspects and bio-remediation. Crit Rev Environ Sci Technol 28(1):1–40. https://doi.org/10.1080/10643389891254188

Wang X, Li Q, Liao Q, Yan Y, Xia J, Lin Q, Wang Q (2020) Arsenic (III) biotransformation to tooeleite associated with the oxidation of Fe (II) via Acidithiobacillus ferrooxidans. Elsevier. https://www.sciencedirect.com/science/article/pii/S0045653520302733

Zhou XY, Wang XR (2019) Impact of industrial activities on heavy metal contamination in soils in three major urban agglomerations of China. J Clean Prod 230:1–10. https://doi.org/10.1016/J.JCLEPRO.2019.05.098

Chapter 10
Analysis of Hedychium Flavum Waste Powder as a Potent Heavy Metal Adsorbent

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Abstract *Hedychium flavum,* a medicinal, edible, and ornamental plant, is widely cultivated in India. Essential oils from the plant are used for various medical purposes and the leftover plant residue after oil extraction is discarded as a waste. An attempt was made to explore the used of this waste to remove heavy metals from wastewater was analysed. Result shows that the waste powder can be used effectively for metal removal. *Hedychium flavum* sequence was submitted to the NCBI database to procure accession numbers MN988619.

Keywords *Hydicum flavum* · *RSM* · Optimization software · Metal removal · Modelling

10.1 Introduction

Agricultural residues are a renewable resource that can help alleviate our planet's pressing environmental issues sustainably and economically. Numerous studies have shown that this type of agricultural waste, both cheap and abundant, has great promise as an adsorbent for removing various contaminants from waste streams. Hazardous metal contamination in water has long persisted as a severe concern to human health and aquatic life due to the persistence of heavy metals in nature. Heavy metals are released into water sources typically caused by leakage of chemical pollution, mineral processing, and many other forms of manufacturing activity. Although trace amounts of various transition metals, e.g., copper, nickel, and lead, are required for live organisms, their excessive levels may induce detrimental effects, including neurological diseases, respiratory failure, birth deformities, or even death in extreme

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cases. Conventional treatment procedures, such as oxidation/reduction, coagulation/flocculation, reverse osmosis/membrane filtering, ion exchange, and electrochemical approaches, are either too expensive or too inefficient to be widely used for the decontamination of heavy metals.

However, the adsorption process using activated carbon as an adsorbent is particularly favored because of its high efficiency, simple operation, immunity to harmful chemicals, reusability, and scope of use. Adsorption process efficiency is very sensitive to adsorbents' properties and the choice of optimal operating conditions. The characteristics of the input materials and the method of manufacture often govern the former. In contrast, the latter demands selecting an optimal combination of several influencing parameters, typically established by extensive experimentation. However, the number of experiments required can be drastically cut down by using optimization techniques. One of the most important multivariate techniques used in analytical optimization is response surface methodology (RSM), which is a collection of mathematical and statistical methods based on fitting empirical models to the experimental data collected from a set of predefined trials. To determine which experiments need to be conducted in the investigated experimental region to assess the independent variable effects and their interactions with the fewest possible trials, BBD is the most frequently used matrix design among those with a wide range of potential properties and characteristics.

The Hedychium plant genus is ornamental and ethnomedicinal, encompassing around 90 species, disseminated across South-East Asia. *Hedychium* species are a potent origin of numerous components with medical and allied industrial appliances. After extraction of oil and other components the wastes of the plant parts are discarded. The current work focused on the use of Hedychium waste on the metal adsorption capabilities (Raj et al. 2013; Thomas et al. 2015).

10.2 Materials and Methods

10.2.1 Materials

Processed Hedychium waste was collected from a local industry. The waste was washed with distilled water and sun dried.

10.2.2 Adsorption Experiments

Adsorption experiments were carried out in the flasks containing 50 mL of aqueous solution of chromium metal ion. The heavy metals levels were confirmed by AAS analysis. The removal efficiency and the adsorption capacity were calculated as follows:

Table 10.1 Design parameters

Factor	Name	Units	Minimum	Maximum
A	pH		1	5
B	Adsorbent dosage	g/L	0.100	1.0
C	Cr concentration	mg/L	50	500

$$\text{Chromium removal } (\%) = [(\text{Ci} - \text{Cf})/\text{Ci}] * 100 \tag{10.1}$$

where Ci and C f are the initial and equilibrium M (II) concentrations (ppm) respectively.

10.2.3 RSM-BBD Experiemntal Design

In order to obtain responses across the entire factor space and determine the region of optimal or near-optimal response via a sequence of designed experiments, response surface methodology is widely applied due to its potency as a powerful tool that combines the benefits of mathematical and statistical techniques. To identify the best conditions for the adsorption process, the current research used response surface methodology (RSM) along with Box Behnken design (BBD). The adsorption capacity of the adsorbent was used as the output variable, with the concentration of metal ions (Chromium) (A), the dosage of the adsorbent (B), and the pH (C) serving as the input factors (Table 10.1).

Analysis of variance (ANOVA) of the quadratic polynomial re- gression models was utilized to identify the significance of input variables as well as the relationship between the responses and the influential factors. In this study, the ANOVA was calculated using Design-Expert version 11.

10.3 Results and Discussions

Experimental design was created to explore the contribution of three influential factors: pH, meatal ion concentration and adsorbant concentration (Factor a, b, c) on the sorption capabilities of *Hedychium flavum* waste powder on chromium metal (Response R) [Table 10.2]. The response metal sorption % was caluated using Eq. 10.1.

To prove the model fitness, the analysis of variance (ANOVA) was done (Table 10.3). The R (%) response was statistically significant with F–value of 56.61 and p–value of 0.0001. The results elucidated that adsorbant dosage (*Hedychium flavum* waste powder) considerably impacts the R (%) response followed by AC: pH with the combination of meatl concentraiton). There was good congruity of the outcome

Table 10.2 Experimental runs and metal sorption response

	Factor A	Factor B	Factor C	Response R
Run	A: pH	B: Adsorbent dosage	C: Cr concentration	%removal
		g/L	mg/L	
1	5	0.55	50	80
2	5	0.1	275	41
3	1	0.55	50	62
4	3	0.55	275	72
5	3	1	50	88
6	5	1	275	90
7	5	0.55	500	69
8	3	0.55	275	73
9	3	0.55	275	73
10	3	0.1	50	40
11	3	0.55	275	73
12	3	1	500	85
13	3	0.55	275	73
14	1	0.1	275	55
15	1	0.55	500	82
16	3	0.1	500	53
17	1	1	275	91

of the 17 experimental runs which can be observed in the normal plot (Fig. 10.1). The relationship between actual and predicted values of R (%) response is illustrated in Fig. 10.2.

For optimization of the variables, the BBD-RSM was utilized, and 17 runs of experiments were created. By the quadratic model (Eq. 10.2) the meatal sorption percentage (R %: response) for optimized values of the factors can be calculated using Eq. 10.2.

$$\%removal = 72.8 + -1.25 * A + 20.625 * B + 2.375 * C + 3.25 * AB + -7.75 * AC + -4 * BC + 1.6 * A^2 + -5.15 * B^2 + -1.15 * C^2 \quad (10.2)$$

The 3D surface and contour plots of the interaction of pH with adsorbant dosage and metal concentration on the metal sorption capacity are depicted in Figs. 10.3, 10.4 and 10.5). For maximal adsorption of the metal using the sorbent the optimal pH was 2.7 and the adsorant dosage was 0.89 g a maximal removal of 97.5% was achieved which was validated with an independent run.

Table 10.3 ANOVA table RSM BBD design

Source	Sum of squares	df	Mean square	F-value	p-value	
Model	3934.07	9	437.12	56.61	< 0.0001	significant
A-pH	12.50	1	12.50	1.62	0.2439	
B-adsorbent dosage	3403.12	1	3403.12	440.74	< 0.0001	
C-Cr concentration	45.13	1	45.13	5.84	0.0463	
AB	42.25	1	42.25	5.47	0.0519	
AC	240.25	1	240.25	31.11	0.0008	
BC	64.00	1	64.00	8.29	0.0237	
A^2	10.78	1	10.78	1.40	0.2760	
B^2	111.67	1	111.67	14.46	0.0067	
C^2	5.57	1	5.57	0.7212	0.4238	

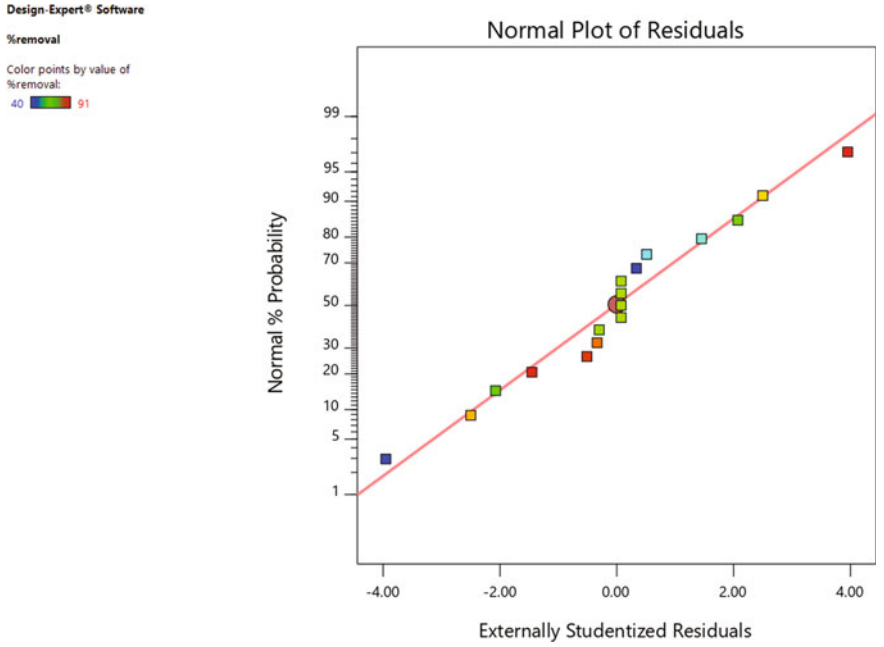

Fig. 10.1 Normal plot

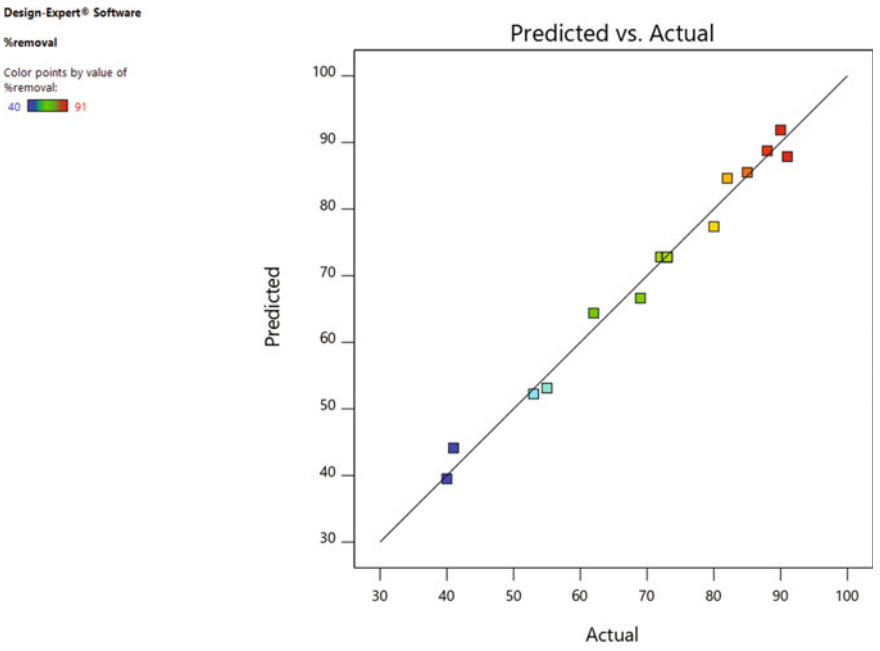

Fig. 10.2 Actual versus predicted

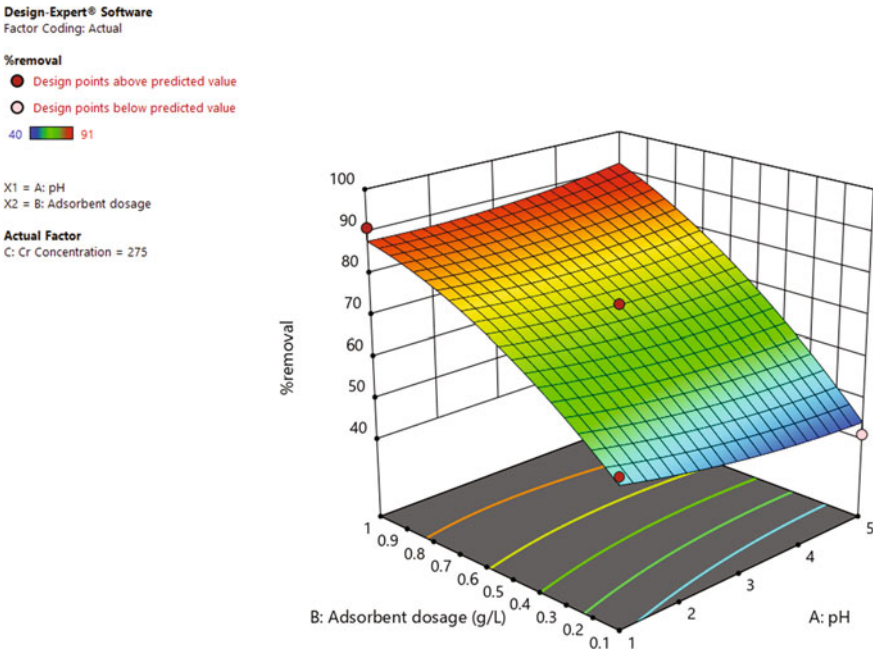

Fig. 10.3 Adsorbant dosage and pH impact on metal sorption.

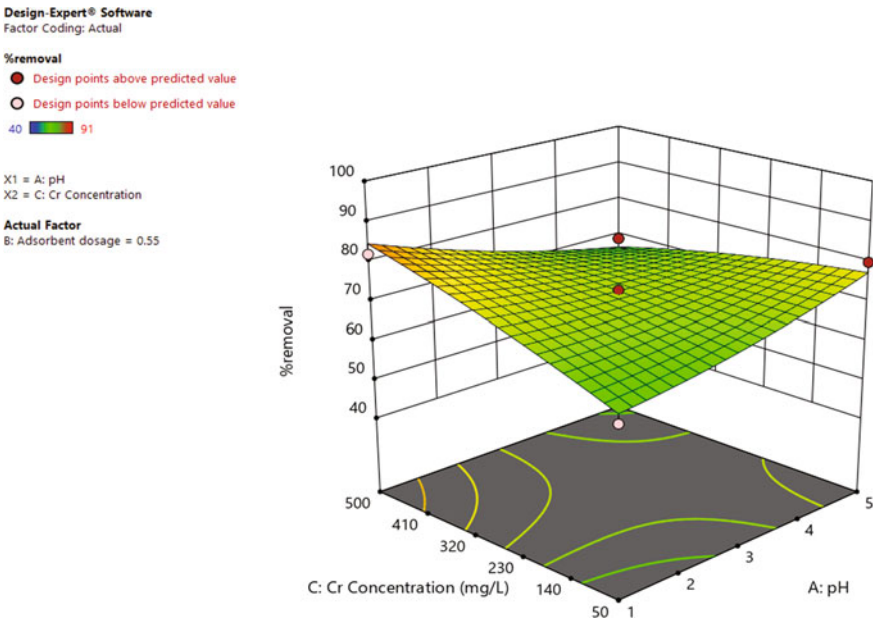

Fig. 10.4 Metal concentration and pH impact on sortion

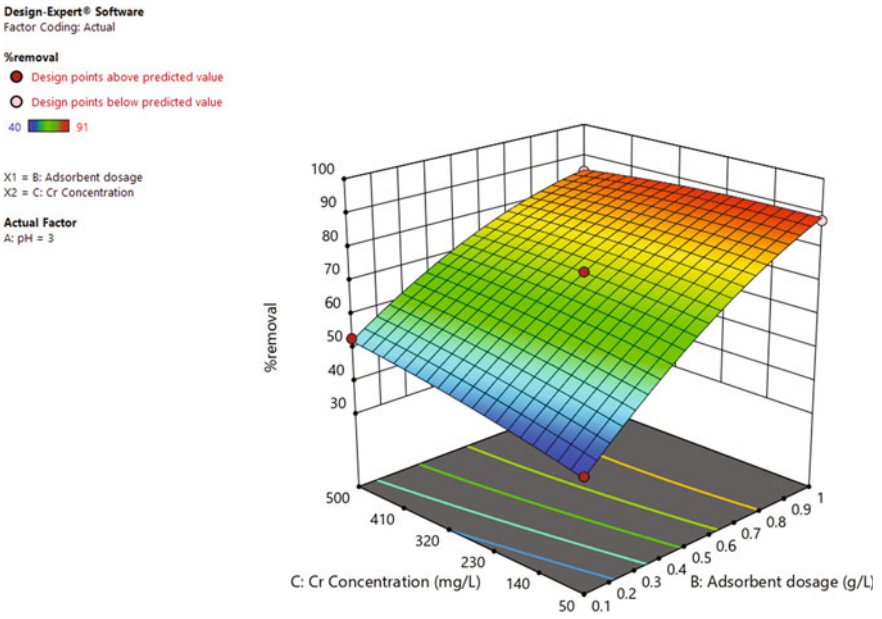

Fig. 10.5 Metal concentration and adsorant dosage impact on sorption

10.4 Conclusion

This study is the first to show that the waste from *Hydicum flavum* can be a good adsorbant to remove metals from waste streams as a maximal removal of 97.5% of metal removal can be achieved under the optimal pH of 2.7 and adsorbant dosage of 0.89 g.

References

Raj G, Dan M, George V, Sethuraman MG (2013) Studies on chemical composition of essential oils from leaf and inflorescence of *Hedychium larsenii*. J Essent Oil Res 25(1):33–38. https://doi.org/10.1080/10412905.2012.744701

Thomas S, Mani B, Britto SJ (2015) A new species of Hedychium (Zingiberaceae) from the southern Western Ghats, India. Webbia 70(2):221–225. https://doi.org/10.1080/00837792.2015.1082270

Chapter 11
Impact of Acinetobacter Baumannii on Dye Degradation and a Molecular Analysis Study

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Abstract *Acinetobacter baumanni* was isolated from polluted soil. An attempt to study the *A. baumannii* to degrade dye was explored. It was found effective against azo dye and was able to completely degrade the dye under 48 h in a shake flask. Molecular analysis on the isolate *A. baumanni* was performed together with *Hedychium flavum*, and the sequence was submitted to the NCBI database to procure accession number MT192652.1. Response surface Methodology-Box-Behnken design (RSM-BBD) was used to optimize the condition and achieve 98–99% dye decolorization.

Keywords *Acinetobacter baumanni* · Dye degradation · Optimization · Molecular analysis

11.1 Introduction

The demand on every industry to provide enough consumer goods for the world's now-staggering 8 billion people was brought on by the Industrial Revolution, which aided in the population expansion that led to that number. The textile sector is vital to meeting the needs of a growing global population. An estimated one million tonnes of dyes and pigments are produced and consumed each year, with a sizable amount of these synthetic colors ultimately finding their way into the environment via wastewater discharges. The environment and human health are in grave danger due to this. Therefore, much water is consumed in the textile industry during the dying process, and the resulting effluent contains a lot of organics and hazardous chemicals. Furthermore, the dissolved oxygen levels of aquatic life are negatively impacted when this wastewater is dumped into water bodies without treatment. Thus, even at 1 ppm, dyes can contribute to diminished oxygen solubility and sunlight dispersion,

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which are essential for aquatic life (Arora 2014; Barikbin et al. 2017). As a result, these effluents must be treated to remove the colors before being released into waterways. Recent reports have highlighted the public health risk posed by wastewater discharges containing the dye, which can cause eye burns, nausea, vomiting, and diarrhea. Numerous treatment technologies have been developed for decolorizing and degrading dyes in wastewater; hence, it is crucial to find an effective treatment method for wastewater containing dyes. Adsorption, reverse osmosis, ultrafiltration, chlorination, and biological approaches, including aerobic and anaerobic treatments, are also examples of such processes. However, most of these conventional pollution control methods only relocate the pollutants from one phase to another rather than transforming them into a less harmful product (Unnikrishnan et al. 2018).

Compared to conventional approaches, bioremediation is a safer, cheaper, greener, and more practical option for dealing with textile waste (Fletcher et al. 2021). Using microbes in the form of bacteria, fungi, algae, yeast, and mixed culture for bioremediation is an exciting new field (Li et al. 2019). The bacteria have an advantage in treating textile effluents due to their capacity to adapt and break down textile effluents at high concentrations. Bacterial methods for degrading synthetic dyes, especially azo dyes, may be helpful (Mishra et al. 2020). Research and development cannot proceed without isolating, identifying, and preserving various dye-degrading bacteria. To decolorize and break down azo dyes, scientists have experimented with a wide range of oxidoreductive enzymes (Srinivasan et al. 2017). Thus, optimizing the significance and interaction among protein ligands can be done cheaply through molecular docking and simulation. Dye decolorizing bacterial systems for real-time wastewater treatment in the textile industries can be effectively screened using this combinatorial approach by first employing bioinformatics tools, then conducting analysis in a wet lab (Gao et al. 2018).

The application of response surface methodology (RSM) has been validated as an efficient way to optimize processes involving several interrelated variables. When designing, modeling, and optimizing a process, the RSM, known as a Box Behnken design (BBD), has proven to be effective in estimating the correlation coefficient for a specific model, resulting in lower experimental errors and better data fitness.

This study focuses on the dye degradation capability of the isolated bacteria, enhancing its potential via optimizing process parameters like the inoculum load, temperature, and dye concentration.

11.2 Materials and Methods

11.2.1 Material

Coimbatore, Tamil Nadu, India, is home to a sizable textile sector, where we sourced our azo dye. The dye has a molecular weight of 967.5 g/mol and an absorption wavelength of 530 nm.

11.2.2 Sample Collection and Isolation of Dye-Degrading Bacteria

Under aerobic circumstances, samples of textile effluent were gathered in Coimbatore, Tamil Nadu, India. Samples that had been adequately diluted were plated on Luria–Bertani (LB) agar that contained azo dye. For additional research, the bacterial strain that could stand up to 10 g/L of the dye was chosen. The chosen bacterial isolates' capacity to discolor was confirmed (Shah 2016).

11.2.3 16S rRNA Sequencing and Submission

Using a kit, genomic DNA was isolated from the bacterium (Gislin et al. 2018). In order to find closely identical sequences, the 16S rRNA sequence was then entered into a National Center for Biotechnology Information (NCBI) blast similarity search engine.

11.2.4 Decolorization Studies

One milliliter of the isolated bacterial strain was added to a flask containing 50 mL 500 mg/L of azo dye amended in LB broth and incubated at 37 °C for 3 days. With the aid of an ultraviolet–visible spectrophotometer, the degree to which a dye lost its color was determined by observing changes in the dye's absorbance at 530 nm (Satar and Husain 2011). The percentage rate of dye decolorization, E, was calculated using the formula:

$$E = [(A_0 - A_t)/ A_0] * 100\% \quad (11.1)$$

where A_0 is the preliminary absorbance and A_t is the ultimate absorbance.

11.2.5 Immobilization of Potential Isolate

Calcium alginate beads were used to immobilize the potential isolate. Beads made of calcium alginate are durable and may be re-used after being immobilized by encapsulation. Hot water was added until wholly dissolved, followed by adding 30 g/L of sodium alginate and 5% of the bacterial culture while stirring frequently. The cell/sodium alginate combination was extruded drop by drop into a chilled, sterile calcium chloride solution using a syringe to produce calcium alginate beads with a diameter of 2 mm. $CaCl_2$ and sodium alginate are combined to create calcium

Table 11.1 Variables chosen for RSM optimization

Factor	Name	Units	Type	Minimum	Maximum
A	Temperature	Celsius	Numeric	25	35
B	Immobilized inoculum	g/L	Numeric	0.1	0.5
C	Incubation time	hours	Numeric	12	36

alginate beads. For increased hardness and durability, calcium alginate beads were resuspended in a calcium chloride solution and chilled in the refrigerator for 24 h.

11.2.6 Optimization of Process Variables

Using the Response Surface Methodology (RSM), statisticians and mathematicians can forecast ideal circumstances with the fewest runs. We studied the connections between temperature, incubation period, and inoculum volume using a three-level factorial Box-Behnken design based on the one factor at a time (OFAT) technique (BBD). The experimental intervals used in the BBD are shown in Table 11.1.

11.3 Results and Discussion

11.3.1 Isolation of Potential Bacteria and Identification

Decolorization effectiveness was evaluated after isolating a bacterial strain that can survive in solutions containing up to 10 g/L of azo dye. It was observed that the bacteria entirely removed maximal color up to 98% (Prasad and Aikat 2014; Permpornsakul et al. 2016). 16S rRNA sequencing helped identify the isolate as *A. baumannii*. *A. baumannii* is a gram-negative bacterium that can be detected in soil and water samples on rare occasions. Few studies have explored the potential of this bacteria for decolorizing dyes. GenBank now contains the sequence in the entry as Accession no. MT192652.1. Results from a phylogenetic study are shown in Fig. 11.1 (seq 1) (Unnikrishnan et al. 2018).

1 cgagcggggg tagggagctt gctactggtc ctagcggcgg acgggtgagt aatgcttatg.
61 aatctgccta ttagtggggg acaacatctc gaaagggatg ctaataccgc atacgtccta.
121 cgggagaaag caggggatct tcggaccttg cgctaataga tgagcctaag tcggattagc.
181 tagttggtgg ggtaaaggcc taccaaggcg acgatctgta gcgggtctga gaggatgatc.
241 cgccacactg ggactgagac acggcccaga ctcctacggg aggcagcagt ggggaatatt.
301 ggacaatggg gggaaccctg atccagccat gccgcgtgtg tgaagaaggc cttatggttg.
361 taaagcactt taagcgagga ggaggctact gtaattaata cctatggata gtggacgtta.
421 ctcgcagaat aagcaccggc taactctgtg ccagcagccg cggtaataca gagggtgcga.
481 gcgttaatcg gatttactgg gcgtaaagcg tgcgtaggcg gctttttaag tcggatgtga.

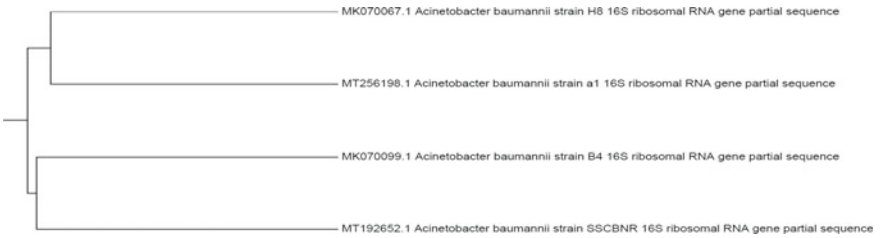

Fig. 11.1 Phylogenetic tree for *Acinetobacter baumanni* (Acc.No. MT192652)

541 aatccccgag cttaacttgg gaattgcatt cgatactggt gagctagagt atgggagagg.
601 atggtagaat tccaggtgta gcggtgaaat gcgtagagat ctggaggaat accgatggcg.
661 aaggcagcca tctggcctaa tactgacgct gga.

Sequence 1: 16 s RNA partial Sequence of Acinetobacter.

11.3.2 Optimization Using Box-Behnken Design

Optimizing decolorization processes with BBD is standard practice. For example, in 2013, Papadopoulou et al. explored the potential of *Pleurotus pulmonaris* AMRL 177 with BBD for decolorizing Remazol brilliant blue, Fazli et al. (2010) employed BBD to enhance Ganoderma species-based reactive blue 19 decolorization. Time, temperature, and inoculum volume were used in the BBD experiment. Table 11.2 details the design, while Table 11.3 provides an ANOVA for the response surface model.

$$\%removal = 82.4 + 0.625 * A + 18 * B + 10.875 * C + 0.25 * AB + -1.5 * AC + 0.25 * BC + -1.95 * A^2 + -7.7 * B^2 + -3.45 * C^2$$

When R1 represents the response, A, B, and C represent temperature, immobilized inoculum amount, and incubation period, respectively, while the other variables represent the interaction of one variable against another. Design expert software was utilized to simulate the design and analyze the results of the testing runs. The model's F-value of 58.26 suggests that it is significant at p0.0001 (Table 11.3). The most critical model terms in the current model are B, C, B2, and C2. Based on the normal-plot, actual vs. predicted plots, the actual and expected outcomes (response- dye decoloration) were good (Figs. 11.2 and 11.3). The outcome was equivalent to that of Unnikrishnan et al. (2018).

It is also possible to predict and compare the interaction of the design parameter on each other and their effect on the dye decolorization evident from the 3-D contour plots (Figs. 11.4a, b, and c) (Papadopoulou et al. 2013).

Table 11.2 Experimental runs with dye decolorization outcome

	Factor 1	Factor 2	Factor 3	Response 1
Run	A: Temperature	B: Immobilized Inoculum	C: Incubation time	% Removal
	Celsius	g/L	Hours	
1	25	0.3	12	61
2	30	0.1	12	45
3	25	0.3	36	88
4	30	0.3	24	82
5	35	0.5	24	91
6	25	0.1	24	55
7	25	0.5	24	93
8	30	0.1	36	64
9	35	0.1	24	52
10	30	0.3	24	83
11	35	0.3	12	69
12	30	0.3	24	83
13	30	0.5	12	78
14	30	0.3	24	82
15	30	0.3	24	82
16	30	0.5	36	98
17	35	0.3	36	90

Table 11.3 ANOVA analysis information

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	3891.11	9	432.35	58.26	<0.0001	Significant
A-Temperature	3.13	1	3.13	0.4211	0.5371	
B-Immobilized inoculum	2592.00	1	2592.00	349.26	<0.0001	
C-Incubation time	946.13	1	946.13	127.49	<0.0001	
AB	0.2500	1	0.2500	0.0337	0.8596	
AC	9.00	1	9.00	1.21	0.3072	
BC	0.2500	1	0.2500	0.0337	0.8596	
A^2	16.01	1	16.01	2.16	0.1853	
B^2	249.64	1	249.64	33.64	0.0007	
C^2	50.12	1	50.12	6.75	0.0355	

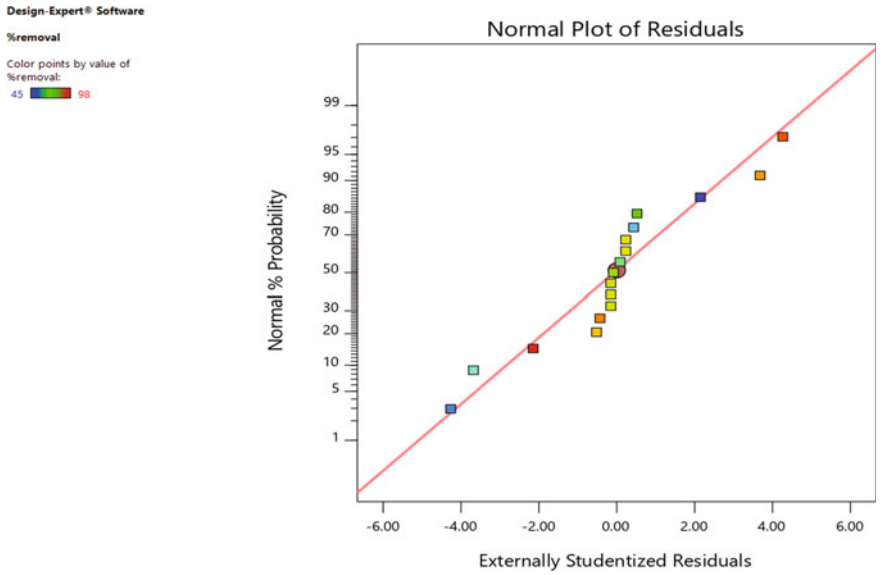

Fig. 11.2 Normal plot

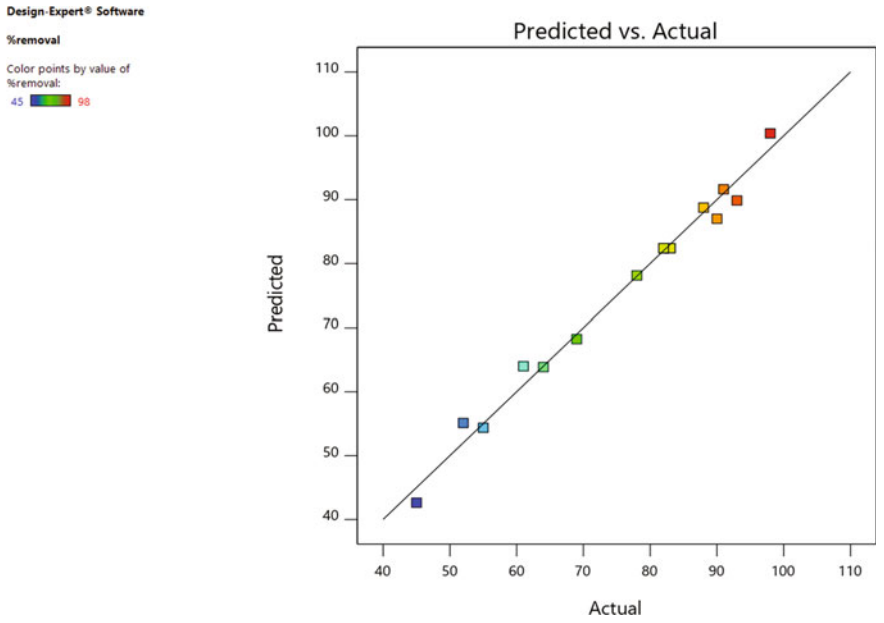

Fig. 11.3 Predicted versus actual plot

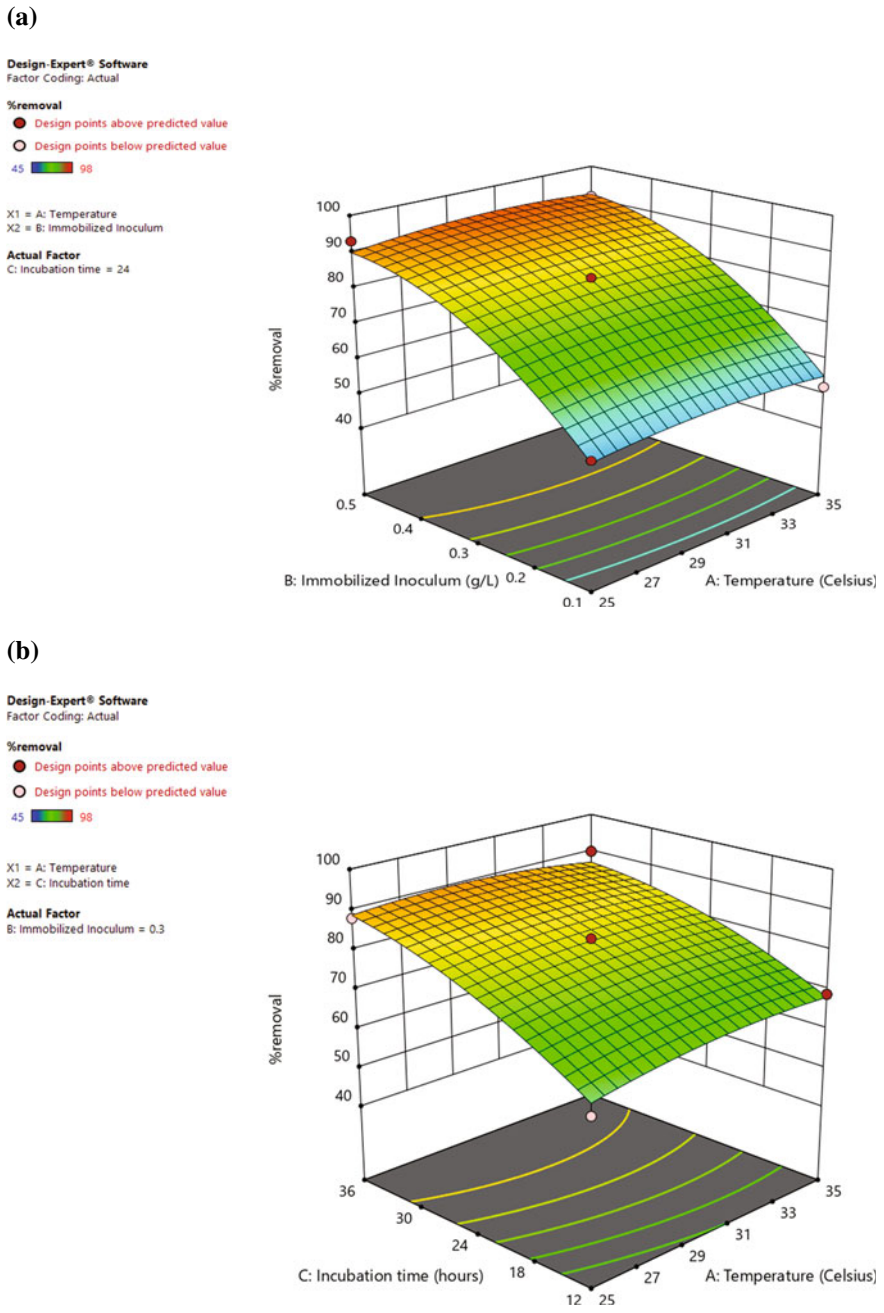

Fig. 11.4 **a** 3D contour plot of temperature versus immobilized inoculum on dye decolorization **b** 3D contour plot of temperature vs. incubation time on dye decolorization **c** 3D contour plot of incubation time vs. immobilized inoculum on dye decolorization

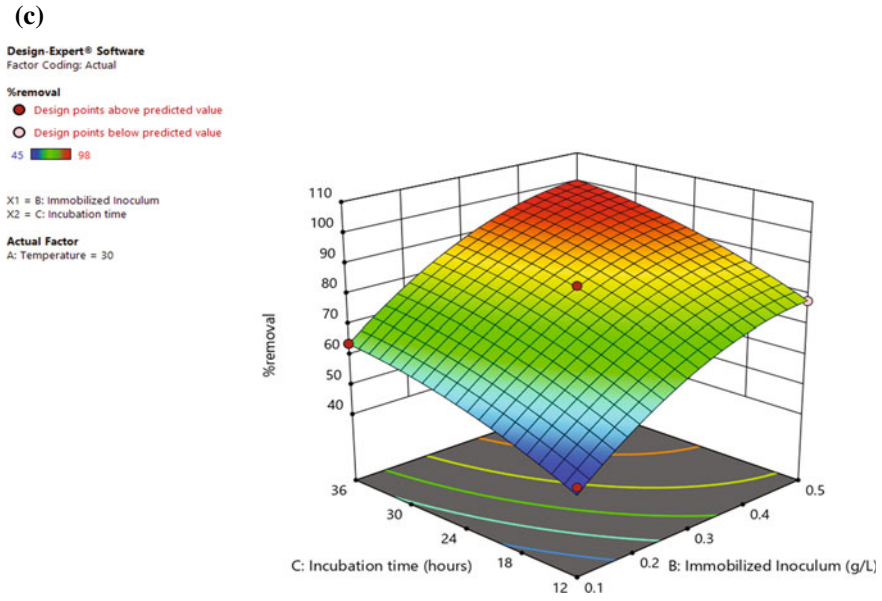

Fig. 11.4 (continued)

11.3.3 Model Validation and Experimental Confirmation

The RSM BBD output was validated with an independent run to achieve peak dye decolorization. For this dye decolorization, the experimental decolorization rate (99.15%) was achieved for an optimized condition of 28 °C with an inoculum load of 450 mg/L and an incubation period of 34 h.

11.4 Conclusion

Acinetobacter baumannii dye decolorization capabilities were evaluated and optimized to the experimental condition via RSM-BBD. As a result, 99% of dye discoloration was achieved for optimized conditions of 28 °C temperature, inoculum load of 450 mg/l, and the maximal dye discoloration was achieved in 35 h. Hence this isolate can be used for larger-scale exploration of dye degradation under optimal conditions.

References

Arora S (2014) Textile dyes: it's impact on environment and its treatment. J Bioremediat Biodegrad 5(3):e146

Barikbin B, Hadinasab S, Nabavian MR (2017) Decolorization of reactive red 198 by ultrasonic process in aqueous solution. J Health Sci Technol 1(2):86–92

Fazli MM, Mesdaghinia A, Naddafi K, et al (2010) Optimization of reactive blue 19 decolorization by Ganoderma sp. using response surface methodology. J Environ Health Sci Eng 7(1):35–42

Fletcher CA, St. Clair R, Sharmina M (2021) A framework for assessing the circularity and technological maturity of plastic waste management strategies in hospitals. J Clean Prod 306:127169

Gao Y, Yang B, Wang Q (2018) Biodegradation and decolorization of dye wastewater: a review. In: IOP conference series: earth and environmental science (vol 78). IOP Publishing, p 012013

Gislin D, Sudarsanam D, Raj GA, Baskar K (2018) Antibacterial activity of soil bacteria isolated from Kochi, India and their molecular identification. J Genet Eng Biotechnol 16(2):287–294

Illanjiam S, Arunachalam KD (2012) Degradation of Azo dyes by immobilized *Pseudomonas aeruginosa* and *Bacillus subtilis*. Discov Life 1:26–31

Li K, Liu Q, Fang F et al (2019) Microalgae-based wastewater treatment for nutrients recovery: a review. Bioresour Technol 291:121934

Mishra S, Nayak JK, Maiti A (2020) Bacteria-mediated bio-degradation of reactive azo dyes coupled with bio-energy generation from model wastewater. Clean Technol Environ Policy 22(3):651–667

Papadopoulou K, Kalagona IM, Philippoussis A, Rigas F (2013) Optimization of fungal decolorization of azo and anthraquinone dyes via Box-Behnken design. Int Biodeterior Biodegr 77:31–38

Permpornsakul P, Prasongsuk S, Lotrakul P, Eveleigh DE, Kobayashi DY, Imai T, Punnapayak H (2016) Biological treatment of reactive black 5 by resupinate white rot fungus *Phanerochaete sordida* PBU 0057. Pol J Environ Stud 25(3):1167–1176

Prasad SS, Aikat K (2014) Study of bio-degradation and bio-decolourization of azo dye by *Enterobacter* sp. SXCR. Environ Technol 35(8):956–965

Satar R, Husain Q (2011) Catalyzed degradation of disperse dyes by calcium alginate-pectin entrapped bitter gourd (*Momordica charantia*) peroxidase. J Environ Sci 23(7):1135–1142

Shah M (2016) Microbial degradation of reactive orange M2R dye by bacterial consortium ETL-A. J Microb Biochem Technol 8(6):483–487

Srinivasan S, Shanmugam G, Surwase SV, Jadhav JP, Sadasivam SK (2017) In silico analysis of bacterial systems for textile azo dye decolorization and affirmation with wet lab studies. Clean Soil Air Water 45(9):1600734

Unnikrishnan S, Khan MH, Ramalingam K (2018) Dye-tolerant marine *Acinetobacter baumannii*-mediated biodegradation of reactive red. Water Sci Eng 11(4):265–275

Chapter 12
Photocatalytic Degradation of Reactive Orange M2R Using Green Route Synthesized Copper Oxide Nanoparticles and Its Optimization Studies

Easvaran Ramya, Alagu Thirumurugan, Natanamurugaraj Govindan, Jayaseelan Aravind, and Sriramulu Gobikrishnan

Abstract The photocatalytic degradation approach for industrial dyes in the presence of appropriate nanocatalyst offers a possible way for the complete removal of various pollutants from the aqueous environment. Therefore, in this study, we have made an attempt on use of bio-synthesized copper oxide (CuO) nanoparticles harnessed by neem extract for the degradation of reactive orange M2R (RO-M2R). The significant catalytic degradation of dye was found to be pH10, catalytic dosage at 10 ppm, and dye concentration at 3 gL^{-1} and irradiation time at 5 h through one factor at a time (OFAT). Further, Box Behnken design was applied to optimize and investigate the interaction between these four variables for the degradation of dye. It was predicted that maximum degradation was 84.04% under optimum conditions of pH at 11.84, catalytic dosage at 2.20 gL^{-1}, dye concentration at 7.49 ppm and irradiation time of 4 h. The validity of the optimal level predicted under the RSM was established by an independent experiment.

Keywords CuO nanoparticles · RSM · Box Behnken design · Photocatalytic degradation

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12.1 Introduction

Water pollution has become a very sensitive issue in the world due to inorganic and organic pollutants. The primary source of contamination of water is the organic dyes discharged by textile industries. There are more than 10,0000 commercially available dyes with over 7×10^5tons of dyestuff produced. In which, concerning 36,000 dye/year are consumed by textile industries across the world. 20% of the dyes in the world production are lost during the dyeing process and is discharged in the textile wastewater (Reza et al. 2017; Esplugas et al. 2002; Houas et al. 2001). In particular, the principal and most adaptable classes of azo dyes are presently utilized in the dyeing of different materials such as textiles, leather, plastics, and cosmetics. The azo dyes containing effluents are released into rivers, lakes, and ground waters for the period of dying process that contains mutagenic and carcinogenic health hazards. As a result, it leads to severe environmental problems due to their good stability under ambient conditions (Mathur et al. 2012).

Organic pollutants are challenging to biological degradation; also, conventional treatment processes are not succeeded to attain their complete elimination of dye molecules. Even though, for a decade, researchers have attempted various methodologies such as membrane filtration, chemical coagulation/flocculation, ion exchange, precipitation, adsorption, biological degradation, and ozonation, etc. Still, these methods are not capable enough and have more limitations. UV light is generally applied to excite a semiconductor surface to produce photoinduced holes or reactive oxygen species (ROS), such as hydroxyl and superoxide radicals in the photocatalytic advanced oxidation technology. Then, the ROS interrelate with organic compounds and guides to their oxidation and overall degradation (Jantawasu et al. 2009). Moreover, for a decade nanocatalyst development has highly attracted researchers to work towards the degradation of industrial wastewater pollutants. Apart from dye degradation studies by nanoparticles, Magnetic Moringa Oleifera (MMO) have also been used as a coagulant for palm oil waste water treatment (Noor et al. 2022).

There are various studies reported on dye degradation by metallic nanoparticles. Chaibakhsh et al. (2016) have reported on photocatalytic degradation of neutral red dye using titanium oxide (TiO_2) nanocatalyst and its optimization by Box-Behnken design. Recently, Uddin and Baig (2019) have studied on the removal of methyl orange dye using cobalt oxide (Co_3O_4) nanoparticles and Dawoud et al. (2020) have also reported on photocatalytic degradation of organic dye of Rhodamine B by using silver (Ag) doped Zirconium oxide (ZrO_2) nanoparticles. Recently, CuO NP's have been prepared from various sources such as *Canthium coromandelicum* leaves extract (Selvam et al. 2022), *Punica granatum* (Kaur et al. 2022), *Pistacia vera L.* hull (Ltaief et al. 2021), *Dictyota dichotoma* endophytic fungi (Kumar et al. 2022), *Elaeagnus indica* (Indhira et al. 2022), *Solanum tuberosum* (Hamami and Javanbakht 2021) were used as a catalyst for textile dye degradation.

Most of the reports on dye removal by magnetic nanoparticles have been performed by 'one variable at a time' (OFAT) approach that perfectly presumed that all variables are independent and do not represent the combined effect of all

the variables involved. Moreover, this method is a prolonged one and also requires a higher number of experiments to determine optimum levels, which may or may not be trustworthy (Farooq et al. 2017). These limitations of the conventional methods can be reduced by response surface methodology (RSM), using this statistical design of experiment.

Thus, herein, biological route synthesized CuO nanoparticles assisted photocatalytic degradation of RO-M2R dye, and their optimization studies were performed using Box-Behnken design to maximize the percentage of dye degradation.

12.2 Materials and Methods

12.2.1 Photocatalytic Degradation Confirmation

RO-M2R was obtained from Precision Scientific Co. Coimbatore, India. The photocatalytic degradation experiment of RO-M2R was performed under sunlight irradiation. The photocatalyst CuO NP's 2 $gL^{-1}$was added to 50 ppm of RO-M2R dye solution at neutral pH to establish the adsorption equilibrium between the dye molecule and CuO NP's. The resulting solution was exposed to sunlight for 4 h, and control was maintained without addition of nanoparticles. These suspensions were measured against absorbance at 495 nm. The percentage of degradation was calculated from the following equation;

$$\% \text{ of Degradation} = (\text{Co} - \text{C}/\text{Co})\,\text{X}\,100$$

where, Co = Initial concentration of dye, C = Final concentration of dye.

12.2.2 Preliminary Experiment

Preliminary experiments were conducted by one factor at a time (OFAT) approach to identify the ranges of four parameters such as pH, dye concentration, catalytic dosage and irradiation time for the degradation of RO-M2R. Statistical design of experiment consists of a variation of several trials at a time when compared to one factor at a time approach in which only one factor is changed and keeping all other factors constant.

12.2.2.1 Evaluation of Process Parameters by OFAT

The dye solution was taken out in reaction vessel, and the pH (3, 4, 5, 6, 7, 8, 9, 10, 11 and 12) was adjusted by using a pH meter (Elico LI 120, India). Similarly, the

different concentrations of RO-M2R working solutions (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 ppm) were prepared and kept the irradiation time for 4 h to study the effect of dye concentration on dye degradation. Likewise, the different concentrations of CuO nanoparticles (0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5 and 5 g/L) were varied and the reaction vessel was kept under sunlight for 4 h. Similarly, the different reaction periods (0, 50, 100, 150, 200, 250, 300, 350, 400 and 450 min) were chosen to check the percentage of degradation. Finally, 2 g/L of CuO nanoparticles was added to various ranges of pH and concentration of RO-M2R dye solution to establish the adsorption equilibrium between the dye molecule and CuO nanoparticles.

12.2.3 Response Surface Methodology for Optimization

Response surface methodology (RSM) was utilized to optimize the four parameters were catalytic dosage, pH, dye concentration and irradiation time. The four parameters were selected as independent variables, and the degradation percentage of RO-M2R was the response variable. Box-Behnken model was preferred to study the combined effect of four independent variables, and Design-expert 11 version was used to explain the response surface. The model was statistically examined. Analysis of variance (ANOVA) employed Fischer's *F*- test to evaluate the overall significance of the model, correlated probability values, and coefficient of determination to estimate the goodness of fit of the regression model. The apt polynomial equation was further conveyed the three-dimensional plots (3D) form, which depicted the interactions graphically. Finally, the central and interaction effects, the 3D plots were analyzed to check the accuracy of the model (Kanmani et al. 2013).

The following second-order polynomial equation explains the connection between the dependent and independent variables:

$$\begin{aligned} Y = {} & \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{22} C^2 \\ & + \beta_{12} AB + \beta_{13} AC + \beta_{23} BC \end{aligned} \tag{12.1}$$

Here the *Y* is the predicted response, β0 is the intercept, β_1, β_2, β_3, are the linear coefficients, β_{11}, β_{22}, β_{33}, are the squared coefficients, and β_{12}, β_{13}, β_{23} are the interaction coefficients.

12.3 Results and Discussion

12.3.1 Confirmation of Photocatalytic Degradation

The previously synthesized CuO nanoparticles (Thirumurugan et al. 2017) were used for the photocatalytic degradation of RO-M2R under sunlight. These

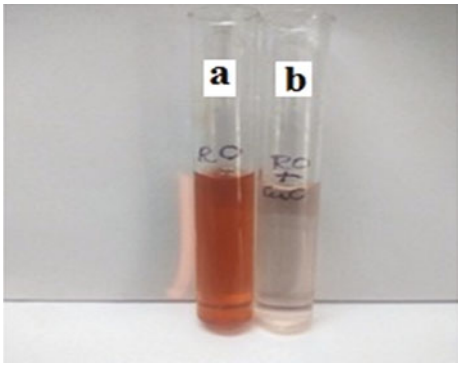

Fig. 12.1 Confirmation of photocatalytic degradation of RO-M2R using CuO NP's under sunlight

nanoparticles were already proved its photocatalytic degradation of 4-nitrophenol as a model (Ramya et al. 2019) and photocatalytic degradation of reactive red 120 (Thirumurugan et al. 2017). Thus, in this study, the photocatalytic degradation of RO-M2R was studied by using green synthesized CuO NP's under sunlight, as shown in Fig. 12.1. After the addition of nanoparticles, the orange color of the solution decreased significantly, become colorless with 74% degradation, and no changes were observed in control. The dye degradation efficiency of nanoparticles may be due to differences in size, shape and catalytic activity (Moon et al. 2015). Kumar et al. (2013), has also reported that metal nanoparticle synthesized by green approach was effectively degraded the dye with periodically increase in time. There are various parameters affecting the photocatalytic degradation, such as pH, dye concentration, catalytic dosage, irradiation time, reaction temperature, light intensity and the presence of electron acceptors. In particular, we investigated the effect of pH, dye concentration, catalytic dosage, and irradiation time through one factor at a time (OFAT) approach to study the impact of each factor ranges for the photocatalytic degradation of RO-M2R.

12.3.2 Evaluation of Process Parameters by OFAT

Effect of pH is one of the main factors for the degradation of industrial dye. To investigate the effect of pH, the experiments were carried out at different pH ranges from 3 to 12 at 2 gL^{-1} of CuO NP's, 50 ppm of RO-M2R and irradiation time of 4 h. The result showed that by increasing the pH of the dye solution, the dye degradation was getting increased, as shown in Fig. 12.2. It was observed that degradation rate increases, with an increase in pH and the maximum photocatalytic degradation, was found to be at pH 10. The cause can be due to efficient formation of hydroxyl radicals in alkaline medium. Excess of hydroxyl anions increases the formation of hydroxyl radicals as the main oxidizing function responsible for dye degradation (Gopalapp et al. 2012). Similarly, Devadi et al. (2014) has observed the maximum degradation

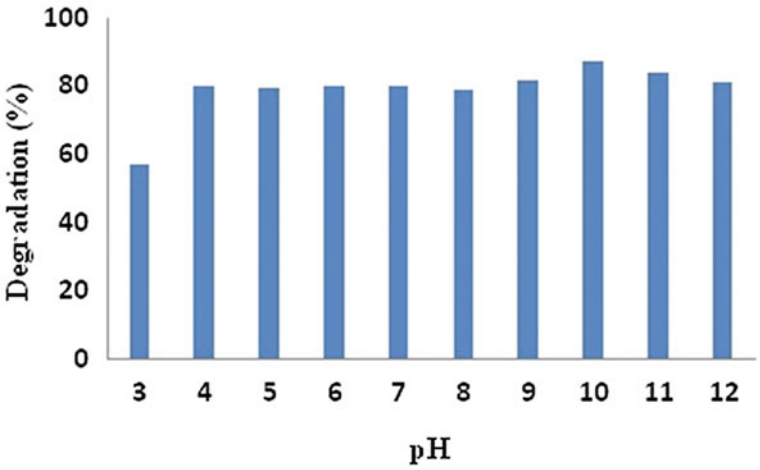

Fig. 12.2 Degradation of RO-M2R at different pH

of methyl blue at pH 11 and at under acidic condition the degradation efficiency get decreased due to the columbic repulsion. Also, Daneshvar et al. (2003) reported that in the presence of ZnO NP's, the photodegradation was considerably improved at high pH (pH = 11), While the lower at low pH is due to the photodecomposition of ZnO to Zn^{2+} takes place in acidic and neutral solutions and that efficient formation of hydroxyl radicals occurs in alkaline solution.

The effect of dye concentration for degradation of RO-M2R was varied between 10 to 100 ppm where pH was kept at 10, catalytic dosage at 2 gL^{-1} and irradiation time for 4 h. The degradation of RO-M2R at different concentration of dye is shown in Fig. 12.3. The result showed that up to 40 ppm the percentage of degradation was approximately the same and after 40 ppm the degradation rate was reduced. While maintaining a constant catalyst dose, it caused the fixed number of catalysis sites to be saturated rapidly, which is due to increasing dye concentration in the solution (Mohan and Pittman 2006). In addition, the light transmittance in the solution may be decreased due to the increased dye concentration; decreased light infiltration can diminish the activation rate of nanoparticles and delay the formation of OH radicals, results in decreased photocatalytic degradation.

Figure 12.4 shows the effects of different concentrations of nanoparticles as catalysts on degradation. The increase in concentration up to 3 gL^{-1} contains a high amount of active site and thus producing more free radicals which uninterruptedly increase the degradation of RO-M2R, and beyond 3 gL^{-1} there was a decrease in degradation rate. According to Akpan and Hameed (2009), this was due to the formation of turbid in the solution, which reduces the penetration of sunlight. Hence, it blocks the radiation to enter into the RO-M2R solution. Recently, Mortazavian et al. (2019) has reported in a study that the amounts of catalyst particles might aggregate leading to a reduced number of active sites because the increase of TiO_2 particles in the solution might supply more active sites for the molecules of dye to be adsorbed

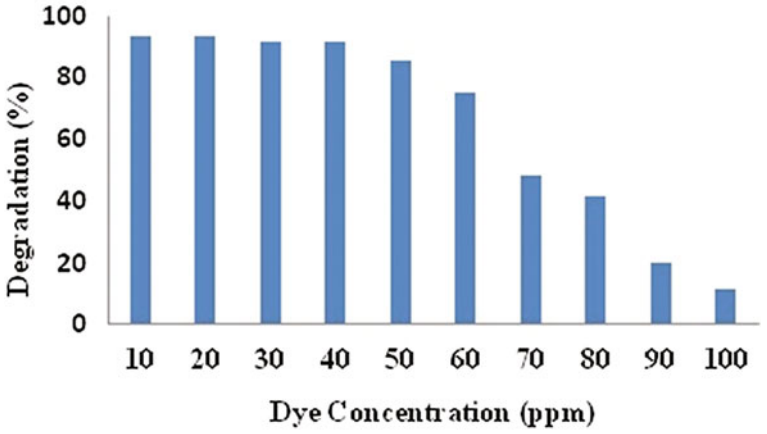

Fig. 12.3 Degradation of RO-M2R at different dye concentration

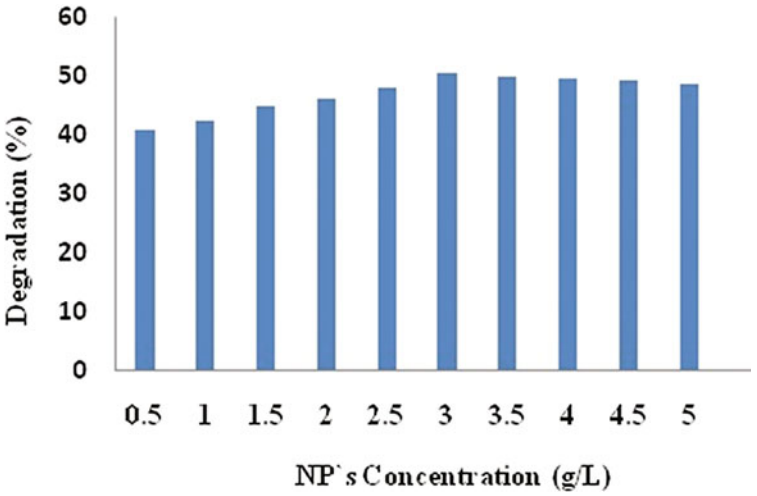

Fig. 12.4 Degradation of RO-M2R at different concentrations of CuO NP‘s

and degraded. This phenomenon might be one of the reasons for decreasing the degradation rate beyond 3 gL^{-1} of CuO NP‘s as a catalyst. Very recently, Hashemi et al. (2022) reported the optimization and assessment of photocatalytic degradation of methyl orange using silver nanoparticles synthesized through green route. As a result, observed that the methyl orange pollutant degradation was increased while increasing the concentration of nanocatalyst.

The effect of irradiation time on degradation was investigated by keeping catalyst amount at 3 gL^{-1}, pH at 10, and dye concentration at 40 ppm and varying the irradiation time of the dye solution under sunlight. Figure 12.5 shows that degradation rate

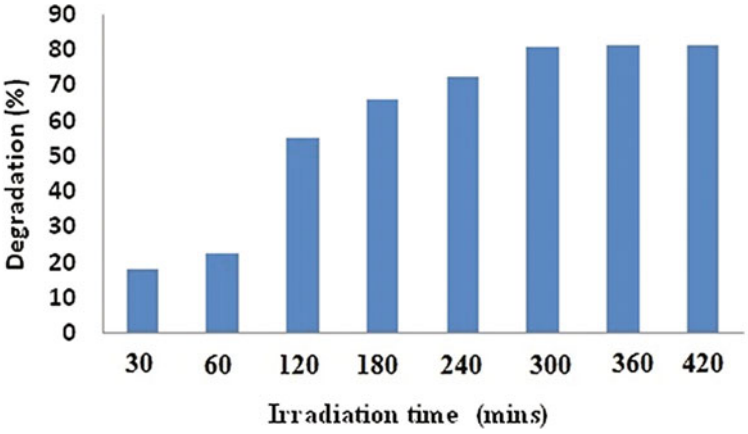

Fig. 12.5 Degradation of RO-M2R at different time intervals

increased as time increased, and this is due to the increased number of photons. The maximum degradation efficiency was observed at 5th h, and after that the degradation remains the same.

12.3.3 Optimization Using Response Surface Methodology

Box Behnken Design (BBD) was employed to evaluate the effect of variables on the degradation of RO-M2R in the presence of sunlight. The influence of operating parameters on the photocatalytic degradation efficiency of RO-M2R were assessed, four main factors were chosen: pH, dye concentration (ppm), catalytic dosage (gL^{-1}), irradiation time (hrs). A total of 29 experiments were used using Design expert software. The experimental outcomes of degradation of dye by nanoparticles were analyzed through RSM as tabulated in Table 12.1 to obtain an empirical model.

Based on the outcomes, an empirical relationship between the responses and independent variables were attained for the dye degradation expressed by the quadratic polynomial equation, as shown below.

$$\begin{aligned}\%\ \text{of Degradation} &= 59.19 + 14.14 * \mathrm{A} - 4.52 * \mathrm{B} + 0.19 * \mathrm{C} - 2.13 * \mathrm{D} \\ &+ 0.38 * \mathrm{AB} - 0.74 * \mathrm{AC} + 0.80 * \mathrm{AD} - 0.71 * \mathrm{BC} \\ &- 1.27 * \mathrm{BD} + 1.74 * \mathrm{CD} + 4.08\mathrm{A}^2 + 0.048\mathrm{B}^2 + 1.94\mathrm{C}^2 + 1.80\mathrm{D}^2\end{aligned}$$

Response surface methodology integrated the interaction effects of variables and helps us in simultaneously optimizing many process parameters within a minimum number of trial runs. Such statistically aided experimental designs can guide to considerably better performance of the nanoparticles. The *P* value acts as a tool

Table 12.1 Box-Behnken design of response surface methodology with real values and their responses

S.NO	A: pH	B: Nanoparticles concentration (g/l)	C: Dye concentration (ppm)	D: Irradiation time (hr)	Degradation (%)
1	8	2	22.5	5.5	54.5
2	12	2	22.5	5.5	80.09
3	8	4	22.5	5.5	47.1
4	12	4	22.5	5.5	74.2
5	10	3	5	4	66.85
6	10	3	40	4	65.18
7	10	3	5	7	58.51
8	10	3	40	7	63.8
9	8	3	22.5	4	53.82
10	12	3	22.5	4	80.22
11	8	3	22.5	7	49.25
12	12	3	22.5	7	78.84
13	10	2	5	5.5	67.3
14	10	4	5	5.5	55.51
15	10	2	40	5.5	69.2
16	10	4	40	5.5	54.57
17	8	3	5	5.5	48.65
18	12	3	5	5.5	80.65
19	8	3	40	5.5	49
20	12	3	40	5.5	78.04
21	10	2	22.5	4	64.73
22	10	4	22.5	4	60
23	10	2	22.5	7	62.35
24	10	4	22.5	7	52.55
25	10	3	22.5	5.5	60.85
26	10	3	22.5	5.5	58.02
27	10	3	22.5	5.5	57.83
28	10	3	22.5	5.5	61.34
29	10	3	22.5	5.5	57.89

for assessing the significance of each coefficient and is indicative of the interaction strength of all independent variable. Low values of P at ($P < 0.05$) indicate the high significance of the corresponding coefficients. In general, larger t, F and smaller P values indicate that the corresponding coefficient terms are significant.

From the ANOVA Table 12.2, the model F-value of the model (41.08) and values of $P > F$(<0.0500) implied that the model was significant statistically. The predicted

Table 12.2 Analysis of variance (ANOVA) and regression analysis for selected model

Source	Sum of squares	Degree of freedom	Mean squares	F value	P value	
Model	2855.57	14	203.97	41.08	<0.0001	Significant
Residual	69.51	14	4.97			
Lack of fit	57.23	10	5.72	1.86	0.2877	Not significant
Pure error	12.29	4	3.07			
Cor total	2925.09					

$R^2 = 0.9762$; Adj.$R^2 = 0.9525$; Predicted $R^2 = 0.8808$

R^2 of the 0.8808 is in reasonable agreement with the adjusted R^2 of 0.9525. Adequate precision measures signal-to-noise ratio (S/N ratio) and detect which experimental parameters generate signals that are large in comparison to the noise. The observed adequate precision ratio was 23.29, greater than 4, which indicated the selected model could be employed to precisely navigate the design space as reported by Chaibakhsh et al. (2016). The accuracy of the model is shown in Fig. 12.6, which evaluates the measured values against the predicted responses of the model for the degradation of RO-M2R. It can be observed that correlation between the experimental data and predicted values ($R^2 = 0.9762$) showed the data fit well with the model in the range studied.

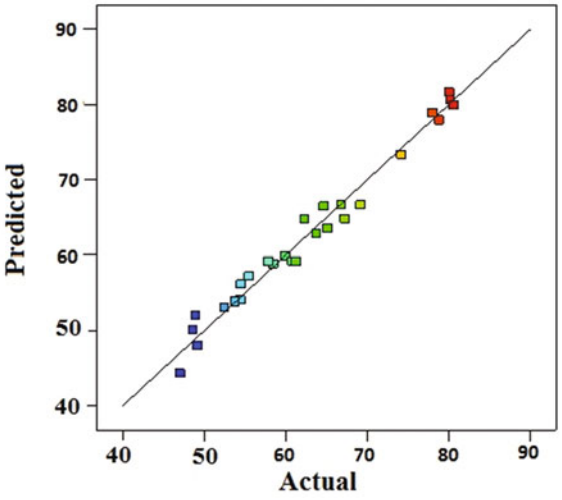

Fig. 12.6 Predicted plotted against the actual experimental values for photocatalytic degradation of RO-M2R

12.3.3.1 Analysis of Response Surface Plots

Three-dimensional surface can be used as a graphical representation of the regression equation to find the optimum levels of the variables studied and widely used to achieve a better understanding of the interactions between variables within the ranges.

Figure 12.7 represents the interaction effect of pH, nanoparticles concentration and dye concentration and irradiation time on the degradation of dye from aqueous solution. There was a significant degradation observed by increasing pH till 12. Similarly, nanoparticles concentrations also influencing the degradation rate of dye molecules up to 2.20 gL^{-1} then degradation rate declines. Whereas, dye concentration and irradiation time were not influencing the degradation rate as pH and nanoparticles concentration. Recently, Chaker et al. (2021), has studied a statistical modelling optimization approach for efficient photocatalytic degradation of azo dye using cerium-doped mesoporous ZnO by central composite design. Their results indicated that all the variable chosen had an impact on the photocatalytic process and pH was the least factor against the effect of catalyst concentration.

The three-dimensional plots based on the interactions between the variables showed an increase in RO-M2R dye degradation as each variable increased to an optimum level, beyond the optimum level; decline rate could be observed (Fig. 12.7a–f). The optimal values obtained from the three-dimensional were also almost equal to the results obtained by the regression analysis (Eq. 12.1). From the model, a maximum dye degradation of 84% under the optimum condition of pH 11.8, nanoparticle concentration of 2.20 gL^{-1}, dye concentration of 7.49 ppm and irradiation time of 4 h were predicted. The experiment was conducted in triplicate to validate the prediction of the model, and the maximum degradation of RO-M2R was found to be 83.41% which was close to the predicted maximum dye degradation of the model.

12.3.4 Mechanism of Photocatalytic Degradation by CuO NP's

For the above observed results, a possible mechanism for the degradation of RO-M2R dye by CuO nanoparticles in aqueous solution can be proposed. A possible photocatalytic mechanism of CuO nanoparticles for the degradation of aqueous RO-M2R is shown in Fig. 12.8. Photocatalytic degradation of organic dyes through the utilization of CuO nanoparticles arises when the photocatalyst absorbs light of a suitable wavelength from sunlight. The light energy from the sun is exciting and promotes electrons in the valence band to conduction band of photocatalyst. As results, creation of positive charges (holes) and negative charges (electrons) on the valence and conduction bands respectively, leads to the formation of electron–hole pairs. This hole oxidizes the water molecule into hydrogen gas and hydroxyl radicals ($OH^{\bullet}$), whereas electron reduces the oxygen molecules to form superoxide radicals ($O_2^{\bullet -}$). Thus radicals formed radicals, attack the dye molecule over the surface of

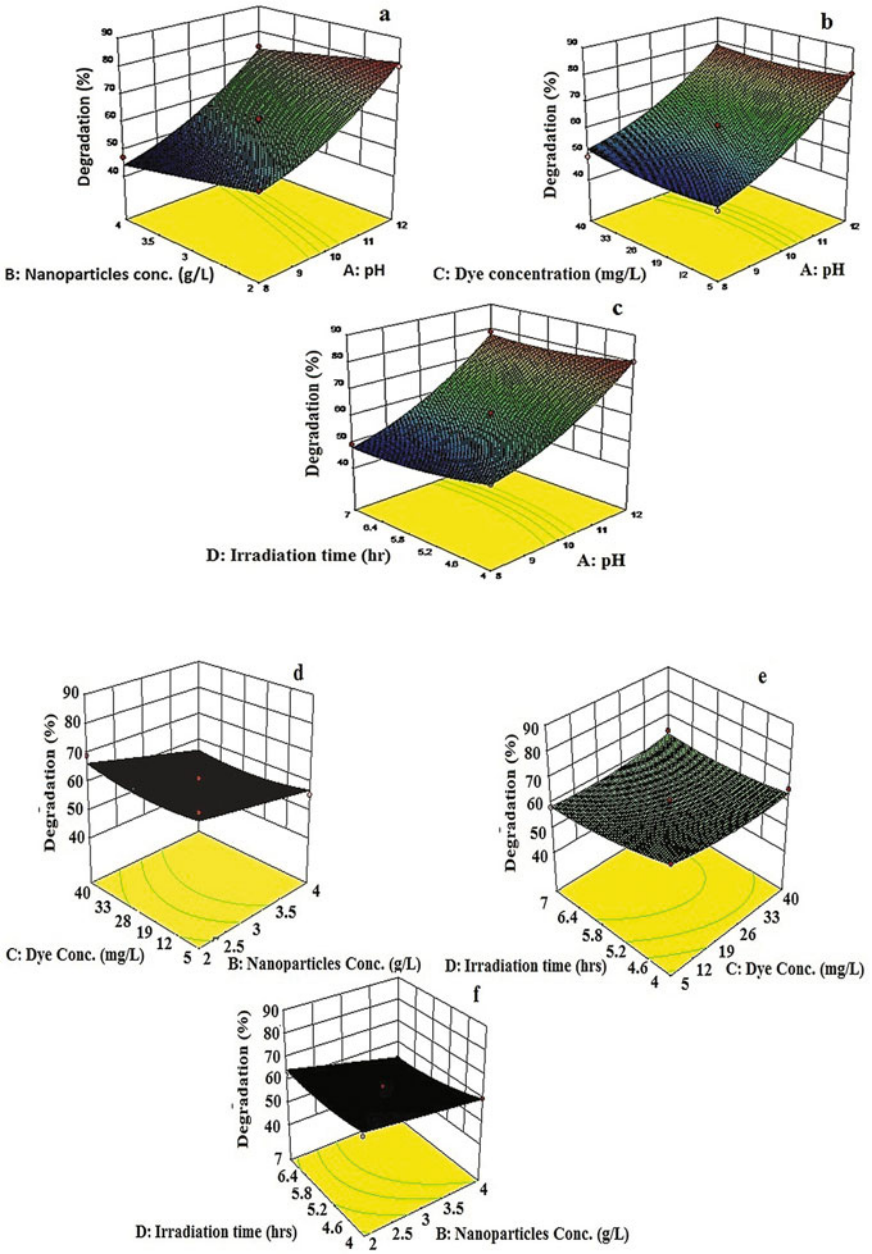

Fig. 12.7 Three dimensional surface plot showing the effect of **a** nanoparticles concentration (gL^{-1}) versus pH **b** dye concentration (mgL^{-1}) versus pH **c** Irradiation time (h) versus pH **d** Dye concentration (mgL^{-1}) versus nanoparticles concentration (gL^{-1}) **e** Irradiation time (h) versus dye concentration (mgL^{-1}) **f** Irradiation time (hrs) versus nanoparticles concentration (gL^{-1}) on degradation of RO-M2R

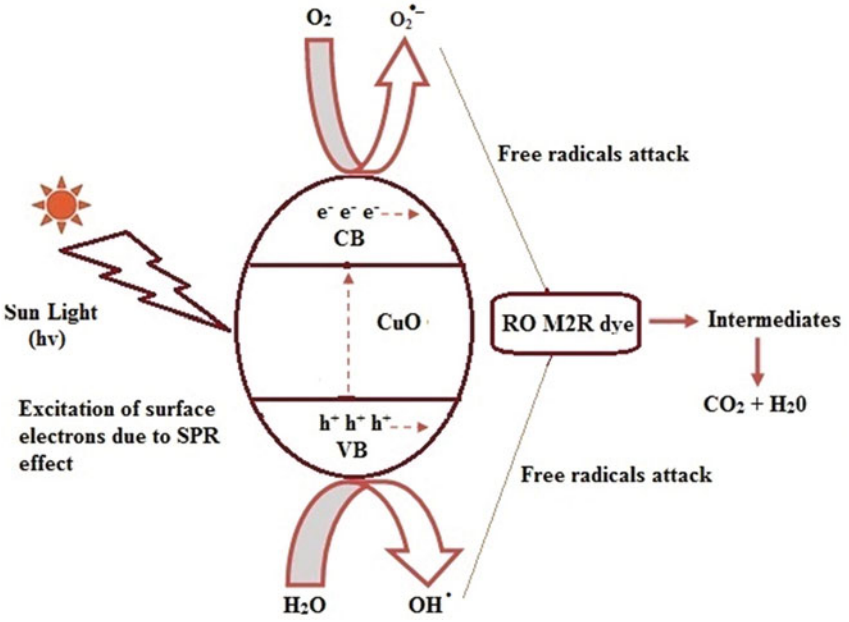

Fig. 12.8 A possible mechanism of photocatalytic degradation by CuO NP's

the CuO NP's and accomplish the dye degradation, and these formed radicals are also responsible for inactivation of bacteria through their cell walls degradation (Dalrymple et al. 2010).

12.4 Conclusions

We conclude that the photocatalytic degradation of RO-M2R by CuO NP's as a catalyst in the aqueous medium was developed a quadratic model using Response Surface Methodology as a functional relationship between catalytic dosage, pH, dye concentration and irradiation time to determine the optimum condition for the degradation of RO-M2R. The optimized degradation of RO-M2R was achieved with 83.41% by Box-Behnken design. Therefore, CuO NP's could be utilized as an efficient photocatalyst for the degradation of dye-containing waste water from textile industries.

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References

Akpan UG, Hameed BH (2009) Parameters affecting the photocatalytic degradation of dyes using TiO2-based photocatalysts: a review. J Hazard Mater 170(2–3):520–529

Chaibakhsh N, Ahmadi N, Zanjanchi MA (2016) Optimization of photocatalytic degradation of neutral red dye using TiO_2 nanocatalyst via Box-Behnken design. Desalin Water Treat 57(20):9296–9306

Chaker H, Attar AE, Djennas M, Fourmentin S (2021) A statistical modeling-optimization approach for efficiency photocatalytic degradation of textile azo dye using cerium-doped mesoporous ZnO: a central composite design in response surface methodology. Chem Eng Res Des 171:198–212

Dalrymple OK, Stefanakos E, Trotz MA, Goswami DY (2010) A review of the mechanisms and modeling of photocatalytic disinfection. Appl Catal B 98(1):27–38

Daneshvar N, Salari D, Khataee AR (2003) Photocatalytic degradation of azo dye acid red 14 in water: investigation of the effect of operational parameters. J Photochem Photobiol A Chem 157(1):111–116

Dawoud TM, Pavitra V, Ahmed P, Syed A, Nagaraju G (2020) Photocatalytic degradation of an organic dye using Ag doped ZrO2 nanoparticles: milk powder facilitated eco-friendly synthesis. J King Saud Univ Sci 32(3):1872

Devadi MAH, Krishna M, Murthy HN, Sathyanarayana BS (2014) Statistical optimization for photocatalytic degradation of methylene blue by Ag-TiO_2 nanoparticles. Proc Mater Sci 5:612–621

Esplugas et al., 2002 Esplugas S, Giménez J, Contreras S, Pascual E, Rodríguez M (2002) Comparison of different advanced oxidation processes for phenol degradation. Water Res 36:1034–1042

Farooq S, Saeed A, Sharif M, Hussain J, Mabood F, Iftekhar M (2017) Process optimization studies of crystal violet dye adsorption onto novel, mixed metal Ni0. 5Co0. $5Fe_2O_4$ ferrospinel nanoparticles using factorial design. J Water Proc Eng 16:132–141

Gopalapp H, Yogendra K, Mahadevan K (2012) Solar photocatalytic degradation of commercial azo dye acid orange 7 by synthesized CaZnO2 nanoparticle as an effective Catalyst. Int J Res Chem Environ 2:39–43

Hamami Z, Javanbakht V (2021) Biosynthesis of copper oxide nanoparticles using biomass, peel, and extract polysaccharides of Solanum Tuberosum for ultrasound-assisted adsorption of azo direct red 80 contaminants. Ceram Int 47(17):24170–24181

Hashemi Z, Mizwari ZM, Mohammadi-Aghdam S, Mortazavi-Derazkola S, Ebrahimzadeh MA (2022) Sustainable green synthesis of silver nanoparticles using Sambucus ebulus phenolic extract (AgNPs@ SEE): optimization and assessment of photocatalytic degradation of methyl orange and their in vitro antibacterial and anticancer activity. Arab J Chem 15(1):103525

Houas A, Lachheb H, Ksibi M, Elaloui E, Guillard C, Herrmann JM (2001) Photocatalytic degradation pathway of methylene blue in water. Appl Catal B 3:1145–1157

Indhira D, Krishnamoorthy M, Ameen F, Bhat SA, Arumugam K, Ramalingam S, Kumar GS (2022) Biomimetic facile synthesis of zinc oxide and copper oxide nanoparticles from *Elaeagnus indica* for enhanced photocatalytic activity. Environ Res 212:113323

Jantawasu P, Sreethawong T, Chavadej S (2009) Photocatalytic activity of nanocrystalline mesoporous-assembled TiO2 photocatalyst for degradation of methyl orange monoazo dye in aqueous wastewater. Chem Eng J 155:223–233

Kanmani P, Karthik S, Aravind J, Kumaresan K (2013) The use of response surface methodology as a statistical tool for media optimization in lipase production from the dairy effluent isolate Fusarium solani. Int Schol Res Notices 528708

Kaur H, Singh J, Rani P, Kaur N, Kumar S, Rawat M (2022) A novel and one-pot synthesis of *Punica granatum* mediated copper oxide having flower-like morphology as an efficient visible-light driven photocatalyst for degradation of textile dyes in waste water. J Mol Liq 355:118966

Kumar RV, Vinoth S, Baskar V, Arun M, Gurusaravanan P (2022) Synthesis of zinc oxide nanoparticles mediated by *Dictyota dichotoma* endophytic fungi and its photocatalytic degradation of fast green dye and antibacterial applications. S Afr J Bot. https://doi.org/10.1016/j.sajb.2022.03.016

Kumar P, Govindaraju M, Senthamil selvi S, Premkumar K (2013) Photocatalytic degradation of methyl orange dye using silver (Ag) nanoparticles synthesized from Ulva lactuca. Coll Surf B Biointerf 103:658–661

Ltaief S, Jabli M, Abdessalem SB (2021) Immobilization of copper oxide nanoparticles onto chitosan biopolymer: application to the oxidative degradation of Naphthol blue black. Carbohyd Polym 261:117908

Mathur N, Bhatnagar P, Sharma P (2012) Review of the mutagenicity of textile dye products. Univers J Environ Res Technol 2(2):1–18

Mohan D, Pittman CU (2006) Activated carbons and low cost adsorbents for remediation of tri- and hexavalent chromium from water. J Hazard Mater 137:762–811

Moon SA, Salunke BK, Alkotaini B, Sathiyamoorthi E, Kim BS (2015) Biological synthesis of manganese dioxide nanoparticles by Kalopanax pictus plant extract. IET Nanobiotechnol 9(4):220–225

Mortazavian S, Saber A, James DE (2019) Optimization of photocatalytic degradation of acid blue 113 and acid red 88 textile dyes in a UV-C/TiO_2 suspension system: application of response surface methodology (RSM). Catalysts 9(4):360

Noor MHM, Azli MFZM, Ngadi N, Inuwa IM, Opotu LA, Mohamed M (2022) Optimization of sonication-assisted synthesis of magnetic Moringa oleifera as an efficient coagulant for palm oil wastewater treatment. Environ Technol Innov 25:102191

Ramya E, Thirumurugan A, Rapheal VS, Anand K (2019) CuO@SiO_2 nanoparticles assisted photocatalytic degradation of 4-nitrophenol and their antimicrobial activity studies. Environ Nanotechnol Monit Manag 12:100240

Reza KM, Kurny ASW, Gulshan F (2017) Parameters affecting the photocatalytic degradation of dyes using TiO_2: a review. Appl Water Sci 7(4):1569–1578

Selvam K, Albasher G, Alamri O, Sudhakar C, Selvankumar T, Vijayalakshmi S, Vennila L (2022) Enhanced photocatalytic activity of novel *Canthium coromandelicum* leaves based copper oxide nanoparticles for the degradation of textile dyes. Environ Res 211:113046

Thirumurugan A, Harshini E, Marakathanandhini BD, Kannan SR, Muthukumaran P (2017) Catalytic degradation of reactive red 120 by copper oxide nanoparticles synthesized by *Azadirachta indica.* In: Bioremediation and sustainable technologies for cleaner environment. Springer, Cham, pp 95–102

Uddin MK, Baig U (2019) Synthesis of Co_3O_4 nanoparticles and their performance towards methyl orange dye removal: characterisation, adsorption and response surface methodology. J Clean Prod 211:1141–1153

Chapter 13
Vermicomposting—An Effective Method for Sustainable Agriculture and Environmental Impact

R. Vinoth Kumar, Farheen Fathima, R. Jaishree, and G. Priya

Abstract Societal waste materials from the landfill are considered as one of the major contributors affecting the ecosystem and environment. Composting and vermicomposting are the commonly used methodologies to treat and convert these waste materials into the useful manure by the collective action of earthworms and other beneficial microbes. Waste management is considered as an integral part of a sustainable society, thereby necessitating the conversion of these biowastes into alternative management processes such as vermicomposting. Vermicompost produced by the activity of earthworms is rich in nutritive organic macro and micronutrients, vitamins, growth hormones, beneficial soil microbes and enzymes such as proteases, amylases, lipase, cellulase and chitinase etc. Such microbes and enzymes continue to disintegrate organic matter even after they have been excreted from the earthworms. In this review, we have summarized the importance of vermicomposting, effect of process-enhancing supplements, the detoxification process of industrial wastes by earthworms and the role of vermicompost in plant growth and development. Further, the recent advancements in innovative vermicomposting technologies employed for small- and large-scale production are highlighted. Collectively, it highlights vermicompost as a powerful crop nutrient in sustainable agriculture.

Keywords Biowaste · Beneficial microbes · Composting · Earthworms · Plant growth and development · Process-enhancing supplements · Technological innovations · Waste management

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13.1 Introduction

Urbanization and advances in industrial processes have increased the amount of garbage produced globally. To mitigate pollution originating from the garbage, developed nations have implemented practical measures like limiting waste production at the source, increasing recycling, and reuse. Globally, at least 2 billion tons of non-hazardous waste materials are generated each year (Chalmin and Gaillochet 2009). Particularly, in low- and middle-income nations, bio-waste is the major source of municipal and industrial wastes (Kaza et al. 2018). Circular economy has gained recognition in current scientific literature and among international and national authorities as a key strategic environmental policy framework (Jain et al. 2022). The most popular methods used for treating and converting these waste materials into valuable end-products are composting and vermicomposting (Adhikary 2012; Zhou et al. 2022a, b). Vermicompost research has had impressive positive growth trends in the previous 25 years with >2000 papers published connected to vermicompost and with more nations participating in this subject globally (Ghorbani and Sabour 2021). This study emphasizes on vermicomposting as an effective method for attaining sustainable agriculture.

13.2 What Is Vermicomposting?

Vermicomposting is a bio-oxidation and stabilisation method that uses the earthworms' biological activity to break down organic waste into a nutrient-rich organic biofertilizer. Sewage sludge and solids from wastewater, brewery materials, paper trash, urban leftovers, food and animal wastes, as well as horticultural residues from processed potatoes, dead plants, and the mushroom business, have all been treated successfully using this method (Garg and Gupta 2009; Lim et al. 2015; Datta et al. 2016; Bhat et al. 2018). Earthworm activity results in the production of vermicompost, which is rich in macro- and micronutrients, vitamins, growth hormones, enzymes including lipase, cellulase, and chitinase, as well as beneficial microflora (Sanchez-Hernandez 2019; Singh et al. 2020).

In the vermicomposting process, the microbial community's composition is greatly changed by the earthworm gut transit (Romero-Tepal et al. 2014). The earthworms aerate, fragment the substrate there by increasing the surface area for microbes and modify the microbial activity in organic waste residues for decomposition (Lavelle et al. 2006). The stomach of earthworms secrete a variety of enzymes, including lipases, proteases, cellulases, amylases, and chitinases, to convert the inaccessible form of minerals, such as nitrogen, phosphorus, potassium, and calcium, etc., (in organic waste materials) into forms that are available to plants (Hand 1988). Due to the faster rate of mineralization and humification by earthworms during vermicomposting than during thermal composting, the finished product (vermicompost) has a higher nutritional value (Albanella et al. 1988). In the process of vermicomposting,

earthworms excrete coelomic fluids, which kill the parasites and bacteria present in the waste and produce pathogen- and odour-free end-product, vermicompost (Kale and Krishnamoorthy 1981).

13.3 Classification of Vermicomposting

Vermicomposting can be broadly divided into two categories: primary vermicomposting and secondary vermicomposting (Garg and Gupta 2009). Directly feeding raw materials to the worms is the primary method of vermicomposting. Because all of the nutrients in organic matter are used to raise worms, a primary system has a high stocking density (number of worms per surface area). The drawback is that worms need to be fed frequently and in small amounts.

Prior to being fed to worms, raw materials are composted by fungus and microorganisms in secondary vermicomposting. In piles of secondary vermicompost, autoheating doesn't happen. Because the organic matter has already undergone some digestion before the worms can consume it, secondary vermicompost systems have relatively high feeding rates (mass of organic matter fed per surface). This implies that secondary bins are supplied in thick layers less frequently.

13.4 Characteristics of Vermicompost

Vermicompost, which is comprised of compost that has been digested, is a great soil enhancer (Garg and Gupta 2009). Worm castings are regarded as a higher value product since they contain significantly more nutrients and microbial life (Table 13.1). Vermicompost includes nutrients that are necessary for plants and other microorganisms, as well as a variety of organic or inorganic minerals like oxygen, nitrogen, and phosphorus. They also have very little heavy metal content. Vermicompost contains a 3:1:2 ratio of NPK in its NPK makeup (Garg and Gupta 2009; Adhikary 2012). This makes it as an excellent nitrogen fertiliser for plants, particularly those with green leaves and those that develop quickly. Vermicompost can also be applied to soil that lacks organic matter to improve it. It includes 1.5 to 2.2% nitrogen, 1.8 to 2.2% phosphorus and 1.0 to 1.5% potassium on an average. There are micronutrients such as sodium, calcium, zinc, sulphur, magnesium and iron in the organic carbon, which ranges in concentration from 9.15 to 17.98 (Garg and Gupta 2009; Olle 2019). The physical characteristics of the soil may also be significantly impacted by worm compost. It was demonstrated that adding 20 tha of vermicompost over the course of two years greatly increased soil porosity and aggregate stability (Table 13.2).

Table 13.1 Population of different microorganisms present in vermicompost (Reproduced from Pramanik et al. 2007)

Treatments	Total bacterial count ($X10^8$)		Cellulolytic fungal count ($X10^6$)		Nitrifier population ($X10^4$)	
	L_0	L_1	L_0	L_1	L_0	L_1
Cow dung						
M_0	29	32	50	52	4	3
M_1	20	169	72	55	10	13
M_2	16	110	59	45	3	28
M_3	49	49	76	59	49	5
Grass						
M_0	17	19	34	35	2	3
M_1	29	31	44	76	12	11
M_2	25	38	41	52	7	15
M_3	35	30	51	38	13	9
Aquatic weeds						
M_0	13	18	41	43	4	4
M_1	27	33	47	49	9	10
M_2	29	37	43	63	8	12
M_3	35	28	51	48	11	9
MSW						
M_0	9	10	19	17	0.7	1
M_1	17	19	23	20	1.5	2.1
M_2	15	23	21	22	1.7	2.9
M_3	20	17	25	21	2	2.3

L_1 is lime at 5 g/kg and L_0 is control; M_0, M_1, M_2, and M_3 are control and incubation of *Trichoderma viridae*, *Bacillus polymxa* and *Phenerocrete crysosporium*, respectively at 50 ml/kg

Table 13.2 Optimal conditions required for breeding *E. fetida* and *E. andrei* in organic wastes

Parameters	Optimal conditions required
Temperature	15–20 °C
pH	5–9
Moisture content	60–80%
Ammonium content of the waste	<1 mg/g
Salt content	<0.5%
Oxygen	Aerobic

13.5 Sources of Organic Wastes for Vermicomposition

Organic wastes are substances that come from living beings such as plants, animals, and microorganisms and are biodegradable—that is, they can be converted into more basic organic molecules. Depending on the amount of moisture present, they can be found in either a solid or a liquid state in nature (Ansari et al. 2020). Vermicompost is made from a variety of organic waste sources, including both small- and large-scale production. The following are the common sources:

Municipal solid trash, which includes food leftovers, recycled newspaper, and grass clippings. Animal waste includes everything from egg shells to vegetable skin to animal dung.

Plant waste, which includes rotting and dead plants, leaves, flowers, etc.

Food waste includes ruined food goods, fruit peels, and vegetable skin.

13.6 Role of Earthworms in Vermicomposting

tEarthworms are nocturnal, terrestrial invertebrates in nature. Globally, there are about 3000 species of earthworms belonging to 9 families, of which 450 species/subspecies have been found in India (Thakur and Yadav 2018). Indian blue worms, *Metaphire posthuman*, and *Lampito mauritii* are a few examples of common Indian earthworms. Except for few species like *Pontodrilus burmudensis*, which thrives in estuary water, the majority of earthworm species are found in soil. These worms eat organic soil surface matter, including bacteria, fungi, dead plants, living protozoa, rotifers, nematodes, and other microbes. The organic materials that earthworms consume are transformed into nutrient-rich inorganic and organic compounds, which are then excreted (Singh et al. 2020).

The commonly used earthworms for the process of vermicomposting are *Eisenia fetida*, *Eisenia hortensis* and *Perionyx excavates*. Red wigglers, or *Eisenia fetida*, are ideal for vermicomposting because of their quick digestion and efficient breakdown. They may be readily maintained, and require moisture and shade to grow. Compared to vegetable and mixed food waste, *Eisenia fetida* favoured tea waste. Vermicomposting was more productive by nearly 40% in tea trash (Garg et al. 2006; Yadav and Garg 2011; Mohapatra et al. 2019). The European nightcrawler, *Eisenia hortensis*, is larger than other earthworms and is generally grey or brown in colour. They are prevalent in heavily forested areas. They can withstand a broad range of temperatures and reproduce quickly. They are hence recommended in equatorial areas.

Native to sub-Saharan Africa, *Perionyx excavatus* is a species of nightcrawler. They efficiently aerate and decompose organic materials. Because they can withstand a wide variety of temperatures and moisture levels, they are ideal for vermicomposting. Additionally, they serve as fish bait. *Perionyx excavatus*, sometimes known as Indian or Malaysian Blues Worms, is a native species to Southern Asia.

They effectively break down organic debris, and are simple to cultivate (Martin and Eudoxie 2018; Ananthavalli et al. 2019).

13.7 Basic Environmental Requirements for Earthworms

The earthworms in the vermicomposting process require the following environmental conditions—temperature, moisture, acidity and alkalinity, aeration, salts and carbon: nitrogen ratio (Peigne and Girardin 2004; Singh et al. 2016) (Table 13.3).

Temperature: Red wigglers grow at their fastest between 27 and 35 °C. Composting worms can withstand temperatures above 85 F for brief periods of time, but they perish if exposed to extreme heat for an extended amount of time. Lower temperatures (below 65F) are more tolerable to them (Liang et al. 2003; Amaravathi and Reddy 2015). As long as the pile's core temperature stays above freezing, composting worms will survive. The worm eggs known as cocoons can endure extreme cold for long periods of time before hatching.

Moisture: Water is a must for worm life at all costs. The ideal range for moisture content for growth is between 70 and 85%. This is a little moister than microbial composting piles, which typically have a moisture content of between 50 and 60% (Liang et al. 2003; Singh et al. 2004). Red wigglers can briefly survive in entirely saturated bins, but if the oxygen supply starts to diminish, they will leave.

Carbon to Nitrogen Ratio (C:N): Compost worms digest just about any type of organic matter, but they prefer higher amounts of carbon than is normal for microbial composting (Ndegwa and Thompson 2000). Organic wastes such as manure, kitchen wastes, coffee grounds, and freshly cut grass contain lots of Nitrogen (N). You need to dilute these materials with byproducts containing high amounts of Carbon (C), such as straw, paper or fallen leaves. The ratio of carbon to nitrogen in worm food is called the Carbon to Nitrogen Ratio (C:N). Worms thrive when their food has C:N greater than 50. The main reason for this is microorganisms outcompete worms at lower C:N ratios. At best, microorganisms take food from the worms' mouth. At worst, they cause the pile to heat, which kills the worms.

Acidity and alkalinity: The pH of soil serves as a gauge for its relative acidity or alkalinity. The ideal pH for composting worm growth and reproduction is 7 (Amaravathi and Reddy 2015; Singh et al. 2016). Their survival rate reduces with pH values of 5 or above. Worms may withstand somewhat greater pH ranges than other less desirable compost pile visitors, such flies. Fungus development is also aided by a lower pH. When the pH is low, worms will flee, but once it is close to neutral, they will return to eat the fungi.

Aeration: Earthworms lack specific respiratory organs and instead breathe via their body walls. As a result, earthworms are extremely sensitive to anaerobic environments. High concentrations of *E. fetida* have been observed migrating from water-saturated substrates where oxygen has been reduced or where carbon dioxide or hydrogen sulphide have accumulated (Yadav and Garg 2011).

Table 13.3 Role of vermicompost for sustainable agriculture

Crops	Beneficial activities on plants
Banana, cassava and cowpea	Improved in yield and biometric characteristics
Brinjal	Increased germination, number of fruits per plant, mean fruit weight, yield per plant
Capsicum	Improved plant nutrition, growth, photosynthesis and chlorophyll content of the leaves Vermiwash significantly affected the growth and nutrient utilization
Carrot	Increased root length, root volume, root weight and root yield
Cowpea	Increased seed yield, straw yield, biological yield, total root nodules and leghaemoglobin content, protein content in seeds
Chickpea	Increased plant height, plant shoot biomass, number of pods and photosynthetic pigments of chickpea plants
Hyacinth bean	Increased growth and yield characteristics
Marigold	Growth augmentation, increased overall nitrate– nitrogen concentrations in leaf tissues at flowering stage, improved soil fertility Increased bud formation, number of flowers per plant, plant shoot biomass, plant height and increased diameter of the flower
Mulberry	Increased luxuriant growth of bacteria treated rhizosphere and other micronutrients
Muskmelon	Increased germination rate, plant height, leaf area and plant biomass
Okra	Increased pod yield and soil fertility Reduced fruit borer infection Induced resistance in plants against pests and disease
Peppermint	Increased levels of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids
Paddy	Increased growth, yield, nutrient uptake and soil characteristics
Soybean	Increased seed germination, seedling survival, shoot length and root length
Spinach	Increased plant height
Strawberry	Increased photosynthesis rate, free radical scavenging and soil enzymatic activity
Tea	Increased radical scavenging activity in leaf extracts
Tomato	Improved quality and productivity of tomato Protective agent for improvement of salt stress resistance in tomato Accumulation of plant growth hormones and carbohydrates Increased juiciness, titratable acidity, ascorbic acid content, shelf life Decreased number of nematode-induced galls on tomato plants

Ammonia and salts: Earthworms cannot survive in organic wastes having significant concentrations of cations because they are extremely sensitive to ammonia and salts (Tripathi and Bhardwaj 2004; Wu et al. 2019). Additionally, they die in wastes that include a lot of inorganic salts. Ammonia and inorganic salts both have extremely distinct hazardous and benign ranges (1 mg/g of ammonia and 0.5% salts, respectively). However, if the ammonia has been removed through pre-composting or water leaching, organic wastes with high ammonia levels may become acceptable. Earthworm activity and rates of organic waste processing drastically drop outside of these environmental parameters, hence wastes should be preconditioned to make them acceptable for earthworms in order to maximise vermicomposting effectiveness.

13.8 Vermicomposting Process

The vermicomposting process consists of two phases based on the biological and physiochemical mechanisms in the earthworms and microorganisms (Fig. 13.1):

Active phase: The earthworm interacts with by-products to change their physical and chemical properties as well as their microbial make-up.

Maturation phase: The earthworm moves to a new layer of new substrate, creating space for the emergence of bacteria that break down the biological matter that the earthworm has previously processed.

13.9 Impact of Additives, Bulking Agents and Microbes

Commercial composting supplements are frequently marketed as "starters," "activators," or "accelerators," but they strongly differ in composition and typically contain a combination of chemicals, bulking agents, and microbes. In order to speed up the composting process and enhance the leaching, greenhouse gas emissions, and odour of the composting process, additives are frequently applied (Awasthi et al. 2020b). The use of such chemicals may not only affect important process variables but also raise the final product's agronomic value (Barthod et al. 2018). Increased pH, improved nitrogen retention (both total and nitrate nitrogen), and faster seed germination have all resulted with the addition of 10–15% biochar made from straw, but the bioavailability of heavy metals has decreased (Awasthi et al. 2020a; Zhou et al. 2021; Zhou et al. 2022a). Additionally, in a field vermicomposting experiment,

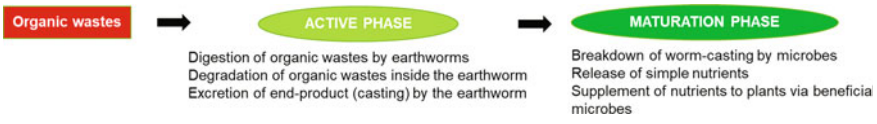

Fig. 13.1 The different phases of vermicomposting by earthworms

biochar was used in conjunction with earthworms to produce vermicompost, which resulted in decreased emissions of ammonia, methane and nitrous oxide (Raza et al. 2022). Similarly, a modest amount of lime (1%), combined with 30% zeolite, may improve the composting process by lowering greenhouse gas emissions and limiting ammonia loss (Awasthi et al. 2016).

Bulking agents are substances that are added to the compost to change its physical composition, including its ability to absorb water, the amount of airflow and space between particles, and the C:N ratio. In order to optimise open air space, C:N ratio, pH, and adjust moisture holding qualities of compost, dry fibrous and carbon-rich materials (such as lignocellulose) are utilised (Sarda et al. 2019). When compared to other comparable bulking agents, sawdust has a stronger potential to minimise leaching and is an efficient regulator of free air space and moisture content (Rich et al. 2018).

Given that peak temperatures during the thermophilic phase can reach up to 70 °C, tolerance to high temperatures is a crucial need for microorganisms that are intended to remain active throughout the composting process. The inoculation of refractory lignocellulose with thermo-tolerant *Actinomycetes* strains such as *Streptomyces* sp. H1, *Mycobacterium* sp. G1, *Micromonospora* sp. G7, and *Saccharomonospora* sp. T9 has demonstrated encouraging results (Wei et al. 2019; Muhammad et al. 2021). The humification process in composted cattle dung was shown to be improved by the addition of thermo-tolerant ammonia oxidising bacteria (Xu et al. 2022).

13.9.1 Effect of Vermicompost on Plant Growth and Development

As macronutrients, micronutrients, and other organic substances found in vermicompost are abundant, they are highly significant for the growth and development of plants (Ceritolu et al. 2018; Annapoorani and Sindhu 2021). Additionally, vermicompost contains enzymes and hormones that promote plant growth (Abbasi and Ramasamy 1999; Hussain et al. 2017). Numerous studies have indicated that bacteria from vermicompost produce plant growth hormones (auxin and cytokinins) (Muscolo et al. 1999; Atiyeh et al. 2002). Many researchers have demonstrated that the final vermicompost made from cow dung, sewage, and paper mill sludge contains significant amounts of humic compounds, which are important for plant growth and development (Senesi et al. 1992; Garcia et al. 1995; Masciandaro et al. 1997; Elvira et al. 1998). The different plant species that have experienced a significant increase in growth include banana, cassava and cowpea (Padmavathiamma et al. 2008), carrot (Chatterjee et al. 2014), Capsicum (Rekha et al. 2018; Khan et al. 2014), cowpea (Khan et al. 2015), chickpea (Yadav and Gard 2015), hyacinth bean (Karmegam and Daniel 2008), mulberry (Mary et al. 2015), muskmelon (Manh and Wang 2014), okra (Oroka 2015; Hussain et al. 2017), peppermint (Ayyobi et al. 2014), paddy

(Jayakumar et al. 2011), tomato (Joshi and Vig 2010; Truong et al. 2018; Benazzouk et al. 2018; Rajya Laxmi et al. 2015; Xiao et al. 2016), soybean (Sheikh et al. 2015), spinach (Peyvast et al. 2008), brinjal (Najar et al. 2015), marigold (Atiyeh et al. 2001; Sangwan et al. 2010), strawberry (Singh et al. 2008; Zuo et al. 2018), groundnut (Mycin et al. 2010), french bean (Hussain et al. 2017) and tea (Bagchi et al. 2015).

13.9.2 Advances in Vermicomposting Technology

In order to obtain high-quality natural fertilisers for sustainable agriculture practises and to improve the role of composting and vermicomposting practises throughout the transition to a circular economy, technology development based on research and innovation activities will be essential (Awasthi et al. 2022). China has the highest percentage of global patents (44%) and that novel composting devices are frequently the subject of compost technology-related inventions (Zheng et al. 2020). Comparing reactor composting to other conventional methods, such as static heaps, windrow composting, and membrane-covered composting, an LCA research found that reactor composting is a potential answer in terms of eco-efficiency (Chen et al. 2020; Liu et al. 2022). Further, pre-degrading organic waste before vermicomposting has resulted in reduced vermicomposting time period of about 2 weeks than traditional vermicomposting methods (Kauser and Khwairakpam 2022) (Fig. 13.2).

Fig. 13.2 Two-step vermicomposting process for organic waste management (Reproduced from Kauser and Khwairakpam 2022)

13.9.3 Small Scale Vermicomposting with Newer Technology

A complementing strategy to raise citizen responsibility and environmental awareness of bio-waste management and composting difficulties might be the community's involvement in constructing practical composting containers (Lunag et al. 2021). Improved composting toilet design may be a solution to the area's sanitation problems; however, this solution is only appropriate in select "green zone" locations where composting processes are likely to be successful (Lopez-Zavala 2019; Zhou et al. 2022a, b). To fasten the transition to a circular economy, on-site vermicomposting must be integrated into a comprehensive strategy. For instance, extra heat energy produced during the thermophilic phase of on-site composting at the home level could be captured and used to heat a greenhouse in the fall. In keeping with the objectives of the circular economy, Neugebauer et al. (2021) employed artificial neural networks to forecast the temperature inside the greenhouse to demonstrate that the vermicomposting process not only decreases bio-waste but also offers a chance for heat recovery. Future studies on small-scale composting techniques should really support such integrative methods (Zhou et al. 2022a, b).

13.9.4 New Technologies for Large-Scale Composting

When additional precautions like insulating methods, additives, and substrate pretreatment are taken into account, in-vessel vermicomposting technologies are viable for colder climates for large-scale production (Kumari et al. 2022). The fact that earthworm extraction is frequently done manually is one of the major issues with large-scale vermicomposting methods. According to a novel technology proposed by Walling et al. (2020), earthworms can be sorted using centrifugation with a worm recovery rate of roughly 84%. A combined strategy, such as vermicomposting with room drying for 10 days, is suggested to improve the stabilisation process of dewatered sewage sludge (Huang et al. 2022). Another technical option to improve operating parameters and hasten the biodegradation process is the vermicomposting reactor (Ramprasad and Alekhya 2021). When compared to a conventional container, a smart vermicompost reactor generated compost in half the time and with a 30% faster worm development rate (Ghorbani and Sabour 2021). The development of mathematical models and sensors to optimise a variety of composting/vermicomposting parameters, including artificial intelligence, is anticipated to be the subject of new study.

13.10 Summary

Without proper treatment, industrial pollutants and sludges may pollute the soil and other wildlife, posing serious health risks. Utilizing earthworms as adaptable natural bioreactors, vermicomposting technology allows for effective nutrient recycling and improved plant development while also decomposing organic matter and maintaining soil fertility. Vermicomposting can be used for a variety of organic solid wastes, including those that are domestic, animal, agro-industrial, human, etc. The value of vermicompost is further increased by the fact that it also provides other advantages. Extra worms can be utilised as a soil cleaner and as protein-rich animal feed, providing they are not grown on polluted wastes. The majority of research also showed that the vermicompost's end product may function as an appropriate medium for plant growth since it has a higher concentration of soil enzymes and growth hormones.

References

Abbasi SA, Ramasamy EV (1999) Biotechnological methods of pollution control. Orient Longman, Universities Press India Ltd., Hyderabad

Adhikary S (2012) Vermicompost, the story of organic gold: a review. Agric Sci 3:905–917

Albanella E, Plaixats J, Cabrero T (1988) Chemical change during vermicomposting (*Eisenia foetida*) of sheep manure mixed with cotton industrial waste. Biol Fertil Soils 6:266–269

Amaravathi G, Reddy RM (2015) Environmental factors affecting vermicomposting of municipal solid waste. Int J Pharm Bio Sci 5(3):81–93

Ananthavalli R, Ramadas V, John Paul JA, Karunai Selvi B, Karmegam N (2019) Seaweeds as bioresources for vermicompost production using the earthworm, *Perionyx excavatus* (Perrier). Bioresour Technol 275:394–401

Annapoorani CA, Sindhu M (2021) Comparative analysis of composting and vermicomposting from peel of *Musa acuminata*. Int J Zool Investig 7:792–800

Ansari AA, Ori L, Ramnarain YI (2020) An effective organic waste recycling through vermicompost technology for soil health restoration. In: Meena RS (ed) Soil health restoration and management. Springer, Singapore, pp 83–112

Atiyeh RM, Arancon N, Edwards CA, Metzger JD (2001) The influence of earthworm processed pig manure on the growth and productivity of marigolds. Bioresour Technol 81:103–108

Atiyeh RM, Subler S, Edwards CA, Bachman G, Metzger JD, Shuster W (2002) Effect of vermicompost and compost on plant growth in horticulture container media and soil. Pedobiologia 44:579–590

Awasthi MK, Wang Q, Huang H, Ren X, Lahori AH, Mahar A, Ali A, Shen F, Li R, Zhang Z (2016) Influence of zeolite and lime as additives on greenhouse gas emissions and maturity evolution during sewage sludge composting. Bioresour Technol 216:172–181

Awasthi MK, Duan YM, Awasthi SK, Liu T, Chen HY, Pandey A, Zhang Z (2020a) Emerging applications of biochar: improving pig manure composting and attenuation of heavy metal mobility in mature compost. J Hazard Mater 389:122116

Awasthi MK, Duan Y, Awasthi SK, Liu T, Zhang Z (2020b) Influence of bamboo biochar on mitigating greenhouse gas emissions and nitrogen loss during poultry manure composting. Bioresour Technol 303:122952

Awasthi MK, Singh E, Binod P, Sindhu SS, Kumar A, Chen H, Duan Y, Pandey A, Kumar S, Taherzadeh MJ, Li J, Zhang Z (2022) Biotechnological strategies for bio-transforming biosolid into resources toward circular bio-economy: a review. Renew Sustain Energy Rev 156:111987

Ayyobi H, Olfati JA, Peyvast GA (2014) The effects of cow manure vermicompost and municipal solid waste compost on peppermint (*Mentha piperita* L.) in Torbat-e-Jam and Rasht regions of Iran. Int J Recycl Org Waste Agric 3:147–153

Bagchi A, Ghosh BC, Swain DK, Bera N (2015) Organic farming practice for quality improvement of tea and its anti-parkinsonism effect on health defense. J Phys Chem Biophys 5:178

Barthod J, Rumpel C, Dignac MF (2018) Composting with additives to improve organic amendments. A review. Agron Sustain Dev 38:17

Benazzouk S, Djazouli ZE, Lutts S (2018) Assessment of the preventive effect of vermicompost on salinity resistance in tomato (*Solanum lycopersicum* cv. Ailsa Craig). Acta Physiol Plant 40(6):121

Bhat SA, Singh S, Singh J, Kumar S, Bhawana VAP (2018) Bioremediation and detoxification of industrial wastes by earthworms: vermicompost as powerful crop nutrient in sustainable agriculture. Bioresour Technol 252:172–179

Ceritoğlu M, Sezer S, Murat E (2018) Effects of vermicompost on plant growth and soil structure. 32:607–615

Chalmin P, Gaillochet C (2009) From waste to resource, an abstract of world waste survey, cyclope, Veolia environmental services. Economica, France

Chatterjee R, Bandyopadhyay S, Jana JC (2014) Evaluation of vegetable wastes recycled for vermicomposting and its response on yield and quality of carrot (*Daucus carota* L.). Int J Recycl Org Waste Agric 3:60

Chen H, Awasthi SK, Liu T, Zhang Z, Awasthi MK (2020) An assessment of the functional enzymes and corresponding genes in chicken manure and wheat straw composted with addition of clay via metagenomic analysis. Ind Crop Prod 153:112573

Datta S, Singh J, Singh S, Singh J (2016) Earthworms, pesticides and sustainable agriculture: a review. Environ Sci Pollut Res Int 23(9):8227–8243

Elvira C, Sampedro L, Benitez E, Nogales R (1998) Vermicomposting of sludges from paper mill and dairy industries with *Eisenia andrei*: a pilot scale study. Bioresour Technol 63:205–211

Garcia C, Ceccanti B, Masciandaro G, Hernandez T (1995) Phosphatase and β-glucosidase activities in humic substances from animal wastes. Bioresour Technol 53:79–87

Garg VK, Gupta R (2009) Vermicomposting of agro-industrial processing waste. In: Pandey A (ed) Singh nee' Nigam P. Biotechnology for agro-industrial residues utilisation. Springer, Dordrecht, pp 431–456

Garg P, Gupta A, Satya S (2006) Vermicomposting of different types of waste using *Eisenia foetida*: a comparative study. Bioresour Technol 97(3):391–395

Ghorbani M, Sabour MR (2021) Global trends and characteristics of vermicompost research over the past 24 years. Environ Sci Pollut Res 28(1):94–102

Hand P (1988) Earthworm biotechnology. In: Greenshields R (ed) Resources and application of biotechnology. The New Wave MacMillan Press Ltd., USA

Huang K, Zhang Y, Xu J, Guan M, Xia H (2022) Feasibility of vermicomposting combined with room drying for enhancing the stabilization efficiency of dewatered sludge. Waste Manage 143:116–124

Hussain N, Abbasi T, Abbasi SA (2017) Enhancement in the productivity of ladies finger (*Abelmoschus esculentus*) with concomitant pest control by the vermicompost of the weed salvinia (*Salvinia molesta*, Mitchell). Int J Recycl Org Waste Agric 6:335–343

Jain A, Sarsaiya S, Awasthi MK, Singh R, Rajput R, Mishra UC, Shi J (2022) Bioenergy and bioproducts from bio-waste and its associated modern circular economy: current research trends, challenges, and future outlooks. Fuel 307:121859

Jayakumar M, Sivakami T, Ambika D, Karmegam N (2011) Effect of turkey litter (*Meleagris gallopavo* L.) vermicompost on growth and yield characteristics of paddy, *Oryza sativa* (ADT-37). Afr J Biotechnol 10(68):15295–15304

Joshi R, Vig AP (2010) Effect of vermicompost on growth, yield and quality of tomato (*Solanum lycopersicum* L.). Afr J Basic Appl Sci 2:117–123

Kale RD, Krishnamoorthy RV (1981) Litter preference in the earthworm *Lampito mauritii*. Proc Indian Acad Sci (Anim. Sci.) 40:123–128
Karmegam N, Daniel T (2008) Effect of vermicompost and chemical fertilizer on growth and yield of hyacinth bean, *Lablab purpureus* (L.) Sweet. Dyn Soil Dyn Plant 2(2):77–81
Kauser H, Khwairakpam M (2022) Organic waste management by two-stage composting process to decrease the time required for vermicomposting. Environ Technol Innov 25:102193
Kaza S, Yao L, Bhada-Tata P, Van Woerden F (2018) What a waste 2.0: a global snapshot of solid waste management to 2050. Urban Development, Washington, DC, World Bank
Khan MH, Meghvansi MK, Gupta R, Veer V, Singh L, Kalita MC (2014) Foliar spray with vermiwash modifies the arbuscular mycorrhizal dependency and nutrient stoichiometry of bhut jolokia (*Capsicum assamicum*). PLoS ONE 3:92318
Khan VM, Manohar RS, Verma HP (2015) Effect of vermicompost and biofertilizer on symbiotic efficiency and yield of cowpea in arid zone of Rajasthan. Asian J Biol Sci 10(1):113–115
Kumari S, Manyapu V, Kumar R (2022) Recent advances in composting and vermicomposting techniques in the cold region: resource recovery, challenges, and way forward. Adv Organic Waste Manage 131–154
Lavelle P, Decaens T, Aubert M, Barot S, Blouin M, Bureau F (2006) Soil invertebrates and ecosystem services. Eur J Soil Biol 42:3–15
Liang C, Das KC, McClendon RW (2003) The influenceof temperature and moisture contents regimes on the aerobicmicrobial activity of a biosolids composting blend. Bioresou Technol 86(2):131–137
Lim SL, Wu TY, Lim PN, Shak KP (2015) The use of vermicompost in organic farming: overview, effects on soil and economics. J Sci Food Agric 95(6):1143–1156
Liu Z, Wang X, Li S, Bai Z, Ma L (2022) Advanced composting technologies promotes environmental benefits and eco-efficiency: a life cycle assessment. Bioresour Technol 346:126576
Lopez Zavala MÁ (2019) Design of composting-type toilet. In: Funamizu N (ed) Resource-oriented agro-sanitation systems. Springer, Tokyo, pp 107–119
Lunag MN, Elauria JC, Burguillos JD (2021) Community-based bin design approach: an initial stage toward urban composting at a hill station, Philippines. Environ Dev Sustain 23:3832–3852
Manh VH, Wang CH (2014) Vermicompost as an important component in substrate: effects on seedling quality and growth of Muskmelon (*Cucumis Melo* L.). APCBEE Proc 8:32–40
Martin M, Eudoxie G (2018) Feedstock composition influences vermicomposting performance of *Dichogaster annae* relative to *Eudrilus eugeniae* and *Perionyx excavatus*. Environ Sci Pollut Res Int 25(18):17716–17725
Mary LCL, Sujatha R, Chozhaa AJ, Navas PMA (2015) Influence of organic manures (biofertilizers) on soil microbial population in the rhizosphere of mulberry (*Morusindica* L.). Int J Appl Sci Biotechnol 3(1):61–66
Masciandaro G, Ceccanti B, Garcia C (1997) Soil agro-ecological management: fertirrigation and vermicompost treatments. Bioresour Technol 59:199–206
Mohapatra D, Sahoo KK, Sannigrahi AK (2019) Impact of *Eisenia fetida* populations on bioconversion of paper mill solid wastes. Int J Recycl Org Waste Agricult 8(1):189–193
Muhammad A, Shi AP, Muhammad A, Zhang MQ, Xu ZH, Abdul S, Muhammad F, Wei W, Ye LH (2021) Ultra-high temperature aerobic fermentation pretreatment composting: parameters optimization, mechanisms and compost quality assessment. J Environ Chem Eng 9:105453
Muscolo A, Bovolo F, Gionfriddo F, Nardi S (1999) Earthworm humic matter produces auxins-like effect on *Daucus carota* cell growth and nitrate metabolism. Soil Biol Biochem 31:1303–1311
Mycin TR, Lenin M, Selvakumar G, Thangadurai R (2010) Growth and nutrient content variation of groundnut *Arachis hypogaea* L. under vermicompost application. J Exper Sci 1:12–16
Najar IA, Khan AB, Hai A (2015) Effect of macrophyte vermicompost on growth and productivity of brinjal (*Solanum melongena*) under field conditions. Int J Recycl Org Waste Agric 4:73–83
Ndegwa PM, Thompson SA (2000) Effects of C-to-N ratio on vermicomposting of biosolids. Bioresou Technol 75(1):7–12

Neugebauer M, Hałacz J, Olkowski T (2021) A compost heating solution for a greenhouse in north-eastern Poland in fall. J Clean Prod 279:123613
Olle M (2019) Vermicompost, its importance and benefit in agriculture. J Agric Sci 2:93–98
Oroka FO (2015) Influence of municipal solid waste vermicompost on soil organic carbon stock and yield of okra (*Abelmoschus esculentus* Moench) in a tropical agroecosystem. J Environ Earth Sci 5(12):61–66
Padmavathiamma PK, Li LY, Kumari UR (2008) An experimental study of vermi-biowaste composting for agricultural soil improvement. Bioresour Technol 99(6):1672–1681
Peigne J, Girardin P (2004) Environmental impacts of farm–scale composting practices. Water Air Soil Pollut 153(1–4):45–68
Peyvast G, Olfati JA, Madeni S, Forghani A (2008) Effect of vermicompost on the growth and yield of spinach (*Spinacia oleracea* L.). J Food Agric Environ 6:110–113
Pramanik P, Ghosh GK, Ghosal PK, Banik P (2007) Changes in organic—C, N, P and K and enzymes activities in vermicomposting of biodegradable organic wastes under liming and microbial inoculants. Bioresour Technol 98:2485–2494
Rajya Laxmi P, Saravanan S, Naik ML (2015) Effect of organic manures and inorganic fertilizers on plant growth, yield, fruit quality and shelf life of tomato (*Solanum lycopersicon* L.) c.v. PKM-1. Int J Agric Sci 5(2):7–12
Ramprasad C, Alekhya D (2021) Design and optimisation of high rate decentralised vermicomposting reactor for the household organic waste. Int J Environ Waste Manage 27(4):420–439
Raza ST, Wu J, Rene ER, Ali Z, Chen Z (2022) Reuse of agricultural wastes, manure, and biochar as an organic amendment: a review on its implications for vermicomposting technology. J Clean Prod 360:132200
Rekha GS, Kaleena PK, Elumalai D, Srikumaran MP, Maheswari VN (2018) Effects of vermicompost and plant growth enhancers on the exo-morphological features of *Capsicum annum* (Linn.) Hepper. Int J Recycl Org Waste Agric 7(1):83–88
Rich N, Bharti A, Kumar S (2018) Effect of bulking agents and cow dung as inoculant on vegetable waste compost quality. Bioresour Technol 252:83–90
Romero-Tepal EM, Contreras-Blancas E, Navarro-Noya YE, Ruiz-Valdiviezo VM, Luna-Guido M, Gutierrez-Miceli FA, Dendooven L (2014) Changes in the bacterial community structure in stored wormbed leachate. J Mol Microbiol Biotechnol 24:105–113
Sanchez-Hernandez JC (2019) Bioremediation of pesticide contaminated soils by using earthworms. Bioremediation of agricultural soils. CRC Press, Boca Raton, pp 165–192
Sangwan P, Garg VK, Kaushik CP (2010) Growth and yield response of marigold to potting media containing vermicompost produced from different wastes. Environmentalist 30:123–130
Sarda LG, Higarashi MM, de Oliveira PAV, Comin JJ (2019) Ryegrass straw and wood shavings as bulking agents on swine slurry co-composting: a case study in Southern Brazil. Int J Recycl Org Waste Agricult 8:55–63
Senesi S, Saiz JC, Miano TM (1992) Spectroscopic characterization of metal humic acid like complexes of earthworms composed organic wastes. Sci Total Environ 117:111–120
Sheikh MA, Dwivedi P, Dwivedi HS (2015) Impact of chemical fertilizer and organic manure on the germination and growth of soybean (*Glycine max* L.). Adv Life Sci Technol 31:73–77
Singh NB, Khare AK, Bhargava DS, Bhattacharya S (2004) Optimum moisture requirement during vermicomposting using *Perionyx excavates*. App Ecol Environ Res 2:53–62
Singh R, Sharma RR, Kumar S, Gupta RK, Patil RT (2008) Vermicompost substitution influences growth, physiological disorders, fruit yield and quality of strawberry (*Fragaria ananassa* Duch.). Bioresour Technol 99:8507–8511
Singh S, Singh J, Vig AP (2016) Effect of abiotic factors on the distribution of earthworms in different land use patterns. J Basic Appl Zool 74:41–50
Singh A, Karmegam N, Singh GS, Bhadauria T, Chang SW, Awasthi MK, Sudhakar S, Arunachalam KD, Biruntha M, Ravindran B (2020) Earthworms and vermicompost: an eco-friendly approach for repaying nature's debt. Environ Geochem Health 42(6):1617–1642

Thakur SS, Yadav S (2018) Exploration of earthworms of India through online digital library. In: Ray S (ed) Earthworms–the ecological engineers of soil. IntechOpen, London [Internet]

Tripathi G, Bhardwaj P (2004) Comparative studies on biomass production, life cycles and composting efficiency of *Eisenia fetida* (Savigny) and *Lampito mauritii* (Kinberg). Bioresour Technol 92:275–283

Truong HD, Wang CH, Kien TT (2018) Effect of vermicompost in media on growth, yield and fruit quality of cherry tomato (*Lycopersicon esculentun* Mill.) under net house conditions. Compost Sci Util 26(1):52–58

Walling E, Tremier A, Vaneeckhaute C (2020) A review of mathematical models for composting. Waste Manage 113:379–394

Wei Y, Wu D, Wei D, Zhao Y, Wu J, Xie X, Zhang R, Wei Z (2019) Improved lignocellulose-degrading performance during straw composting from diverse sources with actinomycetes inoculation by regulating the key enzyme activities. Bioresour Technol 271:66–74

Wu Z, Yin B, Song X, Qiu J, Cao L, Zhao Q (2019) Effects of salinity on earthworms and the product during vermicomposting of kitchen wastes. Int J Environ Res Public Health 16(23):4737

Xiao Z, Liu M, Jiang L, Chen X, Griffiths BS, Li H, Hu F (2016) Vermicompost increases defense against root-knot nematode (*Meloidogyne incognita*) in tomato plants. Appl Soil Ecol 105:177–186

Xu Z, Li R, Wu S, He Q, Ling Z, Liu T, Wang Q, Zhang Z, Quan F (2022) Cattle manure compost humification process by inoculation ammonia-oxidizing bacteria. Bioresour Technol 344:126314

Yadav A, Garg VK (2011) Recycling of organic wastes by employing *Eisenia fetida.* Bioresou Technol 102:2874–2880

Yadav A, Garg VK (2015) Influence of vermi-fortification on chickpea (*Cicer arietinum* L.) growth and photosynthetic pigments. Int J Recycl Org Waste Agric 4:299–305

Zheng X, Aborisade MA, Liu S, Lu S, Oba BT, Xu X, Ding H (2020) The history and prediction of composting technology: a patent mining. J Clean Prod 276:124232

Zhou YW, Awasthi SK, Liu T, Verma S, Zhang Z, Pandey A, Varjani S, Li R, Taherzadeh MJ, Awasthi MK (2021) Patterns of heavy metal resistant bacterial community succession influenced by biochar amendment during poultry manure composting. J Hazard Mater 420:126562

Zhou S, Kong F, Lu L, Wang P, Jiang Z (2022a) Biochar-An effective additive for improving quality and reducing ecological risk of compost: a global meta-analysis. Sci Total Environ 806:151439

Zhou Y, Xiao R, Klammsteiner T, Kong X, Yan B, Mihai FC, Liu T, Zhang Z, Kumar Awasthi M (2022b) Recent trends and advances in composting and vermicomposting technologies: a review. Bioresour Technol 360:127591

Zuo Y, Zhang J, Zhao R, Dai H, Zhang Z (2018) Application of vermicompost improves strawberry growth and quality through increased photosynthesis rate, free radical scavenging and soil enzymatic activity. Sci Hortic 233:132–140

Chapter 14
Remediation of Endocrine Disrupting Compounds and Organic Dye Pollutants Through Biosorbents in a Circular Bioeconomy: Prospects and Constraints

Annu T. Mathew and M. P. Saravanakumar

Abstract The presence of endocrine-disrupting compounds (EDCs) and organic dye pollutants in water may have toxic, hazardous, and carcinogenic effects on exposure. The recent advances in research on biomaterial synthesis, characterization, and applications have attracted a lot of researchers in this area. The physical, chemical, and thermal characteristics of green synthesized materials help to facilitate their interactions with a variety of water pollutants. The use of green synthesized nanomaterials as eco-friendly sorbents increases the value of wastes and naturally available compounds utilized during its synthesis. The major advantage of the adsorption process is that it does not involve the formation of additional toxic by-products, unlike other treatments. Several operational parameters like pH, nanomaterial dose, reaction time, pollutant concentration, other contaminants, and temperature may influence the adsorption process. The adsorptive interactions between bio-based material and water pollutants may be through electrostatic, covalent (H-bond and Van der Waals forces), π- π, and n- π bonds. Many isotherm and kinetic models are used to validate the experimental results for adsorption. The simplicity, eco-friendly, and cost-effective nature of pollutant adsorption through biosorbents provides scope for its utility on a large scale. The circular bioeconomy motivates the *close the loop* concept through the generation of the least waste, utilization of created wastes, and maximum utility of naturally available substances. The prospects regarding the application of biosorbents for the elimination of EDCs and organic dyes in a circular bioeconomy are explained and constraints concerning it are discussed in this work.

Keywords Green synthesized materials · Endocrine-disrupting compounds · Organic dyes · Adsorption · Circular bioeconomy

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14.1 Introduction

EDCs are emerging organic contaminants whose consumption is considered to increase over a while in the future. This is due to its wide range of applications, increasing production, and consumption with the improving standards of living. Micropollutants are a class of compounds that may include pharmaceutical compounds, EDCs, pesticides, personal care products, and biocides. The entry of these compounds into an aquatic ecosystem is through industrial effluents, sewage effluents, animal wastes, landfill leachate etc. Even traces (ng/L-μg/L) of these compounds inside an aquatic ecosystem can prove hazardous (Goswami et al. 2018). Therefore, there is a high risk of exposure and a great need for a detailed study of these toxicants. The existence of these compounds in groundwater, seawater, surface water, and effluents has been detected in various concentrations (Olasupo and Suah 2021). Hence, it becomes important to remove these micropollutants before it transforms into other complex products under the diverse environmental conditions.

In the meantime, dyes can be broadly divided into categories such as acidic, disperse, basic, azo, diazo, and a metal complex. About 20% of industrial wastewater consists of dye pollutants in it. The organic dye release in the environment is a major concern due to potential mutagenic, carcinogenic, and toxic effects (El-Sharkawy et al. 2007). The organic dyes possess chromophores (aromatic rings and azo groups) which make the structure quite stable. Hence, these chemicals become difficult to degrade under natural light, heat, and oxidants in the environment (Qin et al. 2022). Therefore, it is necessary to eliminate dyes from an aquatic environment. Biosynthesis may include the use of plant extracts or sludge or wastes for the generation of nanoparticles (Singh et al. 2018). The extracts of plants or naturally available solutions act as natural reducing and capping agents during nanoparticle synthesis (Jadoun et al. 2021).

The circular water economy aims at providing sustainable production and consumption of water through water reuse and resource recovery. Bioeconomy acts as a catalyst for the transition from a linear economy to a circular one (Ferreira et al. 2018). The linear economy maintains a linear relationship between input and output. It focuses on converting raw materials to final products, distribution, and consumption of these final goods, and disposal of wastes. Bioeconomy is also called circular green economy. An effective bioeconomy must be economical, circular, sustainable, and ecological. The basic strategies towards such a transition involve the least production of waste, and the reuse of the products in a close the loop manner (Aguilar and Twardowski 2022).

14.2 Sources and Effects of EDCs and Organic Dyes in Water

Organic pollutants are released due to anthropogenic activities from domestic, industrial, pharmaceutical, and other sectors. The maximum quantity of various chemicals existing in wastewater treatment plant (WWTP) effluent is mostly present in a range of 1–10^3 μg/L (Rogowska et al. 2020). The report produced by the Department of Water Affairs for wastewater quality management (2012 Green Drop Report) and potable water quality management (2012 Blue Drop Report) indicates that regulatory limits are not met by many municipalities in the North West Province of South Africa. Therefore, this water is not proper for consumption without appropriate treatment (Wanda et al. 2018).

Carpenter et al. screened for about 200 micropollutants collected from 17 sites along the Hudson River estuary. Hudson river serves as a drinking water source for more than 100,000 people and is also used for recreational and commercial purposes. The researchers identified clusters and sub-clusters of micropollutants based on normalized concentration patterns and spatiotemporal occurrence. The major clusters are named 'core micropollutants', 'sourced from diffuse upstream sources', and 'those having sewage treatment plants as sources'. Totally 127 samples are analyzed. A list of 200 target micropollutants is used to detect 168 in at least one of 127 samples. Out of which 116 are derived from wastewater and 52 are sourced from agricultural activities. The concentration of a majority of micropollutants has been found in the range of 1 to 100 ng/L. Few compounds such as atenolol acid, metformin, and sucralose are found in high concentrations of about several mg/L and are from sewage treatment plant outfall samples (Carpenter and Helbling 2018).

The limits of micropollutants present in freshwater have also been exceeded in many other parts of the world. It was reported that high concentrations of acetaminophen, valsartan, codeine, gabapentin, tramadol, etc. have been detected in River Taff and River Ely of Wales, UK. In southern Spain, pharmaceutical concentrations have been detected from compost, sewage sludge, and sediment samples along the Guadiamar River (Ebele et al. 2017). Considering, developing countries like India which show the presence of pesticidal micropollutants in influents of WWTP indicates, that it is an agriculture-based country. The values of pesticide concentration above safety limits can prove to be life-threatening. Similarly, the presence of caffeine in raw sewage of WWTP in China infers great consumption of coffee and tea in the area (Zhou et al. 2010). Therefore, the type of micropollutant is greatly influenced by habitual nature and other associated factors of that particular place. Hence, due to the hazardous nature and increasing risks posed by more than thousands of materials, watch lists have been created for inter-government monitoring. The list includes several priority substances or a group of materials. Some of the compounds like bisphenol A (BPA) and diclofenac are part of the list as they are extremely toxic (Barbosa et al. 2016).

Meanwhile, dyes find broad applications in leather tanning, food processing, cosmetics, paper and printing, and dye manufacturing units. The dyes are present in

industrial wastes and eventually get discharged into water bodies. The total release of dyes from various processes is still unknown but studies suggest that 100 tonnes of dyes are received by water streams per year (Yagub et al. 2012). The processing of fabric requires about 100–200 L of water per kilogram (Holkar et al. 2016). The regulations for textile wastewater emission depend on the product type generated by the factory. Various countries like Malaysia, India, Algeria, etc. are found to release textile wastewater with different physical and chemical properties. The parameters like pH, biochemical oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS), total suspended solids (TSS), turbidity, and color of textile wastewater vary based on the factory processes it undergoes (Dihom et al. 2022). The dyes make the water bodies colored, and their toxicity, structure, and non-degradable nature (sometimes), make even their exposure at small concentrations risky to living organisms (Yagub et al. 2014). Therefore, these water contaminants need to be removed from water and wastewater systems.

Human beings can encounter micropollutants through activities like drinking, bathing, swimming, and recreation in surface water. Sometimes, these toxic compounds get into groundwater, and a lack of proper treatment specific to micropollutant elimination can cause severe health issues (Eggen et al. 2014). Though sometimes they are invisible to the naked eye, even small traces of micropollutants in water are dangerous. From toxicological studies, the presence of micropollutants in drinking water is linked to potential carcinogenic risks which exceed the EPA's negligible risk level (Xue et al. 2021). Additionally, researchers suggest that intermediates generated when a pollutant gets exposed to some organisms or chemicals may be of higher toxicity than parent compounds (Żur et al. 2018).

The presence of diclofenac in quantities less than 1 μg/L can have ill effects on the kidney, liver, and gills of Pacific rainbow trout (*Oncorhynchus mykiss*) (Mehinto et al. 2010). Similarly, chlorpyrifos micropollutants pose a severe threat to living organisms by causing infertility, skin disorders, cancer, etc. (ul Haq et al. 2020). It is reported that parabens induce immunologically mediated systemic hypersensitivity reactions and inhibit estrogenic activity. Also, exposure to N,N-diethyl-meta-toluamide inhibited the enzyme controlling the central nervous system (Keerthanan et al. 2020).

Studies also suggest that the presence of a detrimental type of amines in azo dyes makes them highly toxic (Robinson et al. 2001). The highly soluble and chemically stable nature of dyes makes the wastewater pigmented, posing a severe threat to the bio-system (Asgher and Bhatti 2012). Exposure to dyes can cause allergic skin disorders, in textile workers and people using close-skin fitting clothes made of synthetic fibers. The toxic impacts of prolonged dye contact include chromosomal fractures, respiratory toxicity, carcinogenesis, and mutagenesis (Yagub et al. 2014). The use of dyes like malachite green is banned in many countries due to their highly toxic impact on the aquatic system, yet its illegal utilization is still widespread in some countries (Pathy et al. 2022). Dyes like rhodamine B which are extensively used in the dyeing of paints and fabrics are also reported to endanger the earth due to their hard-to-degrade and high chromatic nature (Xu and Ma 2021). Hence, there is a need

for the removal of these toxic organic compounds from the water and wastewater system.

14.3 Characterization and Use of Biomaterials for Organic Pollutant Removal

The unique properties of green synthesized nanoparticles can be determined by utilizing several tools for characterization. The green synthesized nanoparticles exist in different shapes like spheres, rods, cubes, etc., similar to the nanomaterials obtained using non-green ways. A few examples of morphologies obtained using the green synthesis pathway are mentioned below. The existence of rod-shaped nanoparticles of size 100–150 nm is obtained for hydroxyapatite, green synthesized using sea biowastes (Karvandian et al. 2020). Licorice root extract is utilized to produce hydroxyapatite nanorods in other studies. The aggregated rods appear uniform, similar-sized, and well-crystallized (Ali et al. 2021). In another research, the celery seeds extract acts as a green reducing agent for generating nanocomposites. Silver nanoparticles on the surface of reduced graphene oxide nanosheets are obtained through this process. The TEM images obtained reveal monodispersed, spherical, and fine silver nanoparticles (size 20 nm approx.) on the surface of reduced graphene oxide after microfluidization treatment. The results propose that celery extracts enhance the dispersibility of silver nanoparticles on wrinkled sheets of reduced graphene oxide (Soleymani et al. 2020). Uniform and porous microsphere structures loosely packed by nanocubes are obtained through coconut shells in another study. This structure enhances the active sites and sensitivity (Sivasakthi et al. 2020). Researchers suggest the biosynthesis of Cu_2O nanospheres decorated on graphitic carbonitride using citrus limon leaf extracts through the hydrothermal method. The average particle size of the Cu_2O nanosphere is found to be in the range of 2–10 nm (Induja et al. 2019). Therefore, different morphologies of nanoparticles can be attained using green synthesis methodologies. The derived nanoparticles also possess excellent properties for various field-scale applications.

The surface area of green synthesized nanoparticles is determined using BET analysis. Results indicate that the synthesis of nanotubes using wastes or natural feedstock like coconut oil, rice straw, camphor, palm oil, etc., produces carbon nanotubes of excellent surface area and dimensions (Makgabutlane et al. 2021). For instance- the carbon nanotubes green synthesized using rice straw yield a surface area of 188 m^2/g and a size of 15–40 nm (Lotfy et al. 2018). In another research, the surface area and pore volume of green synthesized iron nanoparticles (using eucalyptus leaves) are compared with raw laterite particles. The surface area and pore volume are higher for iron nanoparticles (36.62m^2/g and 0.0394cm^3/g respectively) than for raw laterite particles (23.18m^2/g and 0.0091cm^3/g, respectively). This can be attributed to polyphenols in the eucalyptus that act as capping and reducing agents (Sangami and Manu 2017). The surface area of α-Fe_2O_3 synthesized using green tea leaf extract

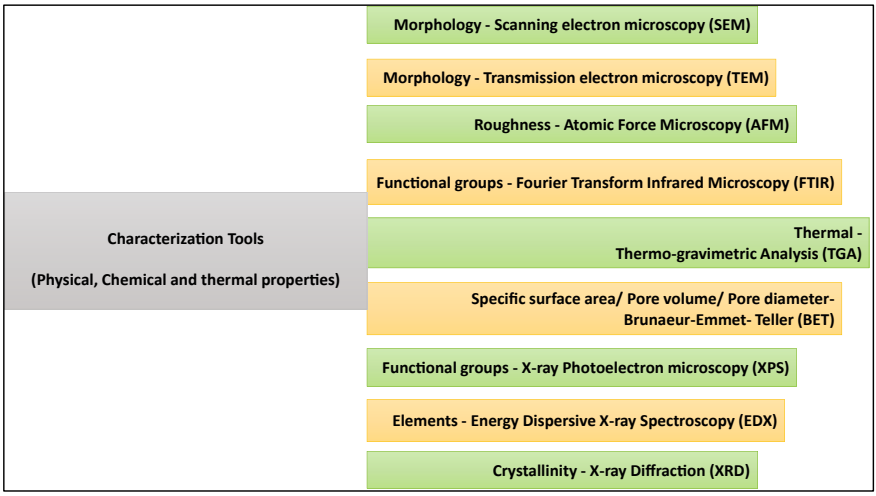

Fig. 14.1 Tools to characterize biosorbents to determine physicochemical properties

through the hydrothermal method is observed to be four times more than traditional commercially available ones. The reactivity is also doubled for green synthesized α-Fe_2O_3 when compared to synthetic nanoparticles (Ahmmad et al. 2013). Therefore, green nanoparticle synthesis helps achieve high surface area and pore volume beneficial for wastewater treatment applications. Hence, the physicochemical properties of bio-based materials found through various tools help to determine the nature and stability of biosynthesized material. The various instrumentation techniques and characterizations that may be used to characterize biomaterial and their origin are presented in Fig. 14.1.

14.4 Adsorption of EDCs and Dyes Using Bio-based Materials

Adsorption is a process employed by most researchers due to its simple operation, high efficiency, and flexibility of design (Crini 2006). The process does not involve the formation of toxic intermediates or by-products unlike other treatments (for example: in the degradation of pollutants in an advanced oxidation process) (Mathew and Saravanakumar 2022a). There are two essential elements in an adsorption process. Firstly, the adsorbent on which the pollutant goes and attaches. Secondly, the adsorbate which is the pollutant itself. Here, the adsorbent is biomaterials and the adsorbate are the EDCs and dyes.

Several factors impact the process efficiency based on the nature of bonds formed between biosorbent and organic pollutants. The presence of other contaminants mostly hinders the removal of EDCs/ dyes in a real water system (Wu et al. 2022).

Still, biosorbents which can perform well in the presence of other pollutants, have been synthesized. The improved performance can be mostly due to better adsorbent properties obtained in the system (Yu et al. 2018). The solution pH, temperature, reaction time, biosorbent dose, and contaminant concentration are a few factors that affect the efficiency of sorbent. The pH of the solution can affect the nature of bonds between biosorbents and pollutants. This interaction is based on multiple factors like the point of zero charge (pH_{zc}) of the adsorbent and the isoelectric point of the pollutant. The biosorbent dose determines the amount of active sites available to pollutant and an increase in the dosage enhances removal till saturation. The pollutant concentration in the solution is a measure of the quantity of a pollutant that needs to be treated by the fixed dose of biosorbent, and this impacts process efficiency. The temperature variation may affect the viscosity of the solution or weaken the bonds for effective adsorption (Mathew and Saravanakumar 2021).

The nature of bonds formed in the system can be analyzed through a deep study of the adsorption mechanism. The types of functional groups on the pollutant and sorbent, with other operational parameters, play a vital role to determine the type of bond in the system. Hence, physisorption or chemisorption can occur in the system. The adsorptive bonds may include electrostatic interaction, π-π bonds, n- π, complexation, H-bonds, hydrophobic interactions, pore filling, etc. Weak physical bonds are formed in the case of physisorption (for example hydrogen bonds), and the formation of strong chemical bonds portrays chemisorption (for example electrostatic bonds) (Heo et al. 2019). The electrostatic bonds of attraction are a result of opposite charges between pollutant and adsorbent. For instance, metal oxides can act as an acid or basic material based on the pH_{zc} of the material. There are –OH groups attached to the surface of metal oxide nanomaterials. The following reactions can occur in such a system that determines the adsorbent charges for electrostatic interactions (Kasprzyk-Hordern et al. 2003).

$$M - OH + H^+ \leftrightarrow M - OH_2^+ (when\ pH < \text{pHzc}) \tag{14.1}$$

$$M - OH + H_2O (when\ pH = \text{pHzc}) \tag{14.2}$$

$$M - OH + OH^- \leftrightarrow M - O^- + H_2O (when\ pH > \text{pHzc}) \tag{14.3}$$

Bonds like Yoshida H-bonds, dipole–dipole, n-π, π-π, etc., can also be found. The Yoshida H-bonds result from bonds between hydrogen from functional groups (like –OH) on the adsorbent and the aromatic rings of the pollutant. The hydrogen on the adsorbent acts as a donor towards hydrogen-accepting groups (for example oxygen and nitrogen) on pollutants forming dipole–dipole H bonds. The n-π bonds are formed when electrons transfer from functional groups (example-carbonyl) of adsorbent to aromatic rings of pollutant. The interaction of π electrons of adsorbent and pollutant leads to the formation of π-π bonds, enhancing adsorption.

The adsorption isotherm curve relates the equilibrium concentration of micropollutant/ organic dye on the surface of the biosorbent to the concentration of these

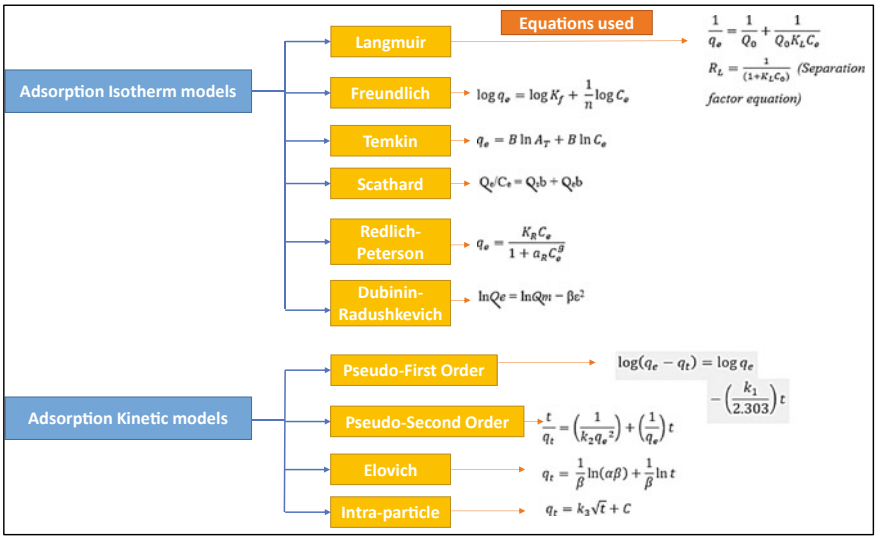

Fig. 14.2 Adsorption isotherm and kinetic models to validate experimental data

pollutants in the reaction solution at a given temperature. Adsorption kinetic models measure the diffusion of pollutants in pores of nanorods and tell about adsorption uptake with respect to time (for constant concentration or pressure). Figure 14.2 shows some adsorption isotherm and kinetic models which helps to validate the adsorption data. The values obtained through experimental analysis are put into equations of isotherm and kinetic models. The isotherm and kinetic plot which yields the highest correlation coefficient and least error function value is the best fit for the set of obtained experimental values (Guillossou et al. 2020).

14.5 Transition to Circular Bioeconomy Through Bio-based Materials

A circular economy is a close the loop approach where the wastes generated in the system are utilized in the system itself. The main strategy toward such a change involves: improving the product's life span, keeping a product in operation, conserving materials, and preserving the energy embodied in materials (Moraga et al. 2019). The circular bioeconomy depends on several factors like environmental, economic, and social factors. The environmental factors cover areas of setting environmental awareness about the toxic effects of water pollutant exposure and regulations set to make these toxin levels under control. Economic factors involve the GDP, income, and job opportunities created if such an approach is adopted. Social

factors include perspectives like the education of people, age, and access to information. The implementation of a circular bioeconomy requires a lot of planning and proper execution to make these toxins under control in an environmentally friendly and economic manner (Mathew and Saravanakumar 2022b).

The transition to a circular bioeconomy for the elimination of organic pollutants on larger scales may involve several steps. The identification of the process involved in pollutant removal is a key step affecting the transition to a circular bioeconomy. This is because the nature of by-products (formed during the treatment) further determines the possibility of process applicability in a circular bioeconomy. If the by-products formed are hazardous, additional treatment should be employed for the removal of such toxins. This may incur additional expenses on large scale. Meanwhile, employing a treatment process that doesn't generate additional by-products or intermediates is advised. This can help to reduce additional costs incurred in the removal of intermediates/ by-products. Hence, adsorption is suggested, as the process does not involve the generation of other by-products (as described in the sections above).

The selection of sorbent used for EDC and dye adsorption is another crucial step for implementing a circular economy. The sorbent should possess certain properties like high efficiency, should be economical, and be synthesized in an eco-friendly manner. The biosorbents can be used to remove organic dyes and EDCs from wastewater. The wastewater obtained after purification can be used to water the plants. These plants/ plant parts act as a source for the synthesis of biosorbents, thereby closing the loop in a circular economy. In such a scenario, the utilization of biomaterial can be an effective option. The treated sludge from wastewater can also enrich the soil, which yields trees, fruits, natural extracts, etc. in a circular bioeconomy. The advantage of utilizing biomaterials is that they can be used in energy storage and generation devices on larger scales. Figure 14.3 shows the sources for the generation of bio-based materials (from sludge and natural components) and their application (pollutant removal and energy devices) in a circular bioeconomy. The major challenge of implementing bio-based materials as adsorbents in a circular bioeconomy is about complete separation of pollutants from sorbents after adsorption.

Several countries (especially European countries) in the world are taking efforts to remove EDCs in effluents. The implementation of lab-scale experiments on large scales can be through considering the costs required for the upgradation of present techniques, availability, and accessibility of resources required. To implement the above in a circular bioeconomy it should be ensured that the biomaterial works for the elimination of multiple types of pollutants (for example EDCs and dyes) on larger scales. Hence, it is crucial to perform pilot scale studies for highly effective bio-based materials obtained at lab scales.

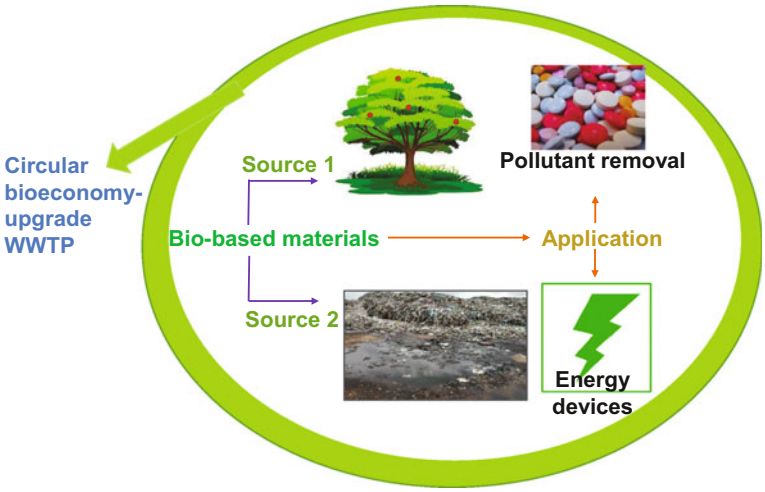

Fig. 14.3 Circular bioeconomy approach: sources and applications of biosorbents

14.6 Conclusion and Future Scope

The biomaterials show excellent morphological, physicochemical, and thermal properties, which help in the adsorption of micropollutants and organic dyes. The bonds formed between biosorbents and contaminants include electrostatic, π-π, n-π, hydrogen, and Vander Waals bonds. The application of biomaterials in energy devices gives scope for its implementation in a circular bioeconomy to upgrade wastewater treatment plants. More pilot-scale studies on energy applications and water pollutant removal using biosorbents should be carried out for a transition to a circular bioeconomy. The properties of biomaterials should be checked using characterization tools after each synthesis, considering the change in properties of sludge/ fruits/ natural components used as pre-cursor. Studies on the disposal/ reuse of pollutant-loaded biosorbents should be encouraged for a complete close the loop approach in treatment plants.

References

Aguilar A, Twardowski T (2022) Bioeconomy in a changing word. EFB Bioecon J 2:100041. https://doi.org/10.1016/j.bioeco.2022.100041

Ahmmad B, Leonard K, Shariful Islam M, Kurawaki J, Muruganandham M, Ohkubo T, Kuroda Y (2013) Green synthesis of mesoporous hematite (α-Fe2O3) nanoparticles and their photocatalytic activity. Adv Powder Technol 24(1):160–167. https://doi.org/10.1016/j.apt.2012.04.005

Ali AF, Alrowaili ZA, El-Giar EM, Ahmed MM, El-Kady AM (2021) Novel green synthesis of hydroxyapatite uniform nanorods via microwave-hydrothermal route using licorice root extract as template. Ceram Int 47(3):3928–3937. https://doi.org/10.1016/j.ceramint.2020.09.256

Asgher M, Bhatti HN (2012) Evaluation of thermodynamics and effect of chemical treatments on sorption potential of Citrus waste biomass for removal of anionic dyes from aqueous solutions. Ecol Eng 38(1):79–85. https://doi.org/10.1016/j.ecoleng.2011.10.004

Barbosa MO, Moreira NFF, Ribeiro AR, Pereira MFR, Silva AMT (2016) Occurrence and removal of organic micropollutants: an overview of the watch list of EU decision 2015/495. Water Res 94:257–279. https://doi.org/10.1016/j.watres.2016.02.047

Carpenter CMG, Helbling DE (2018) Widespread micropollutant monitoring in the hudson river estuary reveals spatiotemporal micropollutant clusters and their sources. Environ Sci Technol 52(11):6187–6196. https://doi.org/10.1021/acs.est.8b00945

Crini G (2006) Non-conventional low-cost adsorbents for dye removal: a review. Bioresour Technol 97(9):1061–1085. https://doi.org/10.1016/j.biortech.2005.05.001

Dihom HR, Al-Shaibani MM, Radin Mohamed RMS, Al-Gheethi AA, Sharma A, Khamidun MHB (2022) Photocatalytic degradation of disperse azo dyes in textile wastewater using green zinc oxide nanoparticles synthesized in plant extract: a critical review. J Water Process Eng 47:102705. https://doi.org/10.1016/j.jwpe.2022.102705

Ebele AJ, Abou-Elwafa Abdallah M, Harrad S (2017) Pharmaceuticals and personal care products (PPCPs) in the freshwater aquatic environment. Emerg Contam 3(1):1–16. https://doi.org/10.1016/j.emcon.2016.12.004

Eggen RIL, Hollender J, Joss A, Schärer M, Stamm C (2014) Reducing the discharge of micropollutants in the aquatic environment: the benefits of upgrading wastewater treatment plants. Environ Sci Technol 48(14):7683–7689. https://doi.org/10.1021/es500907n

El-Sharkawy EA, Soliman AY, Al-Amer KM (2007) Comparative study for the removal of methylene blue via adsorption and photocatalytic degradation. J Colloid Interface Sci 310(2):498–508. https://doi.org/10.1016/j.jcis.2007.02.013

Ferreira A, Marques P, Ribeiro B, Assemany P, de Mendonça HV, Barata A, Oliveira AC, Reis A, Pinheiro HM, Gouveia L (2018) Combining biotechnology with circular bioeconomy: from poultry, swine, cattle, brewery, dairy and urban wastewaters to biohydrogen. Environ Res 164:32–38. https://doi.org/10.1016/j.envres.2018.02.007

Goswami L, Vinoth Kumar R, Borah SN, Arul Manikandan N, Pakshirajan K, Pugazhenthi G (2018) Membrane bioreactor and integrated membrane bioreactor systems for micropollutant removal from wastewater: a review. J Water Process Eng 26:314–328. https://doi.org/10.1016/j.jwpe.2018.10.024

Guillossou R, Le Roux J, Brosillon S, Mailler R, Vulliet E, Morlay C, Nauleau F, Rocher V, Gaspéri J (2020) Benefits of ozonation before activated carbon adsorption for the removal of organic micropollutants from wastewater effluents. Chemosphere 245:125530. https://doi.org/10.1016/j.chemosphere.2019.125530

Heo J, Yoon Y, Lee G, Kim Y, Han J, Park CM (2019) Enhanced adsorption of bisphenol A and sulfamethoxazole by a novel magnetic CuZnFe2O4–biochar composite. Bioresour Technol 281:179–187. https://doi.org/10.1016/j.biortech.2019.02.091

Holkar CR, Jadhav AJ, Pinjari DV, Mahamuni NM, Pandit AB (2016) A critical review on textile wastewater treatments: possible approaches. J Environ Manage 182:351–366. https://doi.org/10.1016/j.jenvman.2016.07.090

Induja M, Sivaprakash KPGP, Karthikeyan S (2019) Facile green synthesis and antimicrobial performance of Cu2O nanospheres decorated g-C3N4 nanocomposite. Mater Res Bull 112:331–335. https://doi.org/10.1016/j.materresbull.2018.12.030

Jadoun S, Arif R, Jangid NK, Meena RK (2021) Green synthesis of nanoparticles using plant extracts: a review. Environ Chem Lett 19(1):355–374. https://doi.org/10.1007/s10311-020-01074-x

Karvandian FM, Shafiei N, Mohandes F, Dolatyar B, Zandi N, Zeynali B, Simchi A (2020) Glucose cross-linked hydrogels conjugate HA nanorods as bone scaffolds: green synthesis, characterization and in vitro studies. Mater Chem Phys 242:122515. https://doi.org/10.1016/j.matchemphys.2019.122515

Kasprzyk-Hordern B, Ziółek M, Nawrocki J (2003) Catalytic ozonation and methods of enhancing molecular ozone reactions in water treatment. Appl Catal B Environ 46(4):639–669. https://doi.org/10.1016/S0926-3373(03)00326-6

Keerthanan S, Jayasinghe C, Biswas JK, Vithanage M (2020) Pharmaceutical and personal care products (PPCPs) in the environment: plant uptake, translocation, bioaccumulation, and human health risks. Crit Rev Environ Sci Technol. https://doi.org/10.1080/10643389.2020.1753634

Lotfy VF, Fathy NA, Basta AH (2018) Novel approach for synthesizing different shapes of carbon nanotubes from rice straw residue. J Environ Chem Eng 6(5):6263–6274. https://doi.org/10.1016/j.jece.2018.09.055

Makgabutlane B, Nthunya LN, Maubane-Nkadimeng MS, Mhlanga SD (2021) Green synthesis of carbon nanotubes to address the water-energy-food nexus: a critical review. J Environ Chem Eng 9(1):104736. https://doi.org/10.1016/j.jece.2020.104736

Mathew AT, Saravanakumar MP (2021) Removal of Bisphenol A and Methylene Blue by α-MnO_2 nanorods: impact of ultrasonication, mechanism, isotherm, and kinetic models. J Hazard Toxic Radioact Waste 25(2):04021005. https://doi.org/10.1061/(ASCE)HZ.2153-5515.0000600

Mathew AT, Saravanakumar MP (2022a) Removal of bisphenol A and methylene blue through persulfate activation by calcinated α-MnO2 nanorods: effect of ultrasonic assistance and toxicity assessment. Environ Sci Pollut Res. https://doi.org/10.1007/s11356-022-23146-x

Mathew AT, Saravanakumar MP (2022b) Removal of micropollutants through bio-based materials as a transition to circular bioeconomy: treatment processes involved, perspectives and bottlenecks. Environ Res 214:114150. https://doi.org/10.1016/j.envres.2022.114150

Mehinto AC, Hill EM, Tyler CR (2010) Uptake and biological effects of environmentally relevant concentrations of the nonsteroidal anti-inflammatory pharmaceutical diclofenac in rainbow trout (*Oncorhynchus mykiss*). Environ Sci Technol 44(6):2176–2182. https://doi.org/10.1021/es903702m

Moraga G, Huysveld S, Mathieux F, Blengini GA, Alaerts L, Van Acker K, de Meester S, Dewulf J (2019) Circular economy indicators: what do they measure? Resour Conserv Recycl 146:452–461. https://doi.org/10.1016/j.resconrec.2019.03.045

Olasupo A, Suah FBM (2021) Recent advances in the removal of pharmaceuticals and endocrine-disrupting compounds in the aquatic system: a case of polymer inclusion membranes. J Hazard Mater 406:124317. https://doi.org/10.1016/j.jhazmat.2020.124317

Pathy A, Krishnamoorthy N, Chang SX, Paramasivan B (2022) Malachite green removal using algal biochar and its composites with kombucha SCOBY: an integrated biosorption and phycoremediation approach. Surf Interfaces 30:101880. https://doi.org/10.1016/j.surfin.2022.101880

Qin P, Chen D, Li M, Li D, Gao Y, Zhu S, Mu M, Lu M (2022) Melamine/MIL-101(Fe)-derived magnetic carbon nanotube-decorated nitrogen-doped carbon materials as sorbent for rapid removal of organic dyes from environmental water sample. J Mol Liq 359:119231. https://doi.org/10.1016/j.molliq.2022.119231

Robinson T, McMullan G, Marchant R, Nigam P (2001) Remediation of dyes in textile effluent: a critical review on current treatment technologies with a proposed alternative. Bioresour Technol 77(3):247–255. https://doi.org/10.1016/S0960-8524(00)00080-8

Rogowska J, Cieszynska-Semenowicz M, Ratajczyk W, Wolska L (2020) Micropollutants in treated wastewater. Ambio 49(2):487–503. https://doi.org/10.1007/s13280-019-01219-5

Sangami S, Manu B (2017) Synthesis of green iron nanoparticles using laterite and their application as a fenton-like catalyst for the degradation of herbicide ametryn in water. Environ Technol Innov 8:150–163. https://doi.org/10.1016/j.eti.2017.06.003

Singh J, Dutta T, Kim K-H, Rawat M, Samddar P, Kumar P (2018) 'Green' synthesis of metals and their oxide nanoparticles: applications for environmental remediation. J Nanobiotechnol 16(1):84. https://doi.org/10.1186/s12951-018-0408-4

Sivasakthi S, Imran H, Karuppasamy G, Sagadevan S, Mohammad F, Dharuman V (2020) Green synthesis of porous carbon nanocubes accumulated microspheres for the simultaneous non-enzymatic sensing of uric acid and dopamine in the presence of ascorbic acid. Synth Met 270:116598. https://doi.org/10.1016/j.synthmet.2020.116598

Soleymani AR, Rafigh SM, Hekmati M (2020) Green synthesis of RGO/Ag: as evidence for the production of uniform mono-dispersed nanospheres using microfluidization. Appl Surf Sci 518:146264. https://doi.org/10.1016/j.apsusc.2020.146264

ul Haq et al., 2020 ul Haq A, Saeed M, Usman M, Naqvi SAR, Bokhari TH, Maqbool T, Ghaus H, Tahir T, Khalid H (2020) Sorption of chlorpyrifos onto zinc oxide nanoparticles impregnated Pea peels (*Pisum sativum* L): equilibrium, kinetic and thermodynamic studies. Environ Technol Innov 17:100516. https://doi.org/10.1016/j.eti.2019.100516

Wanda EMM, Nyoni H, Mamba BB, Msagati TAM (2018) Application of silica and germanium dioxide nanoparticles/polyethersulfone blend membranes for removal of emerging micropollutants from water. Phys Chem Earth Parts a/b/c 108:28–47. https://doi.org/10.1016/j.pce.2018.08.004

Wu Q, Zhang Y, Cui M, Liu H, Liu H, Zheng Z, Zheng W, Zhang C, Wen D (2022) Pyrolyzing pharmaceutical sludge to biochar as an efficient adsorbent for deep removal of fluoroquinolone antibiotics from pharmaceutical wastewater: performance and mechanism. J Hazard Mater 426:127798. https://doi.org/10.1016/j.jhazmat.2021.127798

Xu D, Ma H (2021) Degradation of rhodamine B in water by ultrasound-assisted TiO2 photocatalysis. J Clean Prod 313:127758. https://doi.org/10.1016/j.jclepro.2021.127758

Xue P, Zhao Y, Zhao D, Chi M, Yin Y, Xuan Y, Wang X (2021) Mutagenicity, health risk, and disease burden of exposure to organic micropollutants in water from a drinking water treatment plant in the Yangtze River Delta, China. Ecotoxicol Environ Saf 221:112421. https://doi.org/10.1016/j.ecoenv.2021.112421

Yagub MT, Sen TK, Ang HM (2012) Equilibrium, kinetics, and thermodynamics of methylene blue adsorption by pine tree leaves. Water Air Soil Pollut 223(8):5267–5282. https://doi.org/10.1007/s11270-012-1277-3

Yagub MT, Sen TK, Afroze S, Ang HM (2014) Dye and its removal from aqueous solution by adsorption: a review. Adv Colloid Interface Sci 209:172–184. https://doi.org/10.1016/j.cis.2014.04.002

Yu T, Xue Z, Zhao X, Chen W, Mu T (2018) Green synthesis of porous β-cyclodextrin polymers for rapid and efficient removal of organic pollutants and heavy metal ions from water. New J Chem 42(19):16154–16161. https://doi.org/10.1039/C8NJ03438A

Zhou H, Wu C, Huang X, Gao M, Wen X, Tsuno H, Tanaka H (2010) Occurrence of selected pharmaceuticals and caffeine in sewage treatment plants and receiving rivers in Beijing, China. Water Environ Res 82(11):2239–2248. https://doi.org/10.2175/106143010X12681059116653

Żur J, Piński A, Marchlewicz A, Hupert-Kocurek K, Wojcieszyńska D, Guzik U (2018) Organic micropollutants paracetamol and ibuprofen—toxicity, biodegradation, and genetic background of their utilization by bacteria. Environ Sci Pollut Res 25(22):21498–21524. https://doi.org/10.1007/s11356-018-2517-x

Chapter 15
Role of Non-metallic Fraction Recycled from Waste Printed Circuit Boards for Producing Sustainable Construction Products

Prasanna R. Venkatesan, T. Shanmuga Priya, U. Johnson Alengaram, and Ajayan S. Aswathy

Abstract A large number of waste printed circuit boards are produced annually, which looms large as a serious threat to our environment. When discarded in a landfill, printed circuit boards (PCB) contain poisonous and dangerous metallic and non-metallic fractions (NMF). This study illustrates the viability of using NMF recycled from waste PCB as one of the constituent materials in the manufacture of building goods like fly ash bricks and paver blocks as a possible route to environmentally friendly construction. The raw materials were combined with NMF in a variety of ratios ranging from 5 to 25%. In comparison to conventional products, NMF-incorporated fly ash bricks and paver blocks shown an improvement in compressive strength. Fly ash bricks made with NMF showed no efflorescence. In comparison to conventional products, NMF-incorporated products were observed to absorb less water. When compared to the control specimens, the NMF blended paver block specimens showed a significant resistance to water penetration.

Keywords Printed circuit boards · Non-metallic fractions · Fly ash bricks · Paver blocks

15.1 Introduction

The Global E-Waste Monitor 2020 estimated total e-waste generated globally about 53.6 million tons in 2019. India generated 3.2 million tonnes of e-waste annually in 2019 and is expected to produce 5 million tonnes by 2021 (Cui and Forssberg 2003). The report by Ministry of Environment, Forest and Climate Change (MoEFCC)

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estimates the recovery of metals such as Cu, Ag, Au and Pd is about 30 million tons per annum from waste PCBs. However, recycling PCBs is made difficult by the high non-metal content (up to 70%). Nevertheless, there is evidence about the possibility of using non-metallic fractions for construction applications. The non-metallic powder mainly consists of resins and glass fibres which do not need to be separated and their combined characteristics are very useful for cement composites (Veit et al. 2006). A few studies have been conducted in India related to the use of NMF in composites (Li et al. 2008; Guo et al. 2018). Janardhanan and Ramasamy (2017) used crushed PCB waste in fly ash based geopolymer concrete and suggested that replacing fine sand with 30% of crushed PCBs showed encouraging results. Kunal Kakria et al (2020) studied the addition of NMF by 5, 10, 15, and 20% in geopolymer concrete as an additive material and found that the addition of 15% NMF enhanced the mechanical performance. Using construction fillers, composite panels, bricks, and decorative cement, among other materials, Mou et al. (2007) investigated the effectiveness of incorporating non-metallic materials recovered from waste PCBs in construction. They came to a satisfactory conclusion when compared to conventional materials. Another study conducted in China by Guo et al. (2008) shown that the glass fibres and resin powder found in non-metallic debris from PCBs are also used to improve asphalt through a composition effect. But these are highly uneconomical since there is no commercial value of PCB waste as it is an industrial waste and due to the high costs of recycling.

Generally, PCB is discarded in dump yards and when they are burnt, which emit chemicals that can be harmful to human health. Exposure to the dangerous compounds found in e-waste can cause serious health problems (Qu et al. 2019). Pollutants that enter our environment, such as dioxins and furans from polyvinyl chloride, lead, beryllium, cadmium, mercury, etc., are known to pose a number of health risks, including those related to reproduction, the immune system, the nervous system, kidney damage, lung cancer, chronic beryllium diseases, and others (Guo et al. 2018). Thus, an efficient system to use them for a better purpose will serve the purpose. Senthil Kumar and Baskar (2014) have presented on E- waste as construction material and the results shows that there is decrease in strength for compressive, tensile and flexure but up to certain percentage of replacement it shows a good result and the concrete with partially replaced E-waste is recommended for non-structural elements which lead to reduction of electronic waste (Senthil Kumar and Baskar 2014). Lakshmi and Nagan (2011) have investigated on durability characteristic of E-plastic waste incorporated concrete such as chloride attack test, sulphate attack test, permeability test, Saturated water absorption, Porosity and Sorptivity. For the above test, the concrete with E-plastic waste shows a good result.

Construction materials with minimum damage to the environment is gaining momentum. Urbanization has made cement one of the most consumed materials for large scale production of concrete. Cement demand has topped a whopping 5 billion metric tons worldwide. The inclusion of large amount of raw materials has led to huge amount of CO_2 release in the atmosphere (Adesina 2020). Exhaustive research is being done across globe to find a supplementary cementitious material (SCM) to tackle the damage to environment. Various SCM's such as the Fly Ash

(FA), Ground Granulated Blast Furnace Slag (GGBS), Silica Fume (SF) and Rice Husk Ash (RHA) (Pal et al. 2003; Shanmuga Priya et al. 2021; Corona et al. 2019), etc., which are derived from industrial processes poses a suitable substitute to cement in concrete. On the other hand, with the increasing awareness about the effects of using naturally available resources in the manufacture of construction products, it is important to try and incorporate various other materials into these products to decrease the overall consumption of natural resources (Corona et al. 2019). With the restrictions on the use of natural aggregates like sand, etc. getting tighter day by day, the use of non-metallic fraction from recycled PCB as a replacement to fine aggregates can be thought of as an alternative.

In the present study, the NMF was used to make construction products like fly-ash bricks and paver blocks as additive, and to find the optimum percentage of NMF that can be added to achieve optimum strength. In comparison to conventional materials and fillers, NMF products exhibit improved mechanical properties and durability. Based on the test results it was concluded that optimum replacement of NMF was found to be 30% for fly ash bricks and paver blocks and maximum strength and durability properties were observed. This study suggested that the use of NMF in construction product can be one of the economic ways for their disposal of e-waste in environment friendly manner which contributes to save environment and conserve natural resources.

15.1.1 Objectives

The objective is to study the properties of NMF and its application in construction products. This research also focuses on the impact of NMF on the properties of the products.

- To collect and characterize non-metallic fraction from waste PCB.
- To assess the suitability of recycled non-metallic fraction in construction products.
- To find the mechanical strength of the NMF incorporated mortar samples and pavement blocks.
- To attain the optimum amount of non-metallic fraction to be added to achieve maximum strength output.

15.2 Materials and Methods

OPC of 53 Grade was used in accordance with IS 12269 (2003). The specific gravity of cement was found to be 3.15. The chemical composition of cement are presented in Table 15.1. A low calcium Class F fly ash [confirming with ASTM C311 (2013)] was used and its chemical compositions are given in Table 15.1.

Locally available natural sand conforming to Zone II as per IS 383 (1970), having maximum size of 4.75 mm was used. The specific gravity of fine aggregate was 2.65

Table 15.1 Chemical composition of class f fly-ash

Properties	Cement	Fly ash
SiO_2	15.55	48
Al_2O_3	8.40	24.3
Fe_2O_3	5.87	15.6
SO_3	3.12	0.4
CaO	62.44	3.2
Na_2O	0.16	0.8
K_2O	1.27	–

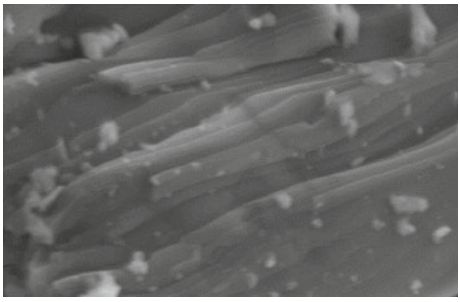


Fig. 15.1 SEM image of NMF

and fineness modulus being 2.36, bulk density was found to be 1675 kg/m^3 with a water absorption of 1.8%. NMF from e-waste was procured from Victory Recycling, Kanchipuram. NMF being both fibrous and particulate in nature had particle size varying from 0.15 to 1.3 microns. NMF is made up of bundles of thermosetting resins and fibre that are loosely intertwined with glass fibres (Fig. 15.1).

Crushed, graded, angular coarse aggregates, of nominal maximum size 12.5 mm with the specific gravity and water absorption being 2.81 and 0.6%, respectively were used. The bulk density of coarse aggregate was 1550 kg/m^3. Fresh potable water, free from organic and acid substances, was used for concrete preparation.

15.2.1 Mixture Proportions for Mortar Mixes

The effects on different percentages of NMF (partial replacement by weight of cement) were evaluated, expecting developments in the strength, durability and microstructural properties. A total of 5 batches of mortar samples were cast. Ordinary portland cement, river sand, NMF and Potable water was used. Mix design was done as per the procedure mentioned in ASTM C311 (2013). The mix proportion in kg/m^3 is presented in Table 15.2.

All the materials were kept at room temperature (27 ± 2 °C) to maintain a uniform mixing temperature. A paddle mortar mixer was used to mix all the materials. The

Table 15.2 Mix proportion

Mix id	Percentage replacement		Quantity of materials (grams)			
	Cement	MBS	Cement	NMF	Sand	Water
CC	100	0	500	0	1375	242
M5	95	5	475	25	1375	242
M10	90	10	450	50	1375	242
M15	85	15	425	75	1375	242
M20	80	20	400	100	1375	242
M25	75	25	375	125	1375	242

mixing of materials was done for around 7 min. After mixing all the materials, the mortar was filled in the mould in 3 layers and compacted using the vibrating table for proper compaction. Tap water was used for curing these specimens for 28 days at room temperature (27 ± 2 °C). The specimens were immersed in water throughout the curing process. Figure 15.3 shows the casted cubes (Fig. 15.2).

(a) Raw material

(b) Mixing process

(c) Bricks after curing

Fig. 15.2 Flyash bricks preparation

Fig. 15.3 Paver blocks preparation

15.2.2 Mixture Proportions for Fly-Ash Bricks

The various raw materials that constitute the fly-ash bricks, along with their proportions used in the industry are: Fly-ash–50%, Lime–15%, Cement–10%, Crusher dust–25% and 150–200 ml of water is used per kilogram of the raw mixture. Fly-ash bricks were cast with varying proportions of NMF replacing fly-ash (0, 5, 10, 15, 20 & 25%). The raw materials were mixed and blended in a miller drum. A fly-ash brick weight about 3.3 kgs. Therefore, a batch of 20 kg of this mixture was taken for 6 bricks (each mix) to get samples with desired proportion of NMF.

15.2.3 Mixture Proportions for Paver Blocks

The various raw materials that constitute the fly-ash bricks, along with the mix proportions used in the industry are:

Cement: Fine aggregates: Coarse aggregates–1: 2.5: 3.25.

Water-cement ratio (w/c)–0.4

Samples of M-35 grade paver blocks were cast with varying proportions of NMF replacing Cement (0, 5, 10, 15, 20 & 25%). The raw materials were mixed and blended in a mixing drum. A paver block weight about 5.4 kgs. Therefore, a batch of 33.75 kg of this mixture was taken for 6 blocks (each mix) to get samples with desired proportion of NMF.

15.2.4 Testing Procedures

Fly ash bricks and paver blocks were used to test the compressive strength as per Codal provisions. For every mix, 6 samples were cast. The strength was determined at

Fig. 15.4 Compression test of paver block

Fig. 15.5 Industrial oven with paver block

the age of 7 and 28 days of curing. The test was conducted using AIMIL compression testing machine with a constant load rate of 2.5 kN on the cubes (Figs. 15.4 and 15.5).

15.2.5 Compressive Strength Testing

Samples of fly ash bricks of size 230 mm X 110 mm X 70 mm were cast. Paver Blocks of size 300 mm X 100 mm X 70 mm were cast. The raw materials with varying proportions of NMF are blended in a blender drum with optimum water content. The mixture is then transferred to hydraulic pressing machine where the blend is fed into the molds and then pressed under pressure. The bricks are then

dried under the sun for 24–48 h. These bricks are then cured by sprinkling water for 21 days for fly ash bricks and paver blocks were cured for 7 and 28 days. At the 7th, 21st and 28th daytests were carried out on the sample utilising a compression testing equipment with a 1200 KN capacity. Three samples per mix were tested and their average was considered in the results. The brick specimens were placed in the compression testing machine on their flat surfaces between plywood sheets. Load was applied axially at a uniform rate of 14 N/mm per minute till failure occurred and the maximum load at failure was noted. The compressive strength was calculated using the equation,

$$\text{Compressive strength}\left(\frac{\text{N}}{\text{mm2}}\right) = \frac{\text{Ultimate load (N)}}{\text{Area of the specimen (mm2)}}$$

15.2.6 Water Absorption Testing

The fly ash bricks and paver blocks were tested in accordance with the procedure laid down in IS3495 (Part 2) for the total water absorption. Specimens were dried in a ventilated oven at a temperature of 105 to 115 °C till it attained substantially constant mass. The specimens were cooled to room temperature and its weight (M_1) was obtained. The completely dry specimens were immersed in clean water at a temperature of 27 °C for 24 h. The specimens were removed and any traces of water were wiped out with a damp cloth. The specimens were weighed 3 min after removing from water, and its weight (M_2) was obtained. Water absorption, percent by mass, after 24-h immersion in cold water is given by the following formula:

$$\text{Water absorption (\%)} = \frac{(\text{M2} - \text{M1})}{\text{M1}} X 100$$

15.2.7 Microstructural Analysis

SEM the raster scan image which is generated can target a resolution higher than 1 nm. Before being considered for SEM analysis, the sample was broken down into smaller sections by grinding, after undergoing the compressive strength test. After which, the samples were cleaned and oven-dried at a temperature of about 96 °C. The samples were prepared in a powdered form, which passed through a 75-micron sieve. Furthermore, the uncoated sieved samples were then kept securely over a Canada Balsam coated glass plate. The samples are then analysed through LEICA microscope and visualized with the aid of QWIN software.

15.3 Result and Discussion

15.3.1 Compressive Strength Results—Mortar Samples

Compressive strength values of NMF incorporated mortar samples shown in Table 15.3. Compressive strength showed an increasing trend till 20% replacement. A compressive strength of 24.02 N/mm^2 was observed in the 28th day mortar sample. On addition of 10% a percentage increase of 1.87 was observed. On 15% replacement a percentage increase of 0.82% was observed and on 20% replacement an increase in strength of 1.45 was observed. Up to 20% of replacement, the compressive strength exhibited an increasing trend. Compressive strength of 25.04 N/mm^2 was found to be the maximum with 20% replacement. The generation of CSH gel is what causes the rise in compressive strength, and the addition of NMF has improved gel formation and increased strength even more. Table 15.3 provides the compressive strength results (Fig. 15.6).

Table 15.3 Compressive strength results

Mix ID	Sectional area	Compressive strength in N/mm^2	
		7 days	28 days
MCC	2500	15.78	24.36
M5	2500	15.34	24.02
M10	2500	15.47	24.47
M15	2500	15.53	24.67
M20	2500	15.87	25.04
M25	2500	15.27	24.01

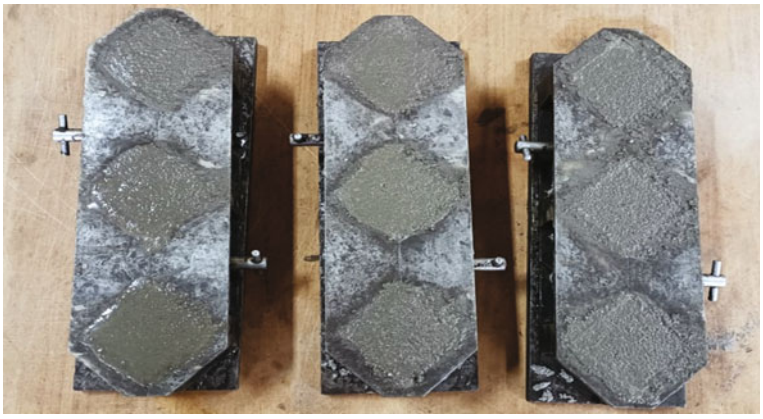


Fig. 15.6 Cubes for compressive strength testing

Table 15.4 Compressive strength for fly ash brick at 21 days

Mix ID	Sectional area (mm^2)	Average load at Failure (kN)	Average strength (N/mm^2)
FAB-CC	25,300	343.34	13.57
FAB-5	25,300	351.67	13.89
FAB-10	25,300	366.67	14.49
FAB-15	25,300	370	15.04
FAB-20	25,300	373.34	16.98
FAB-25	25,300	363.34	16.40

15.3.2 Compressive Strength—Fly Ash Bricks

Average strength values of NMF incorporated fly ash bricks at 21 days. It is observed that on 5% replacement of NMF the strength was increased to 13.89 N/mm^2, as the percentage replacement increased the strength also increased. On 10% replacement there was a percentage increase of 4.32 of compressive strength. The percentage increase on 15% replacement was 3.79 when compared to 10% replacement. Up to 20% replacement, the compressive strength exhibited an increasing trend. The compressive strength was seen to improve to FAB-20. FAB-20, which replaced 20% of the crusher dust with NMF and had a maximum compressive strength of 16.98 N/mm^2, was found to have the highest compressive strength. The formation of cementitious gel around them has imparted strength to the material. Due to addition of NMF, the formation of this gel is further enhanced due to the increased number of particles. The fibrous glass particles present in it contributes to the increase in strength. There was a slight decrease in strength on the addition of 25% NMF. The increase in strength is due to the formation of cementitious gel around them. Due to the addition of NMF, the formation of gel is further enhanced due to the increased number of particles. Also the fibrous glass particles present in it contributes to the increase in compressive strength. The results are given in Table 15.4.

15.3.3 Compressive Strength Results—Paver Blocks

Paver block samples were tested at 7 day and 28 day for compressive strength. For each mix three samples were casted and tested on 7 day and 28 days and the average of the three readings were taken and recorded in the Tables 15.5 and 15.6.

Average strength values of NMF incorporated paver brick at 7 and 28 days is shown in Table 15.3 and 15.4. For both curing age the compressive strength values showed an increase with 5% (PAB-CC) to 0% (PAB-25) replacement of NMF. The percentage increase on 5% replacement was 7.42 compared to conventional mix. On 10% replacement it was 4.73 when compared to 5% replacement. A percentage increase of 2.77 was found in 15% replaced mortar sample. 20% replacement has

Table 15.5 Compressive strength for paver blocks at 7 days

Mix ID	Sectional area (mm^2)	Average load at failure (kN)	Average strength (N/mm^2)
PAB-CC	30,000	643.2	14.4
PAB-5	30,000	673.5	15.6
PAB-10	30,000	675	17.2
PAB-15	30,000	678	18.5
PAB-20	30,000	696	19.4
PAB-25	30,000	538.89	17.6

Table 15.6 Compressive strength for paver blocks at 28 days

Mix ID	Sectional area (mm^2)	Average load at Failure (kN)	Average strength (N/mm^2)
PAB-CC	30,000	1051.5	25.6
PAB-5	30,000	1125	27.5
PAB-10	30,000	1128.3	28.8
PAB-15	30,000	1129.5	29.6
PAB-20	30,000	1148.1	30.2
PAB-25	30,000	1050	29.1

shown a percentage increase of 2.03 when comapred to 15% replaced sample. The paver blocks has shown an increase in strength till 20% addition of NMF. The increase in strength is due to more finer particles than sand in NMF. Due to the addition of NMF the finer particles fills the voids when vibrated in vibrating table. The fibre like glass particles has also contribute to the increase in strength. Similarly a dip in strength was also observed with 25% replacement incorporation of NMF for both curing age. Optimum strength value was observed at 20% replacement of M sand with NMF with maximum compressive strength.

15.3.4 Water Absorption Results—Fly Ash Bricks

The results of all the mixtures water absorption tests were displayed in Table 15.7. It was shown that when NMF was substituted for fly ash brick at various percentages, as percentage replacement increased water absorption decreased. On percentage increase in replacement of NMF the water absorption was decreasing, on 5% replacement the percentage of water absorbed was found to be 21.6% on 10% replacement it was found to be 18.3%. Water absorption was 16.2% and 15.8% on 15 and 20% replacement of NMF respectively. Lowest water absorption percentage was observed in 20% NMF in fly ash brick. The optimum water absorption percentage was 20%.

Table 15.7 Water absorption results–fly ash bricks

Mix ID	Dry weight (Kg)	Wet weight (Kg)	% Water absorbed
FAB-CC	3.303	4.035	22.2
FAB-5	3.213	3.892	21.1
FAB-10	2.970	3.521	18.5
FAB-15	2.789	3.259	16.8
FAB-20	2.817	3.246	15.2
FAB-25	2.532	2.898	14.5

The existence of finer NMF particles and its pozzolanic feature, which led to a discontinuous pore structure, can be a cause of this positive result.

15.3.5 Water Absorption—Paver Block

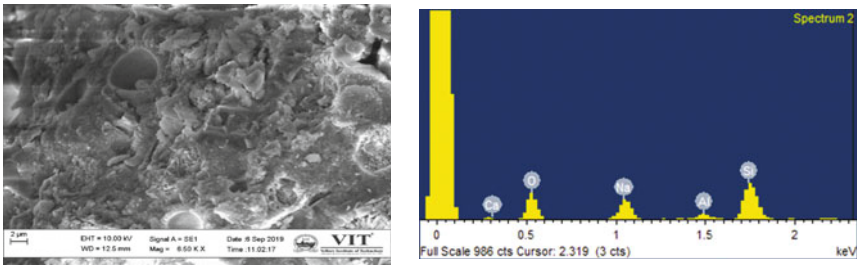
The water absorption performance of the mixes is presented in Table 15.8. Water absorption in paver blocks was found to be decreasing on increasing percentage replacement. 5% replacement has a shown a water absorption of 9.33%. Compare to convention mix the water absorption decreased to 7.5%. On addition of 10% NMF the water absorption decreased to 8.27% and it was observed that on 15% and 20% replacement it was 8.04 and 7.50 respectively. Decrease in the water absorption is observed on the addition of 25% NMF with lowest water absorption. Lowest water absorption percentage was observed in 25% NMF in paver blocks. The optimum replacement percentage was 25%.

15.3.6 Microstructural Analysis—SEM

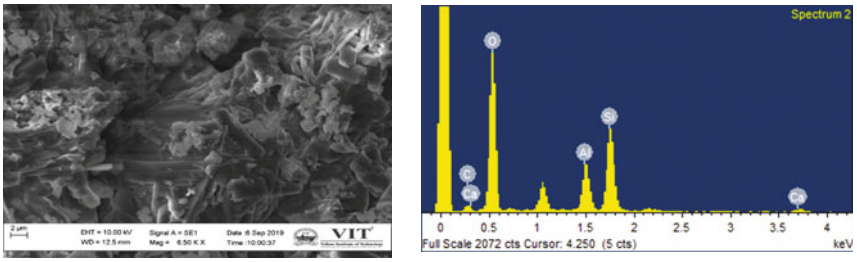
The conventional and 15% replacement of cement by NMF replacement mix were studied through micro-structural analysis and compared with CC to evaluate the

Table 15.8 Water absorption results—paver blocks

Mix ID	Dry weight (kg)	Wet weight (g)	% Water absorbed
PAB-CC	5.327	5.892	10.60
PAB-5	5.035	5.506	9.35
PAB-10	4.962	5.375	8.32
PAB-15	4.627	5.001	8.08
PAB-20	4.280	4.598	7.43
PAB-25	4.105	4.413	7.50



(a) SEM image of Conventional Cement Mortar (CC)



(b) SEM image of NMF

Fig. 15.7 SEM images of CC and NMF

texture and effect of NMF in cement mortar. Figure 15.7a shows the microstructural arrangement of CC, being a uniformly distributed arrangement of materials in absence of any major cracks visually observable. Unreacted particles are visible on the surface of the surface which shows incomplete pozzolanic reaction. Moreover the presence of small amount of Si is reflected in the EDS analysis. Figure 15.7b shows SEM image of NMF and NMF fibers can be seen embedded in the micro-structure of the mix M20. The coexists of calcite and CSH was identified in EDX and white layer of calcite on the top of fibres ie CSH matrix was seen in SEM images. Interstitial voids can be inferred which is found to decrease in the porosity of the materials as reported in waerabsorption test results. An enhanced bonding between the sand and binder is evident as compared to the controlled mix.

15.4 Conclusions

This study shows NMF can be reused in a very efficient manner by using it as a filler material for fly ash bricks and paver blocks. It has been identified that the properties NMF could increase the strength of fly ash bricks and paver blocks. From the various mixes with different proportion of nonmetallic fraction and from various

tests taken to check the strength and durability of the products that have been casted the following inferences were drawn.

- The PCB nonmetallic fraction can be used in construction products.
- The waste PCB nonmetallic fraction improves the compressive strength of fly ash bricks and strength of paver blocks. This increase in strength can be attributed to the glass particles and fibrous materials present in NMF.
- Mortar cubes with 20% replaced NMF showed the maximum compressive strength.
- It is observed that the addition of NMF in fly-ash bricks and paver blocks improved compressive strength up to 20% replacement with cement.
- The optimum replacement of NMF was found to be 25% for fly ash bricks and paver blocks.
- Micro-structural analysis of fly-ash bricks showed the effect of fibrous nature of NMF in increasing the overall performance.
- The NMF addition products can be used for buildings as a sustainable and economical way for disposal of e-waste.

From above experimental results and discussions, it is proved that the replacements are the good alternative materials for reducing crusher dust & fine aggregate. Use of NMF in construction product can be one of the economic ways for their disposal in environment friendly manner which contributes to save environment and conserve natural resources.

References

Adesina A (2020) Recent advances in the concrete industry to reduce its carbon dioxide emissions. Environ Challenges 1:100004

ASTM C 311 (2013) Standard test methods for sampling and testing fly ash or natural pozzolans for use. ASTM International

Corona B, Shen L, Reike D, Carreón JR, Worrell E (2019) Towards sustainable development through the circular economy—a review and critical assessment on current circularity metrics. Resour Conserv Recycl 151:104498

Cui J, Forssberg E (2003) Mechanical recycling of waste electric and electronic equipment: a review. J Hazard Mater 99(3):243–263

Guo J, Rao Q, Xu Z (2008) Phenolic molding compound filled with nonmetals of waste PCBs. Environ Sci Technol 42(2):624–628

Guo Q, Wang E, Nie Y, Shen J (2018) Profit or environment? A system dynamic model analysis of waste electrical and electronic equipment management system in China. J Clean Prod 194:34–42

IS 383 (1970) Specification for coarse and fine aggregates from natural sources for concrete.Bureau of Indian Standards, New Delhi

IS12269 (2003) Specification for 53 grade ordinary Portland cement. Bureau of Indian Standards, New Delhi

Janardhanan T, Ramasamy V (2017). Improvements in the microstructural and mechanical properties of geopolymer concrete containing NMF's of E-wastes as partial replacement of aggregates. Eur J Environ Civ 1–12

Kakria K, Thirumalini S, Secco M, Priya TS (2020) A novel approach for the development of sustainable hybridized geopolymer mortar from waste printed circuit boards. Resour Conserv Recycl 163:105066

Lakshmi R, Nagan S (2011) Utilization of waste E plastic particles in cementitious mixtures. J Struct Eng 38(1):26–35

Li J, Lu H, Liu S, Xu Z (2008) Optimizing the operating parameters of corona electrostatic separation for recycling waste scraped printed circuit boards by computer simulation of electric field. J Hazard Mater 153(1–2):269–275

Mou P, Xiang D, Duan G (2007) Products made from nonmetallic materials reclaimed from waste printed circuit boards. Tsinghua Sci Technol 12(3):276–283

Pal SC, Mukherjee A, Pathak SR (2003) Investigation of hydraulic activity of ground granulated blast furnace slag in concrete. Cem Concr Res 33(9):1481–1486

Qu Y, Wang W, Liu Y, Zhu Q (2019) Understanding residents' preferences for e-waste collection in China-A case study of waste mobile phones. J Clean Prod 228:52–62

Senthil Kumar K, Baskar K (2014) Response surfaces for fresh and hardened properties of concrete with e-waste (HIPS). J Waste Manag 2014

Shanmuga Priya T, Mehra A, Jain S, Kakria K (2021) Effect of graphene oxide on high-strength concrete induced with rice husk ash: mechanical and durability performance. Innov Infrastruct Solut 6(1):1–16

Veit HM, Bernardes AM, Ferreira JZ, Tenório JAS, de Fraga MC (2006) Recovery of copper from printed circuit boards scraps by mechanical processing and electrometallurgy. J Hazard Mater 137(3):1704–1709

Chapter 16
Bioconversion of Organic Waste for Solid Waste Management and Sustainable Agriculture—Emphasized Impact of Bioelectromagnetic Energy

Parul Vats, R. Y. Hiranmai, and Ajay Neeraj

Abstract Municipal solid waste (MSW) management is a pressing issue in developing nations across the globe. Organic fraction makes up a major fraction of the waste composition. Owing to its high organic matter, high abundance and low concentrations of pollutants, MSW is suitable for bioconversion. Old age-established concepts like composting and vermicomposting offer rearrangement and recycling of the organic components of waste through the cyclic pathways in the natural environment lowering the quantity of waste and upcycling it to value-added organic manure. Food production owing to the green revolution has proved to be unsustainable in the longer run. Sustainable agriculture embraces judicious ecosystem management and discourages the use of synthetic inputs. Fortified quality organic manures can be directed for sustainable agricultural practices achieving natural circularity, safe quality food production and upliftment of the physicochemical and biological quality of soil and crops. Engaging the other hidden half of nature- the human world of intents and thoughts, positive-divine thought-based bioelectromagnetic energy generated by meditation during the farming process- Sustainable Yogic Agriculture (SYA) has experimentally shown enhanced levels of agroecosystem health, microbial activity and crop production. The present work reviews the bioconversion of locally available organic waste to manures for nutrient management of agricultural soil and crop production and their quality enhancement by incorporating a transdisciplinary SYA approach. Nature has multidisciplinary intricate linkages and is highly receptive to human emotions. Transdisciplinary practical solutions can become potential solutions to realize the vision of a sustainable nation.

Keywords Agriculture · Bioconversion · Organic waste · Soil fertility · Sustainability · Yogic farming

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16.1 Waste Generation Scenario

The world population has been on the rise and so have developmental activities which is the source of the enormous amount of waste. Approximately 10,000 tons of solid waste are disposed of in Lagos each day, and a large amount of electronic waste is disposed of from the 500 container ships that discharge waste there each month. In the new atlas of the world's biggest dumps, Mbeubeuss is one of 50 giant sites. It is a contender for the title of the largest dump in the world. As a comparison, there is the massive Agbogbloshie dump in Ghana, which is one of the largest in the world and takes 192,000 tonnes of electronic waste every year. Also, the Awotan dump in Ibadan, Nigeria, which has become a breeding ground for diseases. There is a massive tip in Bekasi, Indonesia, called Bantar Gebang, which takes 230,000 tonnes of industrial, medical, and farm waste a year at Dandora outside Nairobi.

About two billion tonnes of municipal solid trash are produced yearly throughout the world, with daily garbage production averaging 0.74 kilos and ranging from 0.11−4.54 kg. Under a "business as usual" scenario, this quantity is projected to increase to 3.40 billion tonnes by 2050. The three high-income nations of Bermuda, Canada, and the United States currently produce the most garbage per person on average, 2.21 kg per day, whereas low-income nations produce 0.74 kg per day (Kaza et al. 2018). Many cities in the world and major cities in India have been struggling due to mountains of waste. Bantar Gebang landfill in Jakarta (Timmerman 2021); Chile's Atacama Desert (Agrawal 2021); Ghazipur, Okhla and Bhalswa in Delhi (Times of India 2022); Bandhwari in Gurugram (Harigovind 2022); Deonar in Mumbai (Hindustan Times 2022); Mittaganahalli landfill in Bangalore (VL 2021) are some examples highlighted in recent years. About 33 percent of the waste worldwide is openly dumped and not disposed of in an environmentally sound manner (Kaza et al. 2018). The basic amenity of sound solid waste management is not yet properly organized in many parts of the world and exhausts quality of life and natural resources.

MSW generation rates are influenced by economic development, industrialization, public habits, and local climate. Income level and urbanization promote increased consumption of goods and services and hence increase the amount of waste generated (Hoornweg et al. 2012; OECD 2021). High-income countries (16% of the world's population) generate about 34 percent of the world's waste. The total quantity of waste generated in South Asia is expected to increase by two times by 2050 (Kaza et al. 2018).

Planning Commission stated that urban residents produce double the waste as rural residents and India is moving into urban areas. 377 million i.e. about 30% of the total population live in towns, it will be more than 50% by 2050. 81% of the total MSW is generated in urban cities. Waste collection efficiency is between 70 and 90% in major Metro cities but the treatment of waste is very improper. Many cities other than composting and refuse-derived fuel (RDF), which is done in limited quantities (Planning Commission 2014). Urban waste management needs urgent attention and priority across the globe and in India.

16.2 Factors Influencing Municipal Solid Waste Generation

MSW generation has been constantly escalating. Several factors influence the generation of waste. Hoornweg et al. (2012) reported that a population of about 3 billion residents generates 1.2 kg per person per day (1.3 billion tonnes per year). The world population is on rise and currently stands at about 7.9 billion (United Nations n.d.). With the population surge, the world generates 2.01 billion tonnes of municipal solid waste annually, per person per day averages 0.74 kg in the range of 0.11 to 4.54 kg and by 2030, waste generation worldwide is expected to reach 2.59 billion tonnes per year (Kaza et al. 2018). MSW generation is affected by economic conditions, industrialization, urbanization, consumer habits, and local climate. Income level, population and urbanization affect waste generation highly by increased consumption of goods, services and materials consumption, and consequently the amount of waste generation (Kawai and Tasaki 2016; Hoornweg et al. 2012).

As per an assessment report by OECD (2021) using United Nations population trends and World Bank development indicators, in a span of 27 years from1990 to 2017, 2.5 billion people are added to the world population to 7.5 billion people at present, this creates an impact on consumption and production rates. The gross domestic product across the globe per capita has increased by fifty percent. As a consequence, the annual material consumption has almost doubled (from 37 billion tonnes to 88 billion tonnes) in 2017 from 1992. The average daily materials used per capita increased from 22 kg in 1990 to 33 kg in 2017. Economic conditions of countries have a distinct impact on waste contribution. High-income countries, although only account for 16% of the world's population, contribute to more than 30 percent, of the world's waste. Other than increased consumption, global trade and exchange come along. As pointed out earlier that presently, Bermuda, Canada, and the United States are the biggest generators of waste per capita with 2.21 kg per day, but in the coming future low-income countries are expected to see waste generation increment by more than three times and South Asia will also witness a doubling of waste generation. In these regions, 33% of the waste is not handled in an environment safe manner and is openly dumped (Kaza et al. 2018), thus requiring urgent action in terms of waste handling, management and disposal. Trade influence is high in this aspect. According to OECD (2021) projections, a relative decoupling between economic growth and materials use will take place in the coming decades with the annual economy growth rate of 2.8%, global materials use is set to increase on average by 1.5% every year. This relative decoupling will happen owing to technical advances and structural changes in the economy. Increasingly, countries are expected to shift toward a service-based economy at the global level, which will reduce the material intensity and global material use and improved technological advancements will help save more than 60 billion tons of materials. However, additional policy measures are required to support the benefit obtained from structural and technological changes.

World bank reports that waste composition differs across income levels, reflecting varied patterns of consumption. High-income countries generate lesser organic, biodegradable waste (32% of the total) and more dry recyclable waste like plastic,

paper, cardboard, metal glass etc. (about 50% of the total waste). Middle- and low-income countries have bigger fraction of organic waste (53% and 56% respectively) (Kaza et al. 2018). Across the globe as well, by 2050, 55% population is likely to move into urban regions automatically promoting higher levels of material use in cities (OECD/European Commission 2020). Globally cities produce half of the solid waste and 70% of greenhouse gas emissions (IEA 2016) but cities hold important facilities for resource efficiency and if proper coordination between authorized bodies is executed, cities can progressively move towards circularity of solid waste management (OECD 2021). Low-income countries are still figuring out proper waste disposal mechanisms and global trade of waste has promoted economically efficient recycling, but also increased waste burden in the recipient countries and pose environmental risks if not handled carefully. Trade influences waste management and treatment. Recycling sector is highly influenced by trade. Trade of waste between nations has promoted economically efficient recycling, with that comes the risks of environmental pollution if the management of waste is not under checks and regulations. The trade has increased by around 30% (by weight) and the main categories are papers and metals (Yamaguchi 2021; Garsous 2019). The largest exporters of waste and scrap in 2018 were the United States, Germany, Japan and France. Import of waste was highest in China along with India and Turkey. Regulatory policies and regulatory instruments, recycling targets, product standards, recycled content requirements, lifetime warranties, bans and restrictions and deposit-refund systems are useful policies to increase resource efficiency and economically efficient and environmentally sound waste processing and disposal (OECD 2021).

16.3 Present Status of Waste Generation in India

Urbanization and sloppy waste management directly contribute to garbage production and pollution. An expanding economy produces more solid trash, such as household, commercial, institutional, and industrial garbage, which substantially raises the volume of waste produced from diverse sources. Garbage, junk (package materials), waste from building and demolition, leaf litter, hazardous wastes, etc. are only a few of the wastes produced in a normal metropolitan civilization (Rajput et al. 2009). In August 2009, FICCI carried out a study on solid waste management in Indian cities. Cities that produce more than 1000 tonnes of solid trash per day, such as Ahmedabad, Kanpur, and Pune, each have just one dumpsite, but Faridabad and Jamshedpur, which produce less than 450 tonnes of solid garbage per day, each have two pits.

16.3.1 Waste Generation and Disposal Status of Indian Cities

There are eight cities where 36% (8 out of 22) generated more than 1000 tons of waste per day (Ahmedabad, Delhi, Greater Mumbai, Jaipur, Kanpur, Lucknow, Pune,

Surat). Indore, Ludhiana and Vadodara generated 13.6% of the waste generated in 22 cities (500–1000 TPD). 11 cities (Agartala, Asansol, Chandigarh, Faridabad, Guwahati, Jamshedpur, Kochi, Kozhikode, Mangalore, Mysore and Shimla) generated less than 500 TPD of waste of 22 cities surveyed, 63.6% (14 out of 22) dispose of more than 75% of their rubbish in dump yards (Ahmedabad, Asansol, Chandigarh, Delhi, Faridabad, Greater Mumbai, Jaipur, Jamshedpur, Kanpur, Lucknow, Ludhiana, Mangalore, Pune, and Vadodara). On the whole, 47.05% (8) of the 17 class I cities had a single dump site, 29.4% (5) had two dump sites, 5.88% (1) had three dump sites, and 11.76% (2) have 4 dumpsites. A total of 100% of the waste collected in Greater Mumbai and Ludhiana is dumped at the dumpsite.

16.4 Problems Caused Due to MSW Mismanagement: Waste Decomposition in Open Dumpsites and Their Impacts

Waste production across the globe and India is ever-increasing and it is impossible to control and manage liquid and gaseous by-products of waste decomposition at open dumpsites without the necessary facilities and measures.

By burning plastics and other materials openly (usually to reduce volume), toxic substances are released into the atmosphere. As a result of these toxic fumes, the chronic respiratory disease can be caused and air pollutant levels are increased, including nitrogen oxides (NOx), sulfur oxides (SOx), heavy metals (mercury, lead, chromium, cadmium, etc.), dioxins and furans, and particulate matter. Open dumpsites have a negative impact on health and the environment because of:

1. Unplanned sitting.
2. Operations are chaotic since there are no overarching standards defining how the facility should be run and many owners and operators of these dumpsites lack the essential tools and knowledge.
3. Ineffective waste input control, either in terms of amount or composition (or both).
4. Random garbage dumping at the dump site.
5. Lack of regulation of the pollutants emitted during waste decomposition and waste burning.

16.4.1 Effect on Health

Dumping of solid waste is hazardous to human health and causes various diseases, waterborne as well as vector borne diseases. It is due to the standing of water in an area and the flow of water is stopped due to dumping near the area and can also result in flooding by obstructing storm water. This waste is dumped because

people frequently leave their trash on the roadways, where it is then dispersed by rag pickers who are looking for food. There have been reports of a greater incidence of congenital birth defects, malignancies, and respiratory illnesses in people who live or work close to landfill dumps. World Health Organization (WHO 2000, 2007) says there is insufficient data on the health effects of landfills and incinerators, including cancer, reproductive issues, and mortality. In contrast, studies from Ray et al. (2005, 2009) have confirmed this at the Okhla dump site in Delhi. Comparing workers of the same age, gender, and socioeconomic status to controls of similar age, gender, and socioeconomic status, 62% had lung infections. Workers in landfills are more likely to suffer cardiovascular disease and tissue damage due to leukocytes, platelets, and inflammation in the air (Ray et. al. 2009). These and other types of issues are maintained by a vicious cycle that includes municipal solid wastes and the pollutants emitted as a result:

(i) Chemical poisoning through chemical inhalation
(ii) Low birth weight
(iii) Cancer
(iv) Congenital malformations
(v) Neurological disease
(vi) Nausea and vomiting
(vii) Mercury toxicity from eating fish with high levels of mercury.

Non-Hodgkin lymphoma and soft tissue sarcomas are the illnesses that are frequently examined in epidemiological studies near MSW incinerators. This is the case because abandoned materials, like old tyres, often contain standing water that serves as a breeding ground for fleas and mosquitoes that can spread illnesses like malaria.

16.4.2 Effect on the Environment

Open dumping is a persistent issue in our nation and around the world, as particular areas turn into common places and dump piles draw new dumping. Disposing of garbage produced by communities and industry on land, in the sea, or in low-lying areas is a highly widespread activity. Sites may be entirely or primarily made up of home or industrial garbage.

Biodegradable waste components (food and yard waste) generally degrade anaerobically at landfills and dumpsites. Additionally, methane (about 50–65%) and carbon dioxide (about 35–45%) are generated as a result of the decomposition of the waste. Several researchers have measured the rates of atmospheric gas release via a landfill cover. Methane and carbon dioxide emissions from landfill surfaces have a big impact on the greenhouse effect and global warming. Methane released into the atmosphere significantly contributes to global warming since its global warming potential is about 21 times that of carbon dioxide (Esakku et al. 2005). Extensive characterization studies of the world's methane sources and sinks have been prompted by recent

increases in atmospheric methane concentrations. This study found that atmospheric methane concentrations were rising on average between 1 and 2% per year. Methane is estimated to be responsible for around 18% of all global warming (Church and Shepherd 1989). Methane is flammable since it is lighter than air. Sparks or flames are likely to cause serious explosions in closed buildings if its concentration reaches as high as about 5–15 percent in the air (Esakku et al. 2005). The dumping and landfilling of waste are estimated to contribute between 5–15% to methane emissions into the atmosphere. Multiple environmental and health risks are produced by the open disposal of solid trash.

The management of all produced garbage must be done appropriately. In India, the location for waste disposal is chosen based on convenience rather than taking the environment into account first. Communities near upstream plants and those around the waste dumpsite may be impacted in different ways. Diversity is referred to by scientists as a technique to determine whether a location has been disturbed. The ecology expert examined the region around the dumps and a "reference site" that was distant from the dumps but had similar topography and water flow patterns. The reference location and the dump region should have had a lot of the same flora if the dump wasn't having any effects on the soil. The local plant population did not significantly differ from that of the reference region without a dump. However, the waste areas have less plant diversity than they ought to have due to worse management and design. The plant diversity at the YK Delta landfill, which was also poorly maintained, was higher than at the reference site but was partially brought on by weedy plants like pet sites, fireweed, grass, and sourdock. Additionally, there were few of each kind of plant. As a result, just a few numbers of plants would predominate in poorly maintained wastes, and it would be more difficult to find other kinds of plants.

The impact of improper solid waste management is finding its way into water bodies as well and impacting marine and terrestrial life. About 80 percent of ocean plastic comes from land and of that 75 percent comes from poorly operating MSWM systems (Kaza et al. 2018).

16.4.3 Effect on Soil

In order for plants to develop, the soil must be "a dynamic natural body on the surface of the earth, made of mineral and organic components and living forms". It also serves as a layer of protection and filtration that is placed over groundwater to lessen the effects of many dangerous pollutants (Venkatesan and Swaminathan 2009). The weight of MSW on land has increased due to urbanization and industrialization, which is negatively affecting the soil's yield and biotic and abiotic qualities. In comparison to control sites, the soils at the disposal sites had higher pH, TDS, and electrical conductivity regimes. Lead (Pb), Copper (Cu), Nickel (Ni), Chromium (Cr), and Zinc (Zn) were among the heavy metal concentrations that were found to be greater at the waste dumping sites, except for Cadmium (Cd), which had a higher

value at the control site. Salinity is another major danger to soil; it results from incorrect human land use, including deforestation, construction work, and industrialization. Salinity has an impact on the physical and chemical makeup of soil, leads to soil degradation, and raises the salinity of subsurface waters (Iwai et al. 2013). Additionally, (Biro et al. 2013) shown in their research that a decrease in soil productivity may be caused by a change in soil characteristics, which may have been 25 related to a change in land use and cover. In natural ecosystems, the restoration of degraded soil is a challenging and protracted process that involves the development of a vegetative community and the resumption of microbial activity (De Souza et al. 2013). Total nitrogen, pH, and the degree of erosion are critical variables that affect soil growth. Soil richness reduced as these factors rose (Pallavicini et al. 2015). Quantifying the ecotoxicological risk of composted residuals is a topic of growing attention. The review by Kapanen and Itavaara (2001) discusses the wide range of ecotoxicity test techniques for compost that use microbes, enzymes, soil fauna, and plants. An early overview of the impacts of MSW-compost incorporation on soil biological characteristics is provided by Gallardo-Lara and Nogales (1987). These typically included rises in the populations of bacteria and fungi in modified soil.

16.5 Municipal Solid Waste Management: Treatment and Practices for Municipal Solid Waste Management

The proper management of solid waste promotes economic growth and a higher standard of living while minimising or eliminating negative effects on the environment and human health. For a municipality, managing garbage successfully involves a variety of procedures. These comprise supervision, gathering, transportation, processing, recycling, and disposal (Fig. 16.1).

16.5.1 Waste Reduction, Reuse and Recycling

Waste reduction and product reuse are examples of trash avoidance strategies. Waste avoidance is given top priority in integrated waste management. Backyard composting can significantly reduce waste volume.

Removing items from the waste stream so that they may be utilized as raw materials to make new goods is the process of recycling (USEPA 2022). Accordingly, recycling happens in three stages: first, waste is separated, recyclables are collected, and then the recyclables are converted into raw materials. These fundamental resources are then used to create new items. It is theoretically feasible to recycle a wide range of materials, including plastics, wood, metals, glass, textiles, paper, cardboard, rubber, ceramics, and leather. Increased diversity of recyclable raw materials is often needed in regions with a broad industrial base and economy. The economy, the environment,

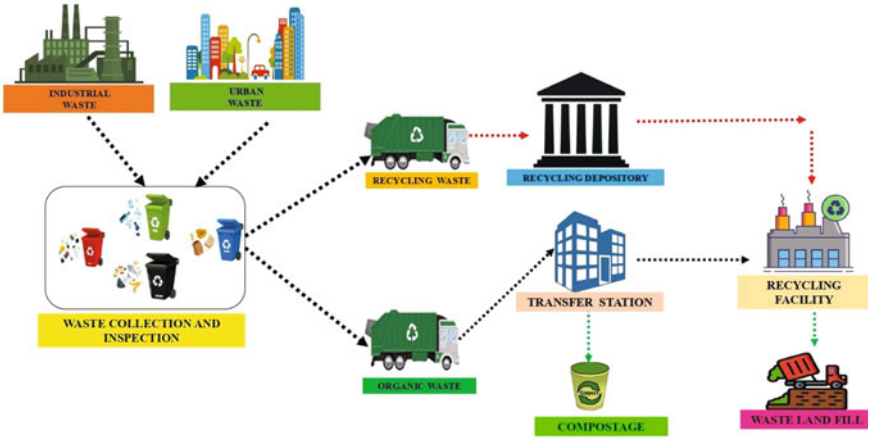

Fig. 16.1 Recycle/composting process of waste in urban area

and society are all benefited by recycling. Because recycling consumes less water, energy, and pollution than obtaining new raw materials, it lowers operating costs (Sandin and Peters 2018).

16.5.2 Treatment and Disposal

With the use of waste treatment procedures, trash may be made simpler to dispose of by being changed into a more manageable form, having its volume reduced, or having its toxicity decreased. In order to select the best treatment option, we need to know the size, shape, and composition of the waste material. Today's waste treatment techniques include using biological processes, exposing the garbage to extremely high temperatures, disposing of it on land or in landfills, and dumping it. Biological degradation can be achieved by aerobic degradation or composting.

16.5.2.1 Anaerobic Digestion

Composting and anaerobic digestion both employ biological processes to break down organic waste. Methane and carbon dioxide are produced as a result of anaerobic respiration. Anaerobic digestion is used to produce biogas, which may be used to produce power, in addition to the humus that is produced and utilised to improve soil. Nutrients like nitrogen, phosphorus, and potassium are needed for the process to run well. The pH must also be kept around 7, the alkalinity must be suitable to buffer pH variations, and temperature must be managed (Christy et al. 2014).

16.5.2.2 Composting and Its Potential in Solid Waste Management

Composting is the controlled aerobic breakdown of organic material by tiny invertebrates and microbes. Aerobic degradation generates better quality by products as compared to anaerobic composting. There are several composting methods in use right now. These include windrow composting, static pile composting, in-vessel composting, and vermicomposting. Municipal solid waste composting is influenced by a number of parameters, including waste source and type, composting design, maturation period, and composting process (Hargreaves et al. 2008). Composition and components of the materials, namely their Carbon/Nitrogen (C/N) ratio, temperature, moisture content, and air volume all affect how quickly compost forms. For the procedure to be effective, the C/N ratio is crucial. Carbon serves as the microbes' energy source, while nitrogen is necessary for the creation of certain proteins. The use of dehydrated mud is useful when the C/N ratio is too high, and the use of cellulose can be used when the ratio is too low. While the moisture content of the compost is significantly affecting the composting process, it is not the only factor.

The wetness is necessary for the microorganisms to carry out their metabolic processes. Composting is not favoured if the trash is too dry. For the purpose of getting rid of harmful germs, a high temperature is preferred. The organisms required to finish the composting process are nevertheless wiped off at temperatures over 75 °C. The procedure performs best at temperatures between 50 and 60 °C, with 60 °C being optimum. It is crucial to ensure that compost is properly aerated and that the amount of air is controlled. When oxygen levels drop, aerobes start to perish and are replaced by anaerobes. A large number of anaerobes will slow down the process, cause smells, and release methane, which is one of the most combustible substances in nature. The first large-scale composting facility, operated by Excel Industries Ltd. in Mumbai, processes 500 tonnes of MSW every day. In Vijaywada, another factory that can process 150 tonnes per day is in operation. The first large-scale composting facility, operated by Excel Industries Ltd. in Mumbai, processes 500 tonnes of MSW every day. In Vijaywada, another factory has been running with a daily capacity of 150 tonnes. End MSW compost is offered for sale for 3.50 INR (Chattopadhyay , 2009).

The MSW rules have established that only non-biodegradable inert trash and compost rejects should be disposed of in landfills; instead, biodegradable wastes should be managed via composting, vermicomposting, and other techniques. Composting is a preferred option in non-hazardous organic waste management and is part of many state SWM Plans. For instance, value added products from compost to be sent to the nearby rural farming lands (Gandhinagar Municipal Corporation 2020; HSPCB 2008; CPCB 2019) achieving circularity in solid waste management. MSW composition in India is on an average 50% compostable in nature. Paper and plastic waste increased by 280% and 1,200%, respectively, during 1996–2011, showing a rapid change in the physical structure of Indian MSW. Utilizing compostable and waste-to-energy (WtE) technologies were the best means to use compostable and combustible waste (Joshi and Ahmed 2016).

16.6 Organic Solid Waste Management as Manure

Manures has been long used as a fertilizer for agricultural systems and if well-managed in terms of storage can boost the crop production and thereby opening the realms of sustainability (Ndambi et al. 2019). Manures are known to improve soil's physical and chemical properties; increase NPK, CEC, Organic carbon, secondary nutrients like Ca, Mg; regulate pH, EC; reclaim soil by alleviating soil salinity; improve harvest index (Das et al. 2016; Wang et al. 2017; Prasad and Singh 1981; Bhatt et al. 2019; Dhar et al. 2015). As the waste enters the natural cyclic pathways via composting, not only will landfill burdens and their associated problems decrease, but also half of the nutritional needs of the plants can be met, resulting in higher crop yields (Singh and Longkumer 2018; Barrera and Hooda 2016) promoting both sustainable agriculture and sustainable development. But thorough waste segregation is the key. So, waste from one becomes a resource for another. For restoring the fertility of soil, organic fertilizer and manure use need to be encouraged. Under the scheme Paramparagat Krishi Vikas Yojana of the National Mission for Sustainable Agriculture, the Indian government promotes the use of organic manures (Press Information Bureau, Government of India, Ministry of Agriculture and Farmers Welfare 2017).

By stimulating enzymatic activities, organic amendments in conjunction with microflora increase mineralization and, therefore, soil aeration (Kang et al. 2021). The increased mineralization in soil improves soil fertility and crop productivity. Compost can be formed from various kinds of waste. Because of high organic matter and low concentrations of pollutants, MSW is used for composting, improving soil physic-chemical and biological characteristics. Bioconversion of organic waste fraction to compost is safe and straightforward answer to costly chemical treatment and fertilization for increasing productivity of salt-affected soil (Meena et al. 2016; Sundha et al. 2018).

There is often concern about heavy metal contamination in food production. Heavy metal can enter the organic materials of agricultural systems from pesticide spray, farm manures from municipal and industrial sludges which can slowly accumulate and cause severe harm to compost producers and handlers, plants and soil biota and end consumers. Through humification, although, organic compounds are made stable and pollution concentrations are reduced (Kumar and Kaushal 2015) and the manure produced can be fortified with additional treatments but strict enforcement of meeting the compost quality standards Fertiliser Control Order (FCO) (2009, 2013) and SWM Rules (2016) should be observed so that the nutrients can be cycled back to the environment for quality and environmentally sound food production in the realm of Sustainable Agriculture.

16.7 Sustainable Agriculture and Organic Farming

The health of our soil is critical to the success of our whole farming industry. Soil fertility refers to a soil's capacity to tolerate plant development while increasing output. To increase crop yields, they amended the soil with organic and inorganic fertilizers. Some soil fertility management strategies for improving agricultural productivity include the use of organic and inorganic fertilizers, legume crop rotation, and expanded germplasm. Soil fertility is critical for long-term agricultural productivity. As a result, organic farming plays a significant role, such as green manure processing. This verse influences soil physicochemically, and biologically, and about soil fertility and nutrient delivery to plants. (Saini et al. 2019). Soil fertility refers to the soil's capacity to deliver needed nutrients for biodiversity. Excessive application of costly chemical fertilizers significantly impacts the decrease of soil physicochemical and biological characteristics (NRCCA 2016).

Earles and Williams (2005) and Trivedi et al. (2016) advocated that for achieving sustainability in agriculture, soil structure, nutritional fertility and management, plant productivity, the healthy diversity and abundance of soil microbial population is an uncompromisable parameter and microbial community also serves as an essential early indicator of changing soil health even before physicochemical parameters show soil degradation.

Microbes in the soil produce polysaccharides and mucins that promote soil fertility and improve soil structure and health. A healthy plant should have a healthy rhizosphere rich with beneficial microorganisms. In contrast, optimum plant growth is challenging in soil contaminated with harmful microbes (Mendes et al. 2013). Microorganisms help to improve soil fertility by transforming available nutrients into usable forms. They consume and generate organic carbon in the Soil (Johns 2017). Organic carbon in the soil enhances soil fertility and water retention capacity. Microorganisms also help to reduce pollution in the environment (Lorenz and Lal 2014). Some of the aspects that are taken care of in Sustainable agriculture are as follows.

16.7.1 Soil Tillage in Sustainable Agriculture

Some of the technologies utilized to integrate amendments are tillage facilities for seeding or transplanting, fertilizer and lime as field-fixed inputs in residues and residues (Bermudez 2002). Deep ploughing promotes soil aeration and breaks up soil clumps to build healthy seed and root beds, improves water infiltration, boosts microbial activity and mineralization rate, and may smash through dense layers that hinder plant development and water movement. Tillage losses can influence the rate and severity of long-term decomposition of soil organic matter raise subsoil compaction problems, and hinder nutrient absorption and drainage (Naresh et al. 2013). Tillage losses can also cause bare pottery, hindering germination and water

infiltration. There are several advantages to low and no-tillage systems: the residue layer on the soil surface protects the soil from wind and water erosion; reduced tillage systems store more moisture in rainfed systems, which endure longer.

16.7.2 Cover Crops in Sustainable Agriculture

Legume cover crops' function in biological nitrogen determination and nutrient budgeting and *rhizobium* bacteria-infected legume cover crops can turn atmospheric nitrogen (N_2) into plant nitrate (NO_3^-) (MacKinnon et al. 2005). As a carbon source, nutrients are released into the soil solution as decaying soil microorganisms break down cover crop leftovers. Cover crops increase microbial activity and would enhance the breakdown of existing soil organic matter, allowing for establishing deep-rooted cover crops (Gougoulias et al. 2014). Temperature and humidity affect microbial activity (at low temperatures and during drought or waterlogging) and the location of cover crop residues on the soil surface. They coordinated the time of nutrient release from cover crops with agricultural productivity, crop demand, and the destiny of essential plant nutrients. Another effect of cover crops on agricultural soil physical characteristics internal (Patil and Lamnganbi 2018). Using cover crops to improve carbon and nutrient cycling results in a short-term improvement in soil physical properties. Many legumes are sensitive and can enhance nematode bug populations, as are cover crops' weed suppressive effects (Clark 2008).

16.7.3 Composts and Animal Manures in Sustainable Agriculture

Compost is used to regenerate soil and restore fertility, store carbon in the soil, and eliminate chemical inputs (fertilisers, pesticides, and fuel), all of which are environmentally friendly and lead to lower processing costs (Diacono and Montemurro 2011). The most suitable composting technology is chosen based on farm assessments (volumes of materials to be composted, matrix sort and supply locations, machinery/facilities already in place), preliminary environmental and economic sustainability assessments using Life Cycle Assessment, Life Cycle Costing, and Energy Analysis methodologies (Pergola et al. 2018). In addition, the global increase in agricultural livestock units is causing an increase in animal waste. Animal waste cannot be applied directly to soil as a soil amendment therefore biological stabilization is a better approach for use. Animal dung may be utilized as a high-value fertilizer for crops and grasslands if distributed correctly and at the right time to minimize losses and maximize nutrient delivery per crop need (Goss et al. 2013).

16.7.4 Crop Rotation in Sustainable Agriculture

This is the process whereby a crop is continuously moved from one place to a different place, principally for ecological reasons. The following are the reasons for crop rotation: Interrupting pest-host interactions, preventing the accumulation of rodents, weeds, and illnesses (Anaya 1999). Various crops (tomatoes, eggplants, peppers and potatoes) share pests and diseases, repeated farming in the same site can lead to pest population growth. Crop rotation to achieve the most fertilizer supplies and to distribute nutrient demand throughout the soil. Multi-year crop rotations also include fallow cycles and perennial cover crop rotations. Areas kept fallow and planted with permanent cover crops (e.g., perennial ryegrass) permit the soil to stay undisturbed and the aggregation processes to continue uninterrupted (Tadesse et al. 2021).

16.8 Basics of Bioelectromagnetic Energy and its Practical Application

Agriculture is open to experimentation from all fields to enhance production for fulfilling the needs of the ever-growing population in a sustainable way. One such approach is Sustainable Yogic Agriculture (SYA) which involves the utilization of basic techniques of sustainable agriculture along with immaterial energy (Brahma Kumaris 2009). In secular terms, immaterial means something that is not perceived with our five senses at first, a dimension that involves vibrational energy, consciousness, ether, sentience, intelligence, and/or electromagnetic waves or sound frequencies. Various disciplines involved in understanding these processes includes quantum mechanics, consciousness and phenomenological studies, bioelectromagnetic, chronobiology, sonochemistry, neuroscience and transpersonal psychology. The concept of "life energy" is prevalent across many cultures from a long time. It is called by various names like Qi (chi), prana, universal fluid, cosmic aether, archaeus, animal magnetism etc. Very less work is done to understand such phenomena, in the meantime, technological advances from here facilitate our daily lives in an increasing number of ways. Various electrical and electromagnetic devices that modify and improve bioenergy fields are employed for better health in energy medicine practices (Wright 2021).

At fundamental levels, atoms and molecules vibrate at various frequencies. It is not that only high vibrations have impact, forces which are at lower frequencies if resonate with something having the same frequency can have powerful impacts. Takoma Bridge incident of 1940 is an infamous example of impact of resonance. The closest we have come to understand the immaterial energy is comparing it with electrical or electromagnetic signals (Rosch 2009). Ravitz (1982) showed the behaviour of the energy to be similar to electrical activity which he studied as life (L) fields, the energy fields encompassing living beings. He reported that mobilization in galvanometer was caused due to stimulus electrical in nature, and emotional activity

evoked stimuli of the same energy. So, he concluded that "Emotions can be equated with energy" and this energy could be influenced by mental and emotional states of mind. Quantum physics suggest we are *electromagnetic* human beings. Our thoughts radiate out from us like broadcast signals like vibrations (Lipton 2012).

The magnetic property of the bioelectromagnetic energy can be inferred by the experiment by laboratory experiments on Wheat and Chickpea at G.B. Pant University of Agriculture and Technology (Pandey et al. 2015). Seeds were exposed to a magnetic field of 100-250mT (milli Tesla) in steps of 50 mT for 1 to 4 h, in steps of 1 h for all field strengths and other batch was of Seeds exposed to metaphysical immaterial energy treatment for 1 to 4 h, in steps of 1 h by trained Rajyoga meditation practitioners. For wheat, slight increase in germination percentage in magnetic field (250 mT) as well as metaphysical energy treatments. Mean root length, shoot length and seedling vigor index was significantly higher in metaphysical energy treatment as compared to magnetic field treatment. Control showed lower root length, shoot length and seedling vigor index. Chickpea Seeds at 150, 250 mT for 1 h and metaphysical energy exposure were at par with all other treatment combinations except 200 and 250 mT for 3 to 4 h. Root length, shoot length and seedling vigor was significantly higher due to metaphysical energy exposed to seeds for 4 h compared with remaining treatment combinations.

These vibrations are subtle in nature and can be measured using electrophotonic emissions/gas discharge visualization (GDV). It is nonintrusive technique that helps capture the physiological and psychoemotional status of a person and the functional status. The sensor used is sensitive enough to catch the change in conductivity of the space in the presence of emotional people which might be due to formation areas of decreased entropy in space, or, associated with the buildup of a negative magnetic charge manifesting in the environment and identify deviations from the normal functional state at early stages and in real time. Biofield aura intensity and related characteristics are dependent on health and emotional state of mind (Kostyuk et al. 2011; Suru Krilian Photography Centre 2022).

Earlier scientists acknowledged the immaterial layer of universe and some have worked and have been working to bring to surface this phenomenon and its impact in the practical physical world. But, in twentieth century many electrotherapeutic energy devices made worthless claims and further doctors viewed anything and everything related to energy medicine as fraudulent which negatively affected the acceptance of immaterial phenomena (Rosch 2009). Otherwise, John van Neuman, mathematician and pioneer in quantum mechanics stated that "measurement chain ends only when the knowledge of the measurement is registered by an extra physical factor, that is the mind (not the brain)". Radin et al. (2012) using his double slit experiment significantly proved that mind actively participates in manifested reality. He showed in his experiments that mental observation of light beam causes interference and collapses the wave function.

In the medical field, Alda et al. (2016) experimented with a group of 40 individuals divided into meditators and non-meditators showed that in comparison with non-meditators, expert meditators' telomere lengths were significantly longer and their percentage of shorter telomeres was lower. Chromosomal protection, end to end

fusion and senescence of cells from degradation from nuclease is extended by degree of intactness of telomeres. Telomeres are reported to decrease in length with age and can help in early detection of diseases like hypertension, atherosclerosis, type 2 diabetes mellitus, cancer mortality, cardiovascular disease, cognitive decline and dementia.

Luders (2014) reviewed and interpreted 3 studies. Data suggested that meditation practice may slow, stall, or even reverse age-related brain degeneration. García-Campayo et al. (2018) conducted an experimental study on 34 participants divided equally into treatment and control suggested that in distinct regions of genomes that are responsible for CpG methylation have shown loss in long-term meditation practitioners. Epigenetic modulations can be impacted by mindfulness which in turn affect biological processes related to lipid or insulin metabolism. By functional analysis, at molecular level, mindfulness practices have crucial roles in signaling pathways of TNF and NF–NF-κB which is also linked to organizing cellular resistance to invading pathogens and enhance immunity (Bonizzi and Karin 2004).

Back in 1999, Harris et al. experimented to assess the effect of positive healing at a distance in a human clinical trial (1013 subjects). Patients that were admitted to a coronary care unit, a team of intercessors on a randomized basis prayed for patients. Severity score when compared with control group was statistically lower in the patients who were subjected to remote, intercessory prayer.

With living systems of plants in 1963, Grad established that plants are receptive to energy; healing energy improved growth, and negative energy stunted it. Backster (2003) demonstrated plants and other living organisms respond to human intention. Emoto (2004) and Radin et al. (2006) have shown impact of thoughts, intentions and words on water crystals, how positive intentions helps form well defined structure in water molecules and negative did the opposite. Oschman (2004) proposed that a photon of electromagnetic energy works the same way as a single antigen, hormone, pheromone, growth factor, neurotransmitter molecule which can provoke a series of signals at cellular levels that affect biological processes by initiating or inhibiting or even accelerating them. Sum of molecular events on the cell surface can in turn activate enzymatic activities which can catalyse and accelerate biochemical processes. Benveniste (2004) proposed that the theory of trial–error method of reception of ligand molecule and receptor can possibly be also that ligand emits an electromagnetic signal with a frequency resonating to the receptor's molecules that causes them to co-resonate and activate the same intracellular responses which can further explain the anomalies that are present despite of the quantitative structure–activity relationship theory for molecular signalling. Some processes are sensitive to these fields and some are not. Electromagnetism needs to be studied and tested more rigorously in order to establish which reactions are sensitive to these fields.

16.9 Potential of Yogic Farming and Related Research Work

Many additive techniques can be applied along with Organic farming to increase the production in fields in a sustainable way (Fig. 16.2). Adding to the fortification of quality of compost, yogic farming, an additive to organic farming is a novel yet ancient approach that harvests the potential of human mind. It refers to the agricultural practices which involve bio-organic and natural inputs along with application of the power of positive thinking and pure feelings, which are immaterial in nature (Brahma Kumaris 2009). Now, subtle, the immaterial counterpart of agroecology in advanced ecology is revealing itself and it needs to be considered in the farming practices and it is not a new concept. Ancient, traditional Indian farming practices well understood and incorporated the material and immaterial science (Wright 2021). Thought based meditation practice of Rajyoga meditation, practitioners create powerful pulses of vibrations by gently guiding the thoughts to a higher level of positive divine thoughts and direct them towards the farming setup–the seeds, water, and/or crops (Brahma Kumaris 2009). Experimental studies reveal that exposing the farming set up to thought based vibration energy helps enhance soil microbiological activity for microbes like Rhizobium, Azotobacter, Azospirillum and PSB under Organic + Yogic Farming module and crop quality in terms of higher values of germination %, vigour, higher dehydrogenase activity and low electrical conductivity and pest damage control when compared with control (Sharma et al. 2014; Pandey et al. 2015; Ndiritu et al. 2016; Yadav and Vats 2021).

This thought-based energy can be applied during the stages of bio conversion of organic waste and crop production to maximize the quality of compost and crop yield.

According to the Ministry of Agriculture and Farmers Welfare, their target is to increase the farmers' income by 2022 twofold, and for this purpose, Agriculture Minister Radha Mohan Singh pointed out that techniques additive to Organic farming, the Sustainable Yogic farming using Rajyoga meditation which works best under organic farming as research shows is being encouraged under the Parampragat Krishi Vikas Yojana (PKVY) which will also have positive societal benefits. (The Economic times 2016; Yadav 2017).

The outcome of some more research works undertaken in agriculture incorporating the bioelectromagnetic energy are tabulated and given in Table 16.1.

A preliminary research, pot scale study was conducted in urban setting of Sector 20, Gurugram with an aim to assess the possibility of using locally available organic wastes for manure production and aiding in SWM and crop production in space constrained urban areas (Fig. 16.3). The locally available waste selected was keeping in mind COVID-19 restrictions. Organic waste from household (fruit and vegetable peels) and paper waste was locally available and is well suited for composting.

The bioelectromagnetic treatment given is the energy from thought-based meditation. The experimental set up consisted treatments on the basis of distance–2 m, 4 m, 6 m and Control. Three types of compost compositions-Paper waste compost,

Fig. 16.2 The additive approaches that are being explored under sustainable organic agriculture and sustainable yogic farming incorporating bioelectromagnetic energy to enhance the agro-production in a sustainable way

Kitchen waste compost and Mix waste compost was produced. Keeping the similar environmental conditions, the similar treatment of average 30 min thought-based meditation energy was given by meditation practitioner The sampling of compost soil was done at 30, 60, 75 days. Highlight of study was that porosity %, organic carbon and organic matter percentage is higher among the compost exposed to thought based energy supporting the use of SYA in organic manure production. The compost was further applied as manure for crop production of Moong beans. The treatment with organic + yogic showed higher germination percentage when compared to inorganic chemical treatment and only organic treatment.

16.9.1 Conclusion

It is one of the basic principles in ecology that waste that we generate will not disappear because cyclic pathways in the natural environment rearrange, cycle and recycle all materials. This circular pattern has been disrupted when we talk about the mismanaged systems of waste management and it is not a problem to ignore anymore. Bioconversion of locally available organic waste to manures for sustainable SWM will reduce a huge burden from landfills. This will aid in nutrient management of agricultural soil and crop production and their quality enhancement by incorporating Sustainable Yogic Agriculture (SYA) approach seems to be a holistic solution harnessing the combined potential of human mind and natural system for agricultural sustainability enabling the prosperity of ecology, economy and society.

Table 16.1 Research works conducted in agriculture using bioelectromagnetic energy

Treatment	Parameter studied	Plant studied	Outcome	References
Pranic energy application	Growth, yield and nutritional content	Finger millet	Plant growth and production parameters were higher as compared to control-plant height, productive tillers count, number of panicles, fingers count and grain yield were 26%, 35%, 54%, 13% and 44% respectively higher statistically (at $p < 0.05$) Protein content in straw and grain was 4.38% and 6.13% compared to control than control with 3.5 and 4.75% Nitrogen and zinc content was also higher in millet grain was higher in treatment	Poornima et al. (2021)
Yogic farming through Brahma Kumaris raja yoga meditation	Crop growth performance	Wheat and groundnut	Seeds exposed to meditative energy showed enhanced germination, seedling growth and vigor. The quality of groundnut and wheat improved, along with increased soil microbial population. Organic + meditation gave comparable results to chemical inputs	Pandey et al. (2015)

(continued)

Table 16.1 (continued)

Treatment	Parameter studied	Plant studied	Outcome	References
Yogic farming (Raja Yoga Meditation)	Germination and seedling vigor + enzymatic activity	Chickpea	Significantly increased root and shoot length, seedling vigor index anddry weight, enzymatic activity of dehydrogenase over control. Electrical conductivity of seed leachate of treated seed significantly decreased over control	Sharma et al. (2014)
Pranic treatment	Plant growth parameters	Cluster beans	For pranic group, significant increase in vegetative growth and higher flowering and yield	Prasad and Jois (2021)
Meditation listening to shamanic double drumming	Germination and growth of plants	Wheat and pea	The seed germination rate exposed to treated water with stimulating intent was about 8.5% higher in treatments at significant p values. Inhibiting intent experiment with wheat showed 4.2% lower germinate rate compared to control seeds. Mass of seedlings was lower in the inhibitory intent	Haid and Huprikar (2001)
Buddhist meditation	Growth of plant due to intentionally treated water and seeds	*Arabidopsis thaliana*	Seeds grown with intentionally treated water had decreased length of hypocotyl and increase in anthocyanin and chlorophyll suggestive of enhanced photomorphogenic growth	Shiah et al. (2021)

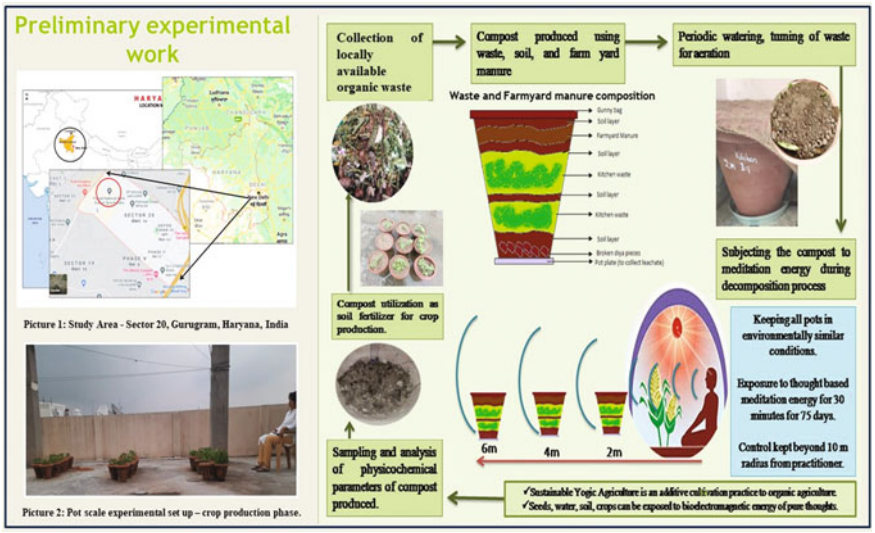

Fig. 16.3 Pot scale study conducted in Sector 20, gurugram showing study area and experimental set up

References

Agegnehu G, Srivastava AK, Bird MI (2017) The role of biochar and biochar-compost in improving soil quality and crop performance: a review. Appl Soil Ecol 119:156–170

Agrawal S (2021) Fast fashion: from environmental damage to poor wages, the dangers of this rising industry. The Indian express. Retrieved on 10 January 2022 from https://indianexpress.com/article/lifestyle/fashion/fast-fashion-dangers-atacama-desert-environment-pollution-workers-7670721/

Akinbile CO, Yusoff MS (2011) Environmental impact of leachate pollution on ground water supplies in Akure Nigeria. Int J Environ Sci Dev 2:81–89

Alda M, Puebla-Guedea M, Rodero B, Demarzo M, Montero-Marin J, Roca M, Garcia-Campayo J (2016) Zen meditation, length of telomeres, and the role of experiential avoidance and compassion. Mindfulness 7(3):651–659

Ali SM, Malik RN (2010) Spatial patterns of vegetation with underlying soil properties prevailing along the drain side areas in Islamabad city. Pakistan J Bot 42:2397–2410

Ali SM, Pervaiz A, Afzal B, Hamid N, Yasmin A (2014) Open dumping of municipal solid waste and its hazardous impacts on soil and vegetation diversity at waste dumping sites of Islamabad city. J King Saud Univ-Sci 26:59–65

Anaya AL (1999) Allelopathy as a tool in the management of biotic resources in agroecosystems. Crit Rev Plant Sci 18(6):697–739

Asefi H, Shahparvari S, Chhetri P (2020) Advances in sustainable integrated solid waste management systems: lessons learned over the decade 2007–2018. J Environ Planning Manage 63(13):2287–2312

Backster C (2003) Primary perception: biocommunication with plants, living foods, and human cells. White Rose Millenium Press, California, USA

Bano A, Fatima M (2009) Salt tolerance in Zea mays (L). following inoculation with Rhizobium and Pseudomonas. Biol Fertil Soils 45(4): 405–413

Benveniste J (2004) A fundamental basis for the effects of emf's in biology and medicine: the interface between matter and function. In: Rosch PJ, Markov MS (eds) Bioelectromagnetic Medicine. Marcel Dekker Inc., New York, NY, pp 207–211

Bermudez M (2002) Impact of variable-rate and starter fertilizer application methods on the crop response to phosphorus. Iowa State University

Bhatt MK, Labanya R, Joshi HC (2019) Influence of long-term chemical fertilizers and organic manures on soil fertility-a review. Univers J Agric Res 7(5):177–188

Biro K, Pradha B, Buchroithner M, Makeschin F (2013) Land use/land cover change analysis and its impact on soil properties in the northern part of Gadarif region, Sudan. Land Degrad Dev 24(1):90–102

Bolan NS, Adriano DC, Naidu R (2003) Role of phosphorus in (im) mobilization and bioavailability of heavy metals in the soil-plant system. In: Reviews of environmental contamination and toxicology. pp 1–44

Bonizzi G, Karin M (2004) The two NF-κB activation pathways and their role in innate and adaptive immunity. Trends Immunol 25(6):280–288

Boutin C, Strandberg B, Carpenter D, Mathiassen SK, Thomas PJ (2014) Herbicide impact on non-target plant reproduction: what are the toxicological and ecological implications. Environ Pollut 185:295–306

Brahma Kumaris: Rural Development Wing Rajyoga Education and Research Foundation (2009) Perpetual yogic agriculture (Shashwat Yogic Kheti) a novel step towards a new era. Retrieved from http://www.yogickheti.org/downloads.html

Bureau of Indian Standards (BIS) (1991) Indian standard specification for drinkingWater, IS 10500. pp 2–4

Buteyn D, Simmons L, Roberts J (2005) The landfill location criteria calculator. A Risk Based Approach to Solid Waste Management. In: Proceedings of 20th International Conference on Solid Waste Technology and Management. Philadelphia, pp 3–6

Chattopadhyay S, Dutta A, Ray S (2009) Municipal solid waste management in Kolkata, India–a review. Waste Manage 29(4):1449–1458

Christy PM, Gopinath LR, Divya D (2014) A review on anaerobic decomposition and enhancement of biogas production through enzymes and microorganisms. Renew Sustain Energy Rev 34:167–173

Clark A (2008) Managing cover crops profitably. Diane Publishing

CPCB (2005) Environmental impact assessment for site selection for common hazardous waste management facility. http://envfor.mic.in/cpcb/hpcrexpert/vol.131.htm. Accessed on January 2005

CPCB (2019) Annual report for the year 2018–19 on implémentation of solid waste management rules, Central Pollution Control. Retrieved ion 11 December 2021 from https://cpcb.nic.in/uploads/MSW/MSW_AnnualReport_2018-19.pdf

Das A (2020). Indian landfill: the ultimate trash kingdom. The times of India. Retrieved on 12 December 2021 from https://timesofindia.indiatimes.com/readersblog/environmental/indian-landfill-the-ultimate-trash-kingdom-25565/

Das A, Patel DP, Lal R, Kumar M, Ramkrushna GI, Layek J, Buragohain J, Ngachan SV, Ghosh PK, Choudhury BU, Mohapatra KP (2016) Impact of fodder grasses and organic amendments on productivity and soil and crop quality in a subtropical region of eastern Himalayas, India. Agr Ecosyst Environ 216:274–282

Dazy M, Béraud E, Cotelle S, Grévilliot F, Férard JF, Masfaraud JF (2009) Changes in plant communities along soil pollution gradients: responses of leafantioxidant enzyme activities and phytochelatin contents. Chemosphere 77:376–383

De la Barrera B, Hooda PS (2016) Greenhouse gas emissions of waste management processes and options: a case study. Waste Manage Res 34(7):658–665

De Souza RG, Da Silva DKA, De Mello CMA, Goto BT, Da Silva FSB, Sampaio EVSB, Maia LC (2013) Arbuscular mycorrhizal fungi in revegetated mined dunes. Land Degrad Dev 24(2): 147–155

Dhar S, Kibria MG, Rahman MM, Hoque MA (2015) Mitigation of the adverse effects of soil salinity in rice using exogenous proline and organic manure. Asian J Med Biol Res 1(3): 478–486. Dhar H, Kumar S, Kumar R (2017) A review on organic waste to energy systems in India. Biores Technol 245: 1229−1237

Diacono M, Montemurro F (2011) Long-term effects of organic amendments on soil fertility. In: Sustainable agriculture. vol 2, Springer, Dordrecht, pp. 761–786

Earles R, Williams P (2005) Sustainable agriculture an introduction. ATTRA

ECS (2001) The sustainable landfill. Environmental Control Systems Inc., Online pdf brochure, South Carolina, USA

El-Fadel M, Findikakis AN, Leckie JO (1997) Environmental impacts of solid waste landfilling. J Environ Manage 50(1):1–25

Emoto M (2004) Healing with water. J Altern Complement Med 10(1):19–21

Esakku S, Kurian J, Nagendran R (2005) Methodological constraints and challenges in sampling and characterization for dumpsite rehabilitation. In: Proceeding Sardinia 2005. Tenth International Waste Management and Landfill Symposium

Gallardo-Lara F, Nogales R (1987) Effect of the application of town refuse compost on the soil-plant system: A review. Biol Wastes 19(1):35–62

Gandhinagar Municipal Corporation (2020) Solid waste management-brief profile of waste management in gandhinagar and gandhinagar municipal corporation. Retrieved on 16 July 2022 from http://npphardoi.co.in/solid-waste-management/index.html

García-Campayo J, Puebla-Guedea M, Labarga A, Urdánoz A, Roldán Pulido M, Mendioroz M (2018) Epigenetic response to mindfulness in peripheral blood leukocytes involves genes linked to common human diseases. Mindfulness 9(4): 1146−1159

Gardner N, Manley BJW, Pearson JM (1993) Gas emissions from landfills and their contributions to global warming. Appl Energy 44(2):165–174

Garsous G (2019) "Trends in policy indicators on trade and environment", OECD trade and environment working papers, No. 2019/01, OECD Publishing, Paris. https://doi.org/10.1787/b8d2bcac-en

Ghasemi MK, Yusuff RB (2016) Advantages and disadvantages of healthcare waste treatment and disposal alternatives: Malaysian scenario. Pol J Environ Stud 25(1)

Glick BR (2016) Impact of soil salinity on the structure of the bacterial endophytic community identified from the roots of caliph medic (Medicago truncatula). PLoS ONE 11:e0159007. https://doi.org/10.1371/journal.pone.0159007

Goss and Tubeileh. and Goorahoo D, 2013 Goss MJ, Tubeileh, Goorahoo D (2013) A review of the use of organic amendments and the risk to human health Adv Agron 120: 275−379

Gougoulias C, Clark JM, Shaw LJ (2014) The role of soil microbes in the global carbon cycle: tracking the below-ground microbial processing of plant-derived carbon for manipulating carbon dynamics in agricultural systems. J Sci Food Agric 94(12):2362–2371

Government of India Ministry of Agriculture and Rural Development. (1985). The Fertiliser (control) Order (FCO) 1985: No.11–3/83-STU. Retrieved on 22 January 2022 from https://agriodisha.nic.in/content/pdf/Fertiliser_Control_Order_1985.pdf

Grad BA (1963) Telekinetic effect on plant growth. Int J Parapsychol.

Haid M, Huprikar S (2001) Modulation of germination and growth of plants by meditation. Am J Chin Med 29(03n04): 393–401

Hargreaves JC, Adl MS, Warman PR (2008) A review of the use of composted municipal soild waste in agriculture. Agric Ecosyst Environ 123:1–14

Harigovind A (2022) Gurgaon civic body fined Rs 2.8 cr over green norms. The Indian Express. Retrieved on 30 September 2022 from https://indianexpress.com/article/cities/delhi/gurgaon-civic-body-fined-rs-2-8-cr-over-green-norms-8174751/

Harris WS, Gowda M, Kolb JW, Strychacz CP, Vacek JL, Jones PG, Forker A, O'Keefe JH, McCallister BD (1999) A randomized, controlled trial of the effects of remote, intercessory prayer on outcomes in patients admitted to the coronary care unit. Arch Intern Med 159(19):2273–2278

Haryana State Pollution Control Board (2008) Annual report for the year 2007–2008. Retrieved from https://hspcb.gov.in/content/annual_report/07_08E.pdf
Hegde DM, Sudhakara Babu SN (2016) Sustainable agriculture. The Indian Society of Agronomy
Hindustan Times (2022) Mumbai's leaky landfills pose 'severe' risk of plastic pollution. Hindustan Times. Retrieved on 10 October 2022 from https://www.hindustantimes.com/cities/mumbai-news/mumbais-leaky-landfills-pose-severe-risk-of-plastic-pollution-101665942939800.html
Hoornweg D, Bhada-Tata P (2012) What a waste: a global review of solid waste management. Urban development series; knowledge papers no. 15. World Bank, Washington, DC. © World Bank. https://openknowledge.worldbank.org/handle/10986/17388 License: CC BY 3.0 IGO
IEA (2016) Energy technology perspectives 2016. Retrieved from https://www.iea.org/reports/energytechnology-perspectives-2016.
Iwai CB, Oo AN, Saenjan P (2013) Soil properties and maize growth in saline and nonsaline soils using cassava-industrial waste compost and vermicompost with or without earthworms. Land Degrad Dev. https://doi.org/10.1002/ldr.2208
Jain S, Sharma MP (2011) Power generation from MSW of Haridwar city: a feasibility study. Renew Sustain Energy Rev 15(1):69–90
Johns C (2017) Living soils: the role of microorganisms in soil health. Fut Direct Intl: 1–7
Johnson BL (1999) A review of the effects of hazardous waste on reproductive health. Am J Obstet Gynecol 181(1):S12–S16
Joshi R, Ahmed S (2016) Status and challenges of municipal solid waste management in India: a review. Cogent Environ Sci 2(1): 1139434
Joshi V, Nachiappan NC (2007) Management of old MSW dumps–challenges and opportunities. In: Proceedings of the International Conference on Sustainable Solid Waste Management, Chennai, India, 5–7 September. pp 3–9
Kang SW, Yun JJ, Park JH, Cho JS (2021) Exploring suitable biochar application rates with compost to improve upland field environment. Agronomy 11(6):1136
Kapanen A, Itävaara M (2001) Ecotoxicity tests for compost applications. Ecotoxicol Environ Saf 49(1):1–16
Kawai K, Tasaki T (2016) Revisiting estimates of municipal solid waste generation per capita and their reliability. J Mater Cycles Waste Manage 18(1):1–13
Kaza S, Yao L, Tata PB, Woerden FV, Ionkova K, Morton J, Poveda RA, Sarraf M, Malkawi F, Harinath AS, Banna F, An G, Imoto H, Levine D (2018) What a waste 2.0-a global snapshot of solid waste management to 2050. International Bank for Reconstruction and Development/The World, Washington, DC
Kostyuk N, Cole P, Meghanathan N, Isokpehi RD, Cohly HH (2011) Gas discharge visualization: an imaging and modeling tool for medical biometrics. Int J Biomed Imaging
Kumar P, Kaushal RK (2015) Avenues of collection and disposal of municipal solid wastes management in India—a review. Int J Eng Sci Inven Res Dev 6:458–465
Lakshmikantha H (2006) Report on waste dump sites around Bangalore. Waste Manage 26(2006):640–650
Lipton B (2012) Quantum physics: spirituality and your life experiences. The biology of Belief. www. abundance-and–happiness. com/quantum-physics. html
Loni R, Najafi G, Bellos E, Rajaee F, Said Z, Mazlan M (2021) A review of industrial waste heat recovery system for power generation with organic rankine cycle: recent challenges and future outlook. J Clean Prod 287:125070
Lorenz K, Lal R (2014) Biochar application to soil for climate change mitigation by soil organic carbon sequestration. J Plant Nutr Soil Sci 177(5):651–670
Luders E (2014) Exploring age-related brain degeneration in meditation practitioners. Ann N Y Acad Sci 1307(1):82–88
MacFarlane DS, Cherry JA, Gillman RW, Sudicky EA (1983) Migration of contaminants in groundwater at a landfill: a case study. J Hydrol 63:1–29
MacFarlane IC (1970) Gas explosion hazards in sanitary landfills. Public Works 101(76–78):138

MacKinnon HL (2005) Are N_2O emissions of soybean crops coupled to N_2 fixation?. Library and Archives Canada= Bibliothèque et Archives Canada, Ottawa
Manara P, Zabaniotou A (2012) Towards sewage sludgebased biofuels via thermochemical conversion–a review. Renew Sustain Energy Rev 16(5):2566–2582
Meena MD, Joshi PK, Narjary B, Sheoran P, Jat HS, Chinchmalatpure AR, Yadav RK, Sharm DK (2016) Effects of municipal solid waste compost, rice-straw compost and mineral fertilisers on biological and chemical properties of a saline soil and yields in a mustard–pearl millet cropping system. Soil Research 54(8):958–969
Mendes R, Garbeva P, Raaijmakers JM (2013) The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiol Rev 37(5):634–663
Mhazo N, Chivenge P, Chaplot V (2016) Tillage impact on soil erosion by water: discrepancies due to climate and soil characteristics. Agr Ecosyst Environ 230:231–241
Ministry of Environment, F. and CC (2016) Solid waste management rules, 2016_S.O. 1357(E) [08–04–2016]: The Gazette of India, 2(REGD. NO. D. L.-33004/9933004/99), 52–90.http://www.moef.nic.in/sites/default/files/SWM. https://cpcb.nic.in/uploads/MSW/SWM_2016.pdf
Mohanty S, Saha S, Santra GH, Kumari A (2020) Future perspective of solid waste management strategy in India. Handbook of Solid Waste Management: Sustainability through Circular Economy. pp 1–36
Mohawesh Y, Taimeh A, Ziadat F (2015) Effects of land use changes and conservation measures on land degradation under a mediterranean climate. Solid Earth Discuss 7:115–145
Monavari M, Arbab P (2005) The environmental evaluation of municipal solid waste landfills of 15 the Tehran province. Environm Sci 2:1–8
Mondal MK, Dasgupta BV (2010) EIA of municipal solid waste disposal site in varanasi using RIAM analysis. Resour Conserv Recycl 54:541–546
Mor S, Ravindra K, Dahiya RP, Chandra A (2006) Leachate characterization and assessment of groundwater pollution near municipal solid waste landfill site. Environ Monit Assess 118(1–3):435–456
Moustakas M, Lanaras T, Symeonidis L, Karataglis S (1994) Growth and some photosynthetic characteristics of field grown Avena sativa under copper and lead stress. Photosynthetica 30:389–396
Naresh RK, Singh SP, Dwivedi A, Sepat NK, Kumar V, Ronaliya LK, Kumar V, Singh R (2013) Conservation agriculture improving soil quality for sustainable production systems under smallholder farming conditions in north west India: a review. Int J Life Sci Bot Pharm Res 2:151–213
Ndambi OA, Pelster DE, Owino JO, De Buisonje F, Vellinga T (2019) Manure management practices and policies in sub-Saharan Africa: implications on manure quality as a fertilizer. Front Sustain Food Syst 3:29
Ndiritu J, Hlongwane J, Baranzika T, Nyembezi L (2016) Applying psychoenergetics to enhance seedling development and crop yields. AfriAlliance Innovation Bridge Event Exhibition, 17th WaterNet/WARFSA/GWP-SA Symposium, Gaborone, Botswana. https://brahmakumaris.org.za/wp-ontent/uploads/2016/07/Applyingpsychoenergetics-to-enhance-seedling-development-and-crop-yields-17th-WaterNet-Conference-2016.pdf. Accessed 15 July 2022
NRCCA Soil Fertility and Nutrient Management–Study Guide. Accessed 26 October 2016
OECD (2021) Towards a more resource-efficient and circular economy-the role of the G20. https://www.oecd.org/env/waste/OECD-G20-Towards-a-more-Resource-Efficient-and-Circular-Economy.pdf. Accessed on 15 September 2022
OECD/European Commission (2020) Cities in the world: a new perspective on Urbanisation, OECD Urban Studies, OECD Publishing, Paris. Retrieved from https://doi.org/10.1787/d0efcbda-en. Accessed on 11 August 2022
Oschman J (2004) Recent developments in bioelectromagnetic medicine. In: Rosch PJ, Markov MS (eds) Bioelectromagnetic Medicine. Marcel Dekker, New York, NY, pp 77–92
Osman KT (2018) Management of soil problems. Springer

Pallavicini Y, Alday JG, Martínez-Ruiz C (2015) Factors affecting herbaceous richness and biomass accumulation patterns of reclaimed coal mines. Land Degrad Dev 26(3):211–217
Panda SS, Minz AP, Dhal NK (2014) Floristic studies of Lajkura coal mines area Jharsuguda, Odisha: an overview. Int J Sci Res Publ 4(12):1–1
Pandey ST, Verma O, Kewalanand PDS, Gill S, Patel JC et al (2015) Yogic farming through Brahma Kumaris Raja yoga meditation: an ancient technique for enhancing crop performance. Asian Agri Hist 19(2):105–122
Pardo T, Clemente R, Epelde L, Garbisu C, Bernal MP (2014) Evaluation of the phytostabilization efficiency in a trace elements contaminated soil using soil health indicators. J Hazard Mater 268:68–76
Patil A, Lamnganbi M (2018) Impact of climate change on soil health: a review. Int J Chem Stud 6(3):2399–2404
Pereira Blanco E, Lloret Maya F, Curiel Yuste J, Barba J (2014) Response of fine root respiration to variations in biotic and abiotic factors in a mixed Mediterranean forest affected by drought-induced secondary succession
Pergola M, Persiani A, Palese AM, Di Meo V, Pastore V, D'Adamo C, Celano G (2018) Composting: the way for a sustainable agriculture. Appl Soil Ecol 123:744–750
Planning Commission, Niti Aayog (2014) Report of the Task Force on Waste to Energy (Volume II) Annexure. https://niti.gov.in/planningcommission.gov.in/docs/reports/genrep/rep_energyvol2.pdf. Accessed 24 October 2021
Poornima R, Prasad KN, Jois SN (2021) Growth, yield and nutritional content of finger millet (Eleusine coracana L.) as influenced by pranic energy application. J Appl Nat Sci 13(1): 42–50
Prasad KN, Jois SN (2021) Effect of pranic agriculture treatment on growth of cluster beans (Cyamopsis tetragonoloba L.). Indian J Agric Res 55(3): 359–363
Press Information Bureau, Government of India and Ministry of Agriculture and Farmers Welfare (2017) Promoting the use of organic manure. https://pib.gov.in/newsite/PrintRelease.aspx?relid=159439. Accessed 15 September 2022
Radin D, Hayssen G, Emoto M, Kizu T (2006) Double-blind test of the effects of distant intention on water crystal formation. Explore 2(5):408–411. https://doi.org/10.1016/j.explore.2006.06.004
Radin D, Michel L, Galdamez K, Wendland P, Rickenbach R, Delorme A (2012) Consciousness and the double-slit interference pattern: six experiments. Physics Essays 25(2):157
Rajput R, Prasad G, Chopra AK (2009) Scenario of solid waste management in present Indian context, Capian. J Environ Sci 7(1):45–53
Rattanaoudom R (2005) Investigation on toxicity and hazardous nature of a municipal solid waste dumpsite. Masters research study No EV-05–22, Asian Institute of Technology
Ravitz LJ (1982) History, measurement, and applicability of periodic changes in the electromagnetic field in health and disease. Ann N Y Acad Sci 98:1144–1201
Ray MR, Roychoudhury S, Mukherjee G, Roy S, Lahiri T (2005) Respiratory and general health impairments of workers employed in a municipal solid waste disposal at an open landfill site in Delhi. Int J Hyg Environ Health 208(4):255–262
Ray MR, Roychoudhury S, Mukherjee S, Siddique S, Banerjee M, Akolkar AB, Sengupta B, Lahiri T (2009) Airway inflammation and upregulation of β2 mac-1 integrin expression on circulating leukocytes of female ragpickers in India. J Occup Health 51(3):232–238
Reinhart DR, McCreanor PT, Townsend T (2002) The bioreactor landfill: its status and future. Waste Manage Res 20(2):172–186
Rosch PJ (2009) Bioelectromagnetic and subtle energy medicine: the interface between mind and matter. Ann N Y Acad Sci 1172:297–311. https://doi.org/10.1111/j.1749-6632.2009.04535.x
Rushbrook P (2001) Guidance on minimum approaches for improvements to existing municipal waste dumpsites
Saini PK, Yadav RK, Yadav GC (2019) Green manures in agriculture: a review. Bhartiya Krishi Anusandhan Patrika 34(1):1–10
Sandin G, Peters GM (2018) Environmental impact of textile reuse and recycling: a review. J Clean Prod 184:353–365. https://doi.org/10.1016/j.jclepro.2018.02.266

Sarang PY, Savoikar PP, Gokhale CS (2012) Engineered landfill a sustainable approach for solid waste management

Sharholy M et al (2005) Analysis of municipal solid waste management systems in Delhi—a review. Book of proceedings for the second International Congress of Chemistry and Environment. Indore, India, pp 773–777

Sharholy M, Ahmad K, Mahmood G, Trivedi RC (2008) Municipal solid waste management in Indian cities–a review. Waste Manage 28(2):459–467

Sharma R, Pandey ST, Verma O, Joshi N, Srivastava RC, Guru SK (2014) Effect of physical and metaphysical energy on germination and seedling vigor of Chickpea. J Basic Appl Agric Res 12(3)

Sharma AR, Singh VP (2014) Integrated weed management in conservation agriculture systems. Indian J Weed Sci 46(1):23–30

Shiah YJ, Hsieh HL, Chen HJ, Radin DI (2021) Effects of intentionally treated water and seeds on the growth of Arabidopsis thaliana. Explore 17(1):55–59

Singh K (2016) Microbial and enzyme activities of saline and sodic soils. Land Degrad Dev 27(3):706–718

Singh RP, Singh P, Arouja ASF, Ibrahim MH, Sulaiman O (2011) Management of urban solid waste: vermicomposting a sustainable option. Resourc Conserv Recycl 55:719–729

Singh RK, Longkumer TE (2018) Compost: the black cold. KrishiVigyan Kendra–Phek, ICAR–NRC on Mithun, Porba, Phek, Nagaland, 112

Smith CJ, Hopmans P, Cook FJ (1996) Accumulation of Cr, Pb, Cu, Ni, Zn and Cd in soil following irrigation with treated urban effluent in Australia. Environ Pollut 94(3): 317–323. Springer, Verlag, New York

Srinivasu DD, Bandyopadhyay M (1998) Solid state acidification of vegetable waste. Indian J Environ Health 40(1998):333–342

Srivastava V, Ismail SA, Singh P, Singh RP (2015) Urban solid waste management in the developing world with emphasis on India: challenges and opportunities. Rev Environ Sci Bio/Technol 14(2):317–337

Stearns RP, Peyotan GS (1981) Active systems for landfill gas control. In: Proceedings of the Technical Program GRCSA, 19th Annual International Seminar, Equipment Show, Salt Lake City Utah 299–309

Sundha P, Basak N, Rai AK, Yadav RK, Sharma DK (2018) Utilization of municipal solid waste compost in reclamation of saline-sodic soil irrigated with poor quality water. J Indian Soc Soil Sci 66(1):28–39

Suru Krilian Photography Centre (2022) GDV Sputnik Sensor. https://www.auraphotographys.com/bio-well-sputnik-sensor.html. Accessed 20 April 2021

Tadesse M, Simane B, Abera W, Tamene L, Ambaw G, Recha JW, Mekonnen K, Demeke G, Nigussie A, Solomon D (2021) The effect of climate-smart agriculture on soil fertility, crop yield, and soil carbon in southern ethiopia. Sustainability 13(8):4515

The Economic times (2016) Govt to promote yogic farming to boost crop yields: Radha Mohan Singh. https://economictimes.indiatimes.com/news/economy/agriculture/govt-to-promote-yogic-farming-to-boost-crop-yields-radha-mohan-singh/articleshow/54735592.cms?from=mdr. Accessed 15 February 2022

Times of India (2022) Delhi: Ghazipur landfill fire continues till evening, no casualty; FIR filed. Times of India. https://timesofindia.indiatimes.com/city/delhi/ghazipur-landfill-fire-continues-till-evening-no-casualty-fir-filed/articleshow/90527690.cms. Accessed 12 April 2022

Timmerman A (2021) Jakarta's poor fear landslides from overflowing waste mountains. Nikkei Asia. https://asia.nikkei.com/Spotlight/Environment/Jakarta-s-poor-fear-landslides-from-overflowing-waste-mountains. Accessed 17 March 2022

Trivedi P, Delgado-Baquerizo M, Anderson IC, Singh BK (2016) Response of soil properties and microbial communities to agriculture: implications for primary productivity and soil health indicators. Front Plant Sci 7:990

United Nations (n.d.) World population trends. https://www.un.org/en/observances/world-population-day. Accessed 29 August 2022
US Environmental Protection Agency (2022) National overview: facts and figures on materials, wastes and recycling. Recycling. https://www.epa.gov/facts-and-figures-about-materials-waste-and-recycling/nationaloverview-facts-and-figures-materials
Vasanthi P, Kaliappan S, Srinivasaraghavan R (2008) Impact of poor solid waste management on ground water. Environ Monit Assess 143(1–3):227–238
Venkatesan G, Swaminathan G (2009) Review of chloride and sulphate attenuation in ground water nearby solid-waste landfill sites. J Environ Eng Landsc Manag 17(1):1–7
VL P (2021) It's too much it. Bangalore Mirror. https://bangaloremirror.indiatimes.com/bangalore/civic/its-too-much-it/articleshow/88327128.cms. Accessed 12 August 2022
Voutsa D, Grimanis A, Samara C (1996) Trace elements in vegetables grown in an industrial area in relation to soil and air particulate matter. Environ Pollut 94:325–335
Wang X, Ren Y, Zhang S, Chen Y, Wang N (2017) Applications of organic manure increased maize (Zea mays L.) yield and water productivity in a semi-arid region. Agric Water Manag 187:88–98
World Health Organization (2000) The world health report 2000: health systems: improving performance. World Health Organization
World Health Organization (2007) The world health report 2007: a safer future: global public health security in the 21st century. World Health Organization
World Bank (2021) World Development Indicators DataBank, https://databank.worldbank.org/source/world-development-indicators. Accessed 17 July 2022
World Health Organization (WHO) (1997) Guideline for drinkingwater quality, Health Criteria and Other Supporting Information. Vol 2, no 2. World Health Organization, Geneva, pp 940−949
Wright J (2021) Subtle agroecologies: farming with the hidden half of nature. Taylor and Francis
Yadav M (2017) Towards a healthier nation: organic farming and government policies in India. Int J Adv Res Dev 2:153–215
Yadav RH, Vats P (2021) Yogic farming: a philosophical vision into Practical farming. Paryavaran perspective, pp 30–31
Yamaguchi S (2021) International trade and circular economy-Policy alignment", OECD Trade and Environment Working Papers, No. 2021/02, OECD Publishing, Paris. https://doi.org/10.1787/ae4a2176-en
Yazdani M, Monavari M, Omrani GA, Shariat M, Hosseini M (2015) Municipal solid waste open dumping, implication for land degradation. Solid Earth Discuss 7:1097–1118
Zahir ZA, Munir A, Asghar HN, Shaharoona B, Arshad M (2008) Effectiveness of rhizobacteria containing ACC deaminase for growth promotion of peas (Pisum sativum) under drought conditions. J Microbiol Biotechnol 18(5):958–963
Zamorano M, Perez JIP, Paves IA, Ridao AR (2007) Study of the energy potential of the biogas produced by an urban waste landfill in Southern Spain. Renew Sustain Energy Rev 11(5):909–922
Zhou X, Shia X, Zhang L, Zhou Y (2012) Effects of pesticide-contamination on population and activity of bacteria in purple paddy soil. Energy Procedia 16:284–289
Zia H, Devadas V (2007) Municipal solid waste management in Kanpur, India: obstacles and prospects. Manag Environ Qual: Int J 18(1):89–108
Zurbrugg C (1999) The challenge of solid waste disposal in developing countries. SANDEC News, EAWAG, No.4, January 1999:10–14
Zurbrugg C (2003) Solid waste management in developing countries. SWM introductory text on www.sanicon.net.5

Chapter 17
Production of Sustainable Building Products Using Micronized Biomass Silica from Rice Husk

S. Thirumalini, T. Shanmuga Priya, Mirijam Vrabec, and Gayathri Chandran

Abstract Micronized biomass silica (MBS) is an agricultural waste obtained by burning and grinding rice husk in a controlled atmosphere to nano scale. The present chapter focuses primarily on the performance assessment of MBS as an ingredient in production of Fly ash brick and paver blocks. The research was carried in two stages. Casting and compressive strength tests of mortar mixtures with variable MBS content in the range of 0, 5, 10, 15, and 20% by weight of cement were conducted in the first step. The optimum percentage of MBS substitution in mortar was found to be 15% and the microstructural properties of hydrated cement mortar mixes using SEM and EDAX shows that the addition of MBS to the hydrated cement mortar mixes resulted in the formation of CSH gels and $CaCO_3$ crystals, which may be credited to improved strength. In second stage, fly ash bricks and paver blocks were casted by replacing fly ash and cement respectively with varying percentage of MBS (0, 10, 20, 30, 40 & 50%). From the compressive strength test it was found out that optimum replacement of MBS was 15% for fly ash bricks and paver blocks. Efflorescence was not observed in fly ash bricks at all proportions of MBS. When compared to control specimens, MBS blended fly ash brick and paver block specimens showed a significant resistance to water penetration. The present study indicates the feasibility of utilizing MBS as a raw material in production of sustainable building materials and products.

Keywords Micronized biomass silica · Cement mortar · Fly ash bricks · Paver blocks

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17.1 Introduction

Concrete is recognized amongst the most important components incorporated in construction sectors. Due to the various mechanical and physical properties it possesses, it is extensively used in construction activities. It is true that its application and usage are touching skies but simultaneously its ill effects on the environment are also touching skies. Amongst the major issues related to concrete structures are carbon dioxide emissions due to large amount of cement usage and cracks-especially micro cracks (Worrell et al. 2001; Coronelli and Gambarova 2004). More than 7% of greenhouse effects are due to carbon dioxide emissions from the extensive cement content in concrete (Turner and Collins 2013).

Global warming is speeding every day and it is consuming every single unit of the atmosphere. It has been caused by extreme increase in greenhouse gas emission, which has now created a blanket of pollution around the planet. Air pollutants are dangerously settling in atmosphere. The more they increase, more is the threat created for each individual's health. Construction industry is one of the main causes of this pollution. Construction companies are the source of many wastes which are untreatable. Additionally, the population is growing and the economy is developing quickly, which has increased the production of industrial and agricultural waste products such fly ash, GGBS, rice husk ash, and silica fume, whose disposal is becoming more difficult. Several research projects are currently being conducted on the use of waste in concrete (Corinaldesi et al. 2002; Mehta 2002; Naik and Moriconi 2005).

Rice is the main source of food in India. This causes a continuous demand requiring production at bulk. While processing the produce for the market, we get a byproduct called rice husk, which is the skin of rice grain. This husk is disposed of without considering the fact that it can be used for our own benefits. An agricultural waste product derived from the processing of paddy is rice husk. Of the 590 million tonnes of paddy produced worldwide, it makes about 20% (Ewais et al. 2014). The rice husk is utilised in India to make partition boards, feed animals, and other things that are considered trash (Gidde and Jivani 2007) Amorphous silica, which is utilized as a mineral additive in concrete, is found in high concentrations in rice husk ash (RHA), which is produced by controlled burning (De Sensale et al. 2008; Dellaet al. 2002). More CSH gel is created when this amorphous silica interacts with hydration products. As a result, concrete's mechanical and durable qualities are improved (James and Subba Rao 1986). It is known that adding RHA to concrete increases strength, decreases permeability, and provides corrosion resistance (Saraswathy and Song 2007). Additionally, numerous studies demonstrate that recycled aggregate concrete with rice husk has superior mechanical qualities to concrete without RHA (Tangchirapat et al. 2008; Akinkurolere 2013).

The performance of regular concrete with RHA has been the subject of numerous studies; however, research on concrete containing micronized biomass silica (MBS) is few. The MBS is made by carefully burning rice husk at a controlled temperature (500–600 C) in a rotary furnace and then grinding it for a short period of time in a jar

mill to minimize the particle size (i.e. micronized). The increased silica concentration and finer particles are the primary distinction between RHA and MBS. In order to produce high performance concrete, RHA and silica fume have been employed in place of cement (Zhang and Malhotra 1996; Appa Rao 2003). Secondary calcium silicate hydrates (CSH) were produced as a result of MBS's pozzolanic reaction with cement hydrates, and MBS's characteristics, which have the ability to lessen the intensity of $Ca(OH)_2$, also improved.

Research carried by Suraya Hani et al. (2015) involved cement replacement with MBS at percentages ranging from 0 to 20%. It was noted that up to 12% of MBS, concrete showed higher strength and lower water permeability when compared with control concrete. Another work by Prameetthaa et al. (2015) incorporated replacement of cement with MBS at 0, 4, 8 and 12% in mortar. An increase in CSH gel formation along with a potential reduction in the intensity of $Ca(OH)_2$ was observed. Optimum results were obtained at 8% MBS on grounds off mechanical and durability properties. The research work carried by Ambily et al. (2015) involved the use of total replacement of ground granulated blast furnace slag against ordinary Portland cement and MBS was replaced at 0, 10, 20, and 30% by volume. Various mechanical and physical properties were analyzed and it was found that 10% replacement of MBS showed a considerable higher compressive strength. The current study has been undertaken to determine the mechanical and durability characteristics of cement mortar with partial replacement of cement and various percentages of MBS, as well as to assess its sustainability, a study has been undertaken.

17.1.1 Objectives

The preeminent goal of this research was to determine the ideal amount of MBS to use in the cement-mortar paste and to assess the partial substitution of cement with MBS in cement-mortar. The project's goals are outlined below in brief: To study the characteristics of Micronized Biomass Silica (MBS) as an effective replacement of cement in cement paste and mortar.

- To study characteristics of Micronizd Biomass Silica as an effective substitute of cement in cement paste and mortar.
- To perform preliminary test on cement replaced with various proportions of MBS to analyze various properties such as its specific gravity, setting time and flowability.
- To design mix proportions with varying contents of MBS as a replacement of cement.
- To study and analyze strength and durability of the rendered mix designs and to compare with the control mix (Traditional cement mortar).
- To perform a cost–benefit analysis to deduce the economic feasibility of using MBS as a cementatious material.

- To undertake an environmental impact assessment to understand the impacts caused by replacing MBS in cement mortar.
- To derive an optimum replacement percentage of MBS to obtain a sustainable cement mortar.

17.2 Materials and Methods

OPC of 53 Grade was used in accordance with IS 12269:2007 and the specific gravity of cement was found to be 3.15. MBS used in this study was obtained from the Astrra Chemicals, a chemical manufacturing company in Chennai. The specific gravity of MBS used is 2.2. MBS has a pozzolanic activity index of 85.05% with a surface area of 20.83 m^2/g. The chemical composition of cement and MBS are presented in Table 17.1 (Fig. 17.1).

A low calcium Class F fly ash (confirming with ASTM C311) was used and its chemical compositions are given in Table 17.1. Locally accessible natural sand with a maximum grain size of 4.75 mm that complied with Zone II was used. According to IS383-2016, the bulk density of fine aggregate was found to be 1675 kg/m3 with water absorption of 1.8%, and the specific gravity was 2.65 and fineness modulus

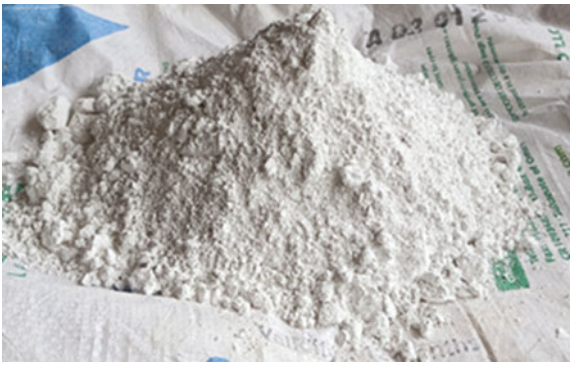

Fig. 17.1 Micronized biomass silica

Table 17.1 Chemical composition (%) of cement and MBS

Compound	Cement	MBS	Flyash
SiO_2	15.55	95.25	48
Al_2O_3	8.40	0.27	24.3
Fe_2O_3	5.87	0.33	15.6
SO_3	3.12	0.21	0.4
CaO	62.44	0.81	3.2
Na_2O	0.16	–	0.8
K_2O	1.27	2.26	–

was 2.36. For the preparation of the mortar, fresh, drinkable water that was devoid of organic and acidic materials was employed.

17.2.1 Mixture Proportions for Mortar Mixes

The effects on different percentages of MBS (partial replacement by weight of cement) were evaluated, expecting developments in the strength, durability and microstructural properties. A total of 5 batches of mortar samples were cast. Ordinary portland cement, river sand, MBS and Potable water was used. Mix design was done as per the procedure mentioned in ASTM C311. The mix proportion in kg/m^3 is presented in Table 17.2 (Fig. 17.2).

To maintain a consistent mixing temperature, all the ingredients were held at ambient temperature (27 °C 2 °C). All the components were mixed using a paddle mortar mixer. The process of combining the ingredients took around 7 minutes. After combining all the ingredients, the mortar was layered three times in the mould and properly crushed using a vibrating table. The casted cubes are shown in Fig. 17.2.

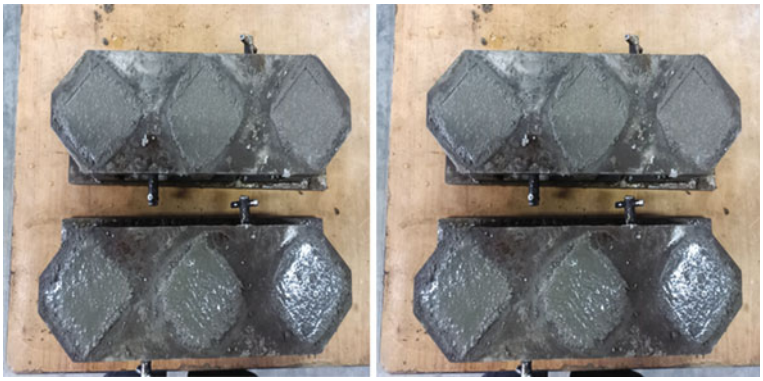

Fig. 17.2 Cubes for compressive strength test

Table 17.2 Mix proportion

Mix ID	Percentage replacement		Quantity of materials (grams)			
	Cement	MBS	Cement	MBS	Sand	Water
M1	100	0	500	0	1375	242
M2	95	5	450	50	1375	242
M3	90	10	400	100	1375	242
M4	85	15	350	150	1375	242
M5	80	20	300	100	1375	242

Tap water was used for curing these specimens for 28 days at room temperature (27 °C ± 2 °C). The specimens were immersed in water throughout the curing process.

17.2.2 Mixture Proportions for Flyash Bricks

The various raw materials that constitute the fly-ash bricks, along with their proportions used in the industry are: Fly-ash−50%, Lime−15%, Cement−10%, Crusher dust−25% and 150−200 ml of water is used per kilogram of the raw mixture.

Fly-ash bricks were cast with varying proportions of MBS replacing flyash (0, 10, 20, 30, 40 & 50%). The raw materials were mixed and blended in a miller drum. A fly-ash brick weight about 3.3 kgs. Therefore, a batch of 20 kg of this mixture was taken for 6 bricks (each mix) to get samples with desired proportion of MBS (Fig. 17.3).

Fig. 17.3 Flyash bricks preparation **a** Mixing of raw materials **b** Bricks emerging from hydraulic press **c** Bricks after curing

Fig. 17.4 Paver blocks preparation

17.2.3 Mixture Proportions for Paver Blocks

Paver blocks of M-35 grade were used in this study. The various raw materials that constitute the fly-ash bricks, along with the mix proportions used in the industry are:

Cement: Fine aggregates: Coarse aggregates−1:2.5:3.25.

Water-cement ratio (w/c) - 0.4

Samples of M-35 grade paver blocks were cast with varying proportions of MBS replacing Cement (0, 10, 20, 30, 40 and 50%). The raw materials were mixed and blended in a mixing drum. A paver block weight about 5.4 kgs. Therefore, a batch of 33.75 kg of this mixture was taken for 6 blocks (each mix) to get samples with desired proportion of MBS. Fig. 17.4 shows the preparation steps of paver blocks.

17.2.4 Testing Procedures

In order to evaluate the compressive strength in accordance with Codal requirements, mortar specimens of 50 × 50 × 50 mm, fly ash bricks, and paver blocks were employed. There were six samples cast for each combination. The strength was assessed after 7 and 28 days of curing With a continuous load rate of 2.5 kN applied to the cubes, the test was carried out using an AIMIL compression testing machine. The produced raster scan picture for SEM can target resolutions greater than 1 nm. The sample underwent a compressive strength test and was then ground into smaller pieces before being evaluated for SEM examination. The samples were then cleaned and dried in an oven at a temperature of about 96 °C. The samples were made into a powder and put through a sieve with a mesh size of 75 microns. Additionally, the uncoated sieved samples were maintained safely over a glass plate that had been coated in Canada Balsam. Following that, the samples are examined using an LEICA microscope and visualised using QWIN software.

Fig. 17.5 Water absorption test

According to the steps outlined in IS3495 (Part 2), the fly ash bricks and paver blocks were evaluated for total water absorption as shown in Fig. 17.5. To achieve basically constant mass, specimens were dried in a vented oven at a temperature of 105 to 115 °C. After the samples had reached room temperature, its weight (M1) was determined. The specimens were fully dry before being submerged in sterile water for 24 h at a temperature of 27 °C. The specimens were taken out, and any water residue was cleaned with a moist cloth. Three minutes after being removed from the water, the specimens were weighed, and their weight (M2) was recorded. Following is the formula for water absorption, expressed as a percentage of mass, following a 24-hour immersion in cold water:

Water absorption = [(M2−M1)/M1] * 100

17.3 Result and Discussions

17.3.1 Compressive Strength Results—Mortar Samples

Compressive strength values of MBS incorporated mortar samples shown in Fig. 17.6. Compressive strength values showed an increase from 5 to 15% replacement of MBS. Decrease in strength was observed with 20% replacement of cement with MBS.

From Fig. 17.6, the compressive strength with 5% MBS showed an increase of 0.56% at 28th day. With 10% MBS addition an increase of 1.09% was observed with respect to reference sample. Compressive strength grows with 15% percentage of MBS addition, after which it rapidly falls. A percentage of 1.45% was observed for this percentage replacement. With 20% addition a percentage decrease of 1.57%

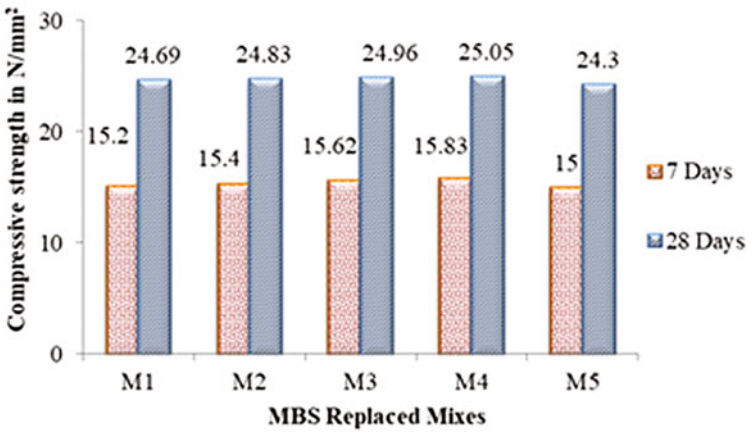

Fig. 17.6 Compressive strength results—mortar samples

was observed. The pozzolanic reaction of MBS is what causes the gain in strength. The MBS contains a lot of silica and it transforms calcium hydroxide into C-S-H molecules when combined which are in charge of developing strength. The constrictive after a certain point, strength decreases as less water is due to the MBS's high water absorption capacity, available for the reaction ability.

17.3.2 Compressive Strength Results—Fly Ash Bricks

Average strength values of MBS incorporated fly ash bricks at 21 days are shown in Fig. 17.7. Compressive strength values showed an increase from 0 (FAB1) to 40% (FAB5) replacement of MBS. With 10, 20, 30, 40% MBS addition a percentage increase of 0.2%, 0.48%, 1.4%, and 2.7% respectively. Decrease in strength was observed with 50% replacement incorporation of MBS. The percentage decrease was around 1.6%.Optimum strength value was observed at 40% of MBS in fly ash brick mix. Up until 40% replacement, the compressive strength grows as the percentage of MBS does, after which it rapidly falls.

17.3.3 Compressive Strength Results—Paver Blocks

Samples of paver blocks had their compressive strength tested after seven and twenty-eight days. For each mix, three samples were cast and evaluated over the course of seven and twenty-eight days. The average of these readings was then taken and is shown in Tables 17.3 and 17.4 below.

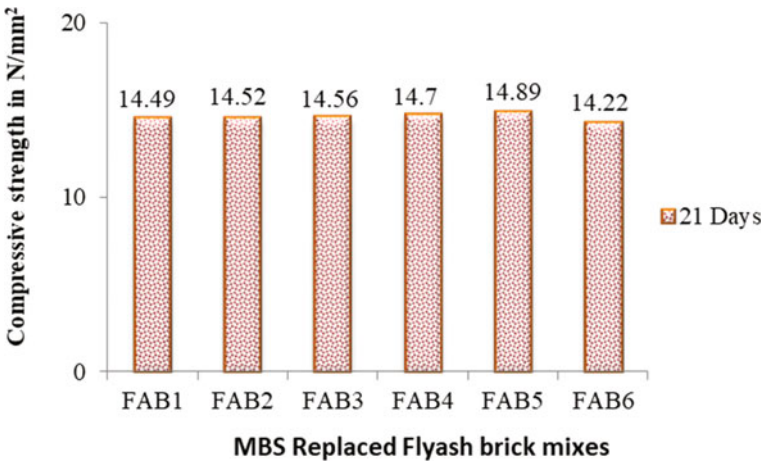

Fig. 17.7 Compressive strength results—fly ash bricks

Table 17.3 Compressive strength for paver blocks at 7 days

eMix Id	Sectional area (mm^2)	Average load at failure (kN)	Average strength (N/mm^2)
PVB1	30,000	643.2	21.44
PVB2	30,000	673.5	22.45
PVB3	30,000	675	22.5
PVB4	30,000	678	22.6
PVB5	30,000	696	23.2
PVB6	30,000	538.89	21.3

Table 17.4 Compressive strength for paver blocks at 28 days

Mix Id	Sectional area (mm^2)	Average load at failure (kN)	Average strength (N/mm^2)
PVB1	30,000	1051.5	35.05
PVB2	30,000	1125	37.5
PVB3	30,000	1128.3	37.61
PVB4	30,000	1129.5	37.65
PVB5	30,000	1148.1	38.27
PVB6	30,000	1050	35

Average strength values of MBS incorporated paver brick at 7 and 28 days is shown in Fig. 17.8. For both curing age the compressive strength values showed an increase with 0% (PAB1) to 40% (PAB4) replacement of MBS. With 10% MBS the percentage increase in strength for 7 and 28th day was 4.7% and 6.9% respectively. For 20%

replacement it was 4.9% and 7.3% respectively. Similarly for 30% replacement it was 5.4% and 7.4% respectively. Maximum hike in strength was observed at 40% replacement. A dip in strength was observed with 50% replacement incorporation of MBS for both curing age. Optimum strength value was observed at 40% of MBS in paver brick mix. Up-to 40% substitution, the compressive strength grows as the percentage of MBS does, after which it rapidly falls till 50% replacement.

17.3.4 Water Absorption Results—Fly Ash Bricks

The results of all the mixtures water absorption tests were displayed in Table 17.5. It was shown that when MBS was substituted for fly ash brick at various percentages, as percentage replacement increased water absorption decreased. A percentage decrease of 1.29%, 20.1, 33.7%, 24.02%, 25.3% was observed for 10, 20, 30, 40 and 50% MBS addition respectively. Lowest water absorption percentage was observed in 30% MBS in fly ash brick. The optimum water absorption percentage was 40%. The existence of finer MBS particles and its pozzolanic feature, which led to a discontinuous pore structure, can be a cause of this positive result.

17.3.5 Water Absorption—Paver Block

Table 17.6 displays the mixes' water absorption capabilities. When MBS is added, which has PVB4's mix having the lowest water absorption, the water absorption decreases? 20% MBS in paver blocks had the lowest recorded water absorption percentage. The recommended replacement rate was 20%.

Table 17.5 Water absorption results−fly ash bricks

Mix ID	Dry weight (kg)	Wet weight (kg)	% Water absorbed
FAB1	3.303	3.813	15.4
FAB2	2.817	3.246	15.2
FAB3	2.970	3.335	12.3
FAB4	3.213	3.540	10.2
FAB5	2.825	3.156	11.7
FAB6	2.713	3.026	11.5

Table 17.6 Water absorption results—paver blocks

Mix ID	Dry weight (kg)	Wet weight (kg)	% Water absorbed
PVB1	5.327	5.521	3.64
PVB2	4.280	4.425	3.38
PVB3	4.627	4.764	2.96
PVB4	4.962	5.062	2.01
PVB5	5.035	5.231	4.90
PVB6	5.125	5.426	5.87

17.3.6 Micro-structural Analysis—Mortar Mix

The conventional and 15% replacement of cement by MBS replacement mix were studied through micro-structural analysis and compared with CC to evaluate the texture and effect of MBS in cement mortar. Fig. 17.8a shows the microstructural arrangement of CC, being a uniformly distributed arrangement of materials in absence of any major cracks visually observable. Unreacted particles are visible on the surface of the surface which shows incomplete pozzolanic reaction. Moreover the presence of small amount of Si is reflected in the EDS analysis.

Figure 17.8b shows the micro-structural matrix for MBS addition mortar mix which shows finished pozzolanic reactions. The SEM image shows a dense matrix due to enhancement in the formation of CSH/CASH gels which is also confirmed by the EDS analysis which shows large amounts of Si and Ca governing the high strength of the mix. Lastly MBS is accounted for higher amounts of Si and Al, thus promoting a high strength mix with desirable properties.

17.4 Conclusions

The below mentioned outcomes were achieved from the above described results

- Cement replacement with MBS in mortar increased compressive strength by up to 15%.
- The optimum replacement of MBS was found to be 20% for fly ash bricks and paver blocks.
- Micro-structural analysis of mortar mixes showed the effect of pozzolanic nature of MBS in increasing the overall performance.
- The MBS addition products can be used for buildings as a sustainable and economical way for disposal of rice husk waste.
- MBS mortar can be applied other primarily places where cement mortar is used leads to paving a way for a cleaner and greener construction.

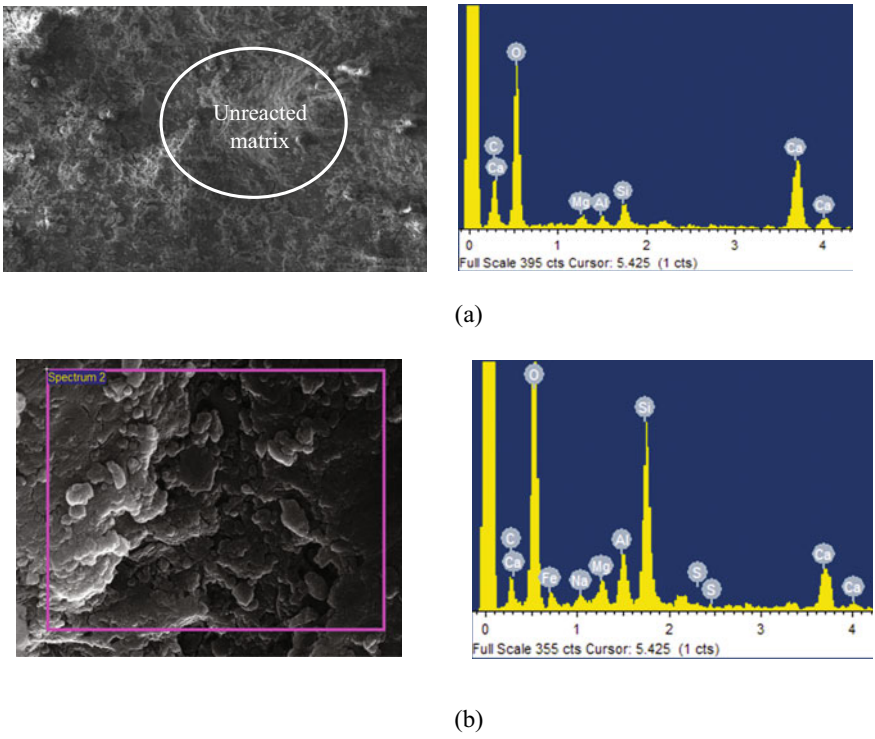

Fig. 17.8 SEM & EDS **a** CC, **b** MBS mix

References

Akinkurolere OO (2013) Effects of rice-husk ash as partial replacement for cement on compressive strength of recycled aggregate concrete. Int J Eng Technol 3(8):777–80.

Ambily PS, Ravisankar K, Umarani C, Kumar SS (2015) Influence of micronised biomass silica on the workability and strength of alkali activated slag concrete

Appa RG (2003) Investigations on the performance of silica fume-incorporated cement pastes and mortars. Cem Concr Res 33:1765–1770

ASTM C 311 (2013) Standard test methods for sampling and testing fly ash or natural pozzolans for use. ASTM International

Corinaldesi V, Giuggiolini M, Moriconi G (2002) Use of rubble from building demolition in mortars. Waste Manage 22(8):893–899

Coronelli D, Gambarova P (2004) Structural assessment of corroded reinforced concrete beams: modeling guidelines. J Struct Eng 130(8):1214–1224

De Sensale GR, Ribeiro AB, Gonçalvesm A (2008) Effects of RHA on autogenous shrinkage of Portland cement pastes. Cem Concr Compos 30(10):892–897

Della VP, Kühn I, Hotza D (2002) Rice husk ash as an alternate source for active silica production. Mater Lett 57(4):818–821

Ewais EM, Ahmed YM, El-Amir AA, El-Didamony H (2014) Cement kiln dust/rice husk ash as a low temperature route for wollastonite processing. Ep-J Silic Based & Compos Mater 66(3).

Gidde MR, Jivani AP (2007) Waste to wealth-potential of rice husk in India–a literature review. In: Proc of the international conference on cleaner technologies and environmental management. Pondicherry Engineering College, Pondicherry, India, pp 586–90.

IS 383, B. I. S (1970) Specification for coarse and fine aggregates from natural sources for concrete. Bureau of Indian Standards, New Delhi

IS12269, I. S. D (2003) Specification for 53 grade ordinary Portland cement. Bureau of Indian Standards, New Delhi

James J, Subba RM (1986) Reactivity of rice husk ash. Cem Concr Res 16(3):296–302

Mehta PK (2002) Greening of concrete industry for sustainable development. Concr Int 24(7):23–28

Naik TR, Moriconi G (2005) Environmental friendly durable concrete made with recycled materials for sustainable concrete construction. In: Malhotra VM, Sakai K (eds) Sustainable development of cement concrete and concrete structures. Proceedings of International, symposium. Toronto, Canada, pp 485–505.

Prameetthaa J, Bharatkumar BH, Iyer NR (2015) Investigation on micronized biomass silica as a sustainable material. Cem Concr Compos 60:25–33

Rodríguez de Sensale G (2006) Strength development of concrete with rice-husk ash. Cem Concr Compos 28(2):158–160

Saraswathy V, Song HW (2007) Corrosion performance of rice husk ash blended concrete. Constr Build Mater 21(8):1779–1784

Suraya Hani A, Rahman IA, Saman HM (2015) Enhancing the performance of recycled aggregate concrete using micronized biomass silica. In: InCIEC 2014. Springer, Singapore, pp 65–77

Tangchirapat W, Buranasing R, Jaturapitakkul C, Chindaprasirt P (2008) Influence of rice husk–bark ash on mechanical properties of concrete containing high amount of recycled aggregates. Constr Build Mater 22(8):1812–1819

Turner LK, Collins FG (2013) Carbon dioxide equivalent (CO2-e) emissions: a comparison between geopolymer and OPC cement concrete. Constr Build Mater 43:125–130

Worrell E, Price L, Martin N, Hendriks C, Meida LO (2001) Carbon dioxide emissions from the global cement industry. Ann Rev Energy Environ 26(1):303–329

Zhang MH, Malhotra VM (1996) High-performance concrete incorporating rice husk ash as a supplementary cementing material. ACI Mater J 93(6).