

Review

Microbial Remediation: A Promising Tool for Reclamation of Contaminated Sites with Special Emphasis on Heavy Metal and Pesticide Pollution: A Review

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Abstract: Heavy metal and pesticide pollution have become an inevitable part of the modern industrialized environment that find their way into all ecosystems. Because of their persistent nature, recalcitrance, high toxicity and biological enrichment, metal and pesticide pollution has threatened the stability of the environment as well as the health of living beings. Due to the environmental persistence of heavy metals and pesticides, they get accumulated in the environs and consequently lead to food chain contamination. Therefore, remediation of heavy metals and pesticide contaminations needs to be addressed as a high priority. Various physico-chemical approaches have been employed for this purpose, but they have significant drawbacks such as high expenses, high labor, alteration in soil properties, disruption of native soil microflora and generation of toxic by-products. Researchers worldwide are focusing on bioremediation strategies to overcome this multifaceted problem, i.e., the removal, immobilization and detoxification of pesticides and heavy metals, in the most efficient and cost-effective ways. For a period of millions of evolutionary years, microorganisms have become resistant to intoxicants and have developed the capability to remediate heavy metal ions and pesticides, and as a result, they have helped in the restoration of the natural state of degraded environs with long term environmental benefits. Keeping in view the environmental and health concerns imposed by heavy metals and pesticides in our society, we aimed to present a generalized picture of the bioremediation capacity of microorganisms. We explore the use of bacteria, fungi, algae and genetically engineered microbes for the remediation of both metals and pesticides. This review summarizes the major detoxification pathways and bioremediation technologies; in addition to that, a brief account is given of molecular approaches such as systemic biology, gene editing and omics that have enhanced the bioremediation process and widened its microbiological techniques toward the remediation of heavy metals and pesticides.

Keywords: heavy metals; pesticides; effects; bioremediation; mechanism

1. Introduction

Bioremediation is the utilization of living entities for the alleviation of hazardous effects of pollutants that are undesirable for living sustenance. Various living organisms have the

capability to remediate pollutants; fungi, bacteria, algae and their oxidative biocatalysts are instrumental in recycling recalcitrant biomolecules and xenobiotics [1,2]. Additionally, plants are involved in eliminating pollutants naturally, transgenically or in relationship with rhizosphere microbes [3,4]. Microorganisms are interconnected with all living beings and play a vital part in biogeochemical cycling, thus forming the basis of a functional ecosphere [5]. Environmental factors such as temperature, moisture, humidity, oxygen, pH and nutrients have a part in modulating the activities of microbes [6]. The variability in environmental factors from one area to another has led to the vast diversification of microorganisms and their proficiencies [7].

Nutrient demand is one reason organisms use xenobiotic compounds (e.g., a source of carbon and nitrogen) [8]. However, they cannot solely depend on xenobiotics for growth and thus need supplementary C and N sources. Then, by co-metabolism, they can modify or degrade pollutants [9]. Microorganisms have diverse catabolic genes promoting their ability to process metabolic reactions for the breakdown and transformation of environmental contaminants into non-toxic ones ([10]). Currently, industries produce a wide range of organic and inorganic pollutants that have a detrimental impact on the environment and humans [11,12]. Pesticides, phenols and dyes are examples of organic pollutants, whereas hazardous heavy metals are examples of inorganic pollutants. Heavy metals affect cellular components and organelles and cause oxidative stress in cells and tissues [13]. Pesticide toxicity contributes to specific pathologies in living beings and also has harmful effects on other non-target organisms [14,15]. Indiscriminate utilization of pesticides can also cause negative impacts on biodiversity [16]. Technologies such as membrane filtration, ion exchange and chemical precipitation have been developed to eliminate heavy metal ions from contaminated areas, which convert heavy metal pollutants to their inactive states [17]. Similarly, conventional technologies for decontaminating the pesticide-polluted sites, such as landfilling, chemical alteration, incineration, composting, etc., are being employed [18]. Due to the various limitations of these methods, such as secondary pollution induction, low-density sludge generation, restricted activity in acidic environments, etc., researchers have focused their attention on using bioremediation technologies that are environmentally friendly, low cost and highly efficient in degrading pollutants [1]. The use of various microbial agents, such as bacteria, fungi, yeasts and algae, has received tremendous attention globally to remediate different matrixes contaminated with heavy metals and persistent organic pollutants [19]. Microbial remediation represents the most suitable and preferential technique in the current times of both environmental and economic crises, chiefly in developing countries. A study conducted by Blaylock et al. [20] reported a 50–60% cost reduction when bioremediation approaches were employed for the remediation of contaminated soils compared to other conventional methods. Several researchers have isolated and characterized promising microbial species for the clean-up of several industrial contaminants including heavy metals and pesticides [21–23]. It is noteworthy to mention here that microbial remediation is listed as one of the safest and most advantageous, reliable and efficient methods to eliminate toxic heavy metals and pesticides [1]. Microbes are omnipresent in an environment and can withstand harsh environmental conditions, thus, making them excellent for the degradation of toxic contaminants [24,25]. The high specificity of these entities towards a variety of contaminants, as well as to functions at lower concentrations, makes microbial remediation an exceptional choice. For example, the microbial biosorption of heavy metals has proven very sound for the remediation of polluted sites [21,26]. Furthermore, due to the small size of microorganisms, microbial biomass provides a larger surface area to volume for adsorption [27] compared to other remediation techniques, including phytoremediation. The complicated structure of microbes efficiently accelerates the bioadsorption of toxic contaminants. For example, the metal removal efficiency of biofilms is reported to be 91.71–95.35%, as compared to plankton cells, which remove 4.79–10.25%, as per the report on *Rhodotorula mucilaginosa* [28]. In addition, the exopolymeric substances present in biofilms make them natural stabilizers [29]. Another advantage of employing microbes for the remediation processes is their fast-multiplying ability, which can be stim-

ulated by the addition of adequate nutrition or engineered microbes [30] compared to phytoremediation. Systemic biology [SB] under diverse environmental pressures permits the assessment of microbial behavior at the community level. By employing the SB approach, important information for the metabolic engineering of microorganisms can be obtained for their augmented bioremediation proficiency. Omics approaches would assist in trailing the novel microbes for bioremediation. Multi-omics studies will help us develop novel hypotheses and theories for the bioremediation of the contaminated environment. This paper contains a broad and updated overview of the bioremediation of heavy metals and pesticides with major emphasis on bacterial, fungal and algal degradation. Moreover, major detoxification mechanisms along with the sophisticated molecular approaches to accelerate this environmentally friendly technique are also discussed.

2. Sources of Heavy Metals and Pesticides

2.1. Sources of Heavy Metals

Globally industrialization, on one side, is meeting the demands of the population and, on the other side, exposes the environment to a diverse variety of contaminants, including heavy metals. These contaminants have negative effects both on the environment and living organisms [31]. Furthermore, these contaminants enter the food chain and manifest a lethal influence on health [32], even at slightly larger concentrations than needed for normal metabolism [33]. Among the heavy metal contaminants, arsenic, chromium, cadmium, lead and mercury have received a lot of attention compared to other metals because their concentrations are higher than the safety threshold in many terrestrial, aquatic and aerial systems [34]. They are generally termed “toxic heavy metals” (THM) or the “most problematic heavy metals” [35]. Current data estimated that mercury, chromium, cadmium and lead—because of human interference—threaten approximately 66 million individuals all over the globe [34]. World Health Furthermore, contaminated drinking water affected approximately 150 million individuals globally [36]. The elimination of heavy metals from polluted areas is a complicated job for environmental conservation.

Heavy metals have their origin both from natural and human-made sources and can be found in the soil, water, atmosphere and biological organisms [37]. From anthropogenic sources, the generation of toxic pollutants is permanent and constant, while natural sources are affected by weather and usually do not cause pollution [38]. The chief sources of human-made pollution are factories, urbanization and agriculture [39,40]. The most important polluter industries include textiles, tanneries, fertilizers, galvanizing factories, metallurgic factories, varnishes, pharmaceuticals and pesticide-producing factories [41]. Metallurgical factories directly produce contamination during the extraction and processing of metals, while most industries also indirectly produce pollution [42]. Tanneries and textile factories produce highly contaminated effluents that reach water sources. For example, in southwestern Dhaka, heavy metal pollution was analyzed in tannery waste-affected water bodies, and the mean concentrations of As(VIII), Cr(VI), Cd(II) and Pb(II) were observed to have surpassed their threshold limits [43]. During mining processes, large quantities of waste rocks are produced containing heavy metals in low concentrations. These metals are carried by biological and chemical leaching to the ground and water areas and are ultimately incorporated into the food chain [44]. For instance, deposits of the Matylda catchment in southern Poland were polluted with Zn(II), Pb(II) and Cd(II) and their levels were approximately 1000 times more than normal values, possibly as a result of the emission of mine waters [45].

Agro-based activities also add to heavy metal contamination because of the continuous consumption of inorganic chemicals. Natural phosphate has impurities in the form of heavy metals. Heavy metals were detected in almost 200 phosphate fertilizers of west European countries and metals such as As(V/III), Cd(II), Ni(II), Cr(VI) and Zn(II) were found in higher amounts [46]. Likewise, pesticides also have impurities in the form of heavy metals. Moreover, numerous inorganic pesticides contain Hg(II), As(V/III), Cu(II) and Pb(II) as active elements. Pesticides containing Hg(II) and Pb(II) as their impurities

are banned because of their high toxicity, and there is still a possibility of finding them in agricultural areas due to the persistent nature of such elements [47]. Land irrigation is a common practice with industrial and municipal wastewater. The concentrations of heavy metals in wastewater are usually below their toxic levels; however, constant irrigation with wastewater results in heavy metal accumulation in soil [48]. Microorganisms are considered a potent source for the remediation of heavy metal polluted wastewater [49,50]. Electronic waste also contributes to heavy metal pollution. In China, surrounding areas of an e-waste recycling site were observed to be heavily intoxicated with Cu(II) and Cd(II), which surpassed their threshold levels [51]. Heavy metal generation from natural sources includes various types of rocks, mineral deposits, volcanic eruption, pathogenic processes and oceanic evaporation [52].

Studies have revealed that mining is a source of heavy metal contamination [53], and it has been reported that in China, mining has generated approximately 12 lakh ha of wasteland and is increasing with a figure of approx. 47,000 ha/year [54]. In gold mining areas of Shaanxi (Xiaoqinling), approx. 49.62% of all soil samples have high ecological risks [55]. In paddy soil, increased levels of Zn, Cu and Cd were observed in concentrations of 498, 502 and 3.92 mg/kg, respectively [56].

2.2. Sources of Pesticides

The global population has been exponentially increasing, and therefore so is the food need. The green revolution witnessed during the early 1960s made a significant contribution to the agricultural sector, and major credit goes to the introduction of agrochemicals (fertilizers and pesticides). The term pesticide generally covers a diverse group of insecticides, herbicides, fungicides, nematicides, defoliants and anti-rodent drugs [57]. In the current scenario, pesticides hold an indispensable place in our farming sector, keeping in view the food security issues, especially in underdeveloped nations. The total annual loss of food production to pests is approximately 45% [58]. Notwithstanding, excessive environmental degradation can be witnessed because of the consumption of such chemicals [59]. The most widely used pesticides worldwide mainly include atrazine, simazine, isoproturon, mecoprop and glyphosate [60,61]. Globally more than two million tons of pesticides are used annually, mainly including herbicides, insecticides and fungicides, which contribute 50%, 30% and 15%, respectively, along with other types (e.g., rodenticides and nematicides) [62]. The picture is more complicated in developing nations, which together account for one-quarter of global pesticide use.

Industries discharge large amounts of pesticide residues; these residual particles contaminate the aquatic ecosystem, which includes the aquatic biota along with sediments, causing deleterious effects on living beings through drinking water and food intake [63]. Presently, many aquatic environs are intoxicated with insecticides. In the last few years, scholars have noted insecticide remnants in drinking and groundwater around the globe. Another example of pesticides in groundwater is in China's Shanxi province, where contamination was revealed with many pesticides, such as endosulfan-sulfate, aldrin, etc., all are organochlorine pesticides [64]. The movement of pesticides in the soil depends on their solubility in water and the adsorption rate. Organic matter content defines the retention of pesticides in the soil, because of the presence of binding sites for these pollutants, particularly for compounds that are hydrophobic in nature. For example, different types of soils retain hexachlorocyclohexane isomers, which are generally governed by organic matter. Retention of contaminants by components of soil leads to reduced bioavailability and restricted degradation [65].

3. Effect of Heavy Metals and Pesticides on Human Health

3.1. Effect of Heavy Metals

Many heavy metals in biological systems perform various functions that are essential for the sustenance of all living beings. However, some heavy metals have no functional role in biological systems [66]. Biological systems are set with homeostatic mechanisms

that tend to regulate the heavy metal concentration and reduce the harmful effects because of excessive heavy metal levels. Toxicity due to heavy metals depends upon concentration, route and length of exposure. Heavy metals cause lethal effects on cells by hampering biological activities, mimicking vital elements, destabilizing biomolecules and generating reactive oxygen species [67]. Lead poisoning due to acute exposure from drinking polluted waters can cause hypertension, arthritis, renal dysfunction, vertigo and hallucinations. Chronic exposure can cause birth defects, allergies, autism, mental retardation, psychosis, hyperactivity, muscular weakness, paralysis or even death [68]. Mercury is one of the five toxic heavy metals known to react with other metals and form inorganic and organic mercury. Mercury is discharged into the environment by industrial activities. In most marine fauna, its concentration is higher with increased trophic levels [69]. Organic mercury, because of its lipophilic nature, is known to permeate across the cell membrane. Increased concentrations of mercury may cause damage to neurons leading to tremors, irritability, memory problems, etc. [70]. Chromium, another heavy metal, has many oxidation states, the most stable forms being Cr(VI) and Cr(III). Cr(III) has a role in glucose metabolism and is regarded as an essential supplement for humans and animals, while Cr(VI) is a pollutant and is highly toxic. The US Environmental Protection Agency has listed it as a human carcinogen. Cr(VI), in contact with broken human skin, can result in ulcer formation. Cr(IV) causes oxidative stress and DNA damage in human liver carcinoma cells and in Sprague–Dawley rats [71]. Heavy metals have a role in the development of various pathologies caused by free radical formation, which results in oxidative stress. The origin of radicals is mainly because of the redox reactions in transition elements such as Zn(II) and Cu(II) metals. Heavy metals such as lead, copper and mercury have been associated with atherosclerosis and schizophrenia [72]. Due to reactive oxygen species, oxidation of low-density lipoproteins and degradation of the vascular wall in the arteries occurs, causing plaque formation. Cadmium has been found to be associated with copper, lead and zinc ores and has been reported to cause damage to the skeletal system and has been associated with Itai-Itai disease [73]. Likewise, several other effects of cadmium toxicity have been reported, such as it causes a disruption in the steroidogenic pathway, glomerular and tubular damage and pneumonitis [74]. Copper is reported to cause various types of cancers and has a role in the BRAF signaling pathway of oncogenes [75]. As a consequence of heavy metal contamination, Alzheimer's disease, Menkes disease and cancers can be induced by heavy metal ion intake [76]. Therefore, pollution by heavy metals is a serious concern, and its remediation is of immense importance.

3.2. Effect of Pesticides

Currently, more than two billion tonnes of pesticides are utilized worldwide to enhance agricultural production [14]. However, these pesticides not only affect the target pests but also cause harm to non-target species, including human beings [14,77,78]. Moreover, when pesticides are applied, below one percent reach the target pests, while the rest pollutes the surrounding environment [14]. Due to the inadequate management system of pesticides, occupational hazards and risks to ecosystems are the main concern [79]. In addition, pesticide remnants retained in the product can directly affect the health of humans through the consumption of food products. Pesticides have resulted in occupational poisoning, with one million cases throughout the world. Evidence shows that pesticides have caused severe pathologies that affect the health of human beings [34]. One of the consequences of pesticide pollution is the rise in cancer rates, termed 'cancer villages' in which the frequency of mortality is considerably more than the average because of pesticide pollution over a large scale [80]. Toxic pesticides that affect most humans include organophosphorus (OP), organochlorine (OC) and carbamates (CB). These compounds act primarily on the nervous system causing disruption of nerve functioning [81]. Carbamates and organophosphorus pesticides act on the inhibitors of acetyl cholinesterase and cause increased levels of acetylcholine, affecting both the central and peripheral nervous systems, muscles, brain, liver and pancreas, whereas organochlorine pesticides can cause alteration

in the ion channels. OP, OC and CB insecticides trigger oxidative stress in cells due to disruption in mitochondrial function and thus affect the hormonal and neuronal status of the body [82]. There is a close relationship between pesticides and health disorders such as Parkinson's disease, Hodgkin's disease, non-Hodgkin's disease, respiratory, endocrine and reproductive disorders [83,84]. Some pesticides, such as paraquat, maneb and rotenone, result in oxidative stress due to reactive oxygen species (ROS) and cause neurodegenerative disorders [84]. Many pesticides act as endocrine disruptor compounds (EDCs), which cause interference with the endocrine system, resulting in distressing effects on the growth and reproduction of an organism. Organophosphorus pesticides cause acute intoxication in high doses; symptoms include bronchospasm, bradycardia, hypertension, gastrointestinal upset, sweating, urination, muscle weakness and central nervous system depression. In chronic conditions, symptoms include headache, blurred vision, nausea, dizziness, chest tightness, abdominal pain and vomiting [85]. Respiratory diseases have been reported in individuals who are exposed to carbamate insecticides. A positive association has been found between carbamate and the development of atopic asthma. This link was found for domestic, environmental and occupational exposures. Studies showed that carbamates and organophosphorus pesticides decrease expiratory flow rate [86]. Organochlorine pesticides also affect human well-being and the environment. Biodegradation of OC is difficult due to its high solubility in lipids and its persistent nature [87]. Harmful effects of organochlorine pesticides mainly include infertility, neurotoxicity, immunotoxicity and cancer of reproductive systems. Various epidemiological studies suggested a close association between pesticide contact and bronchial hyper-reactivity symptoms [88]. Many studies have revealed the role of OCPs in the risk of type 2 diabetes [89].

4. Microbial Remediation for Heavy Metal Cleanup

Heavy metals pollute the environment and endanger public health by contaminating the food chain and drinking water [90]. Various microorganisms, including bacteria, algae, fungi, etc., are employed to clean up sites contaminated with heavy metals. Table 1 depicts the ability of various microbial species taken up for remediation processes.

4.1. Bacteria

Interaction of heavy metals and microbes occurs through direct or indirect mechanisms depending on the microorganisms, metal species and the surrounding environment. Various factors, e.g., temperature, pH, nutrient sources and metal ions, are important for governing the bioavailability and mobility of heavy metals for transformation processes by microorganisms. Bacteria can survive in a wide range of environmental settings due to their small size, rapid growth rate and easy cultivation; they have been extensively utilized for remediation of lethal metals from the environment. Usually, heavy metals attach to the functional groups such as the amino, carboxyl, sulfate and phosphate groups present on the polysaccharide layers of the bacterial cell wall [26,91]. Generally, the heavy metal uptake potential of bacteria ranges from 1 mg/g to 500 mg/g. Mercury-resistant strains of *pseudomonas aeruginosa* absorb mercury ions selectively with a maximum uptake capacity of about 180 mg/g [26]. Due to a high affinity of cysteine for mercury ions, accumulation of mercury ions occurs by cysteine-rich proteins, which have abundant sulfhydryl groups. Pb (II) is known to be adsorbed by *Bacillus* sp. PZ-1 and *Pseudomonas* sp. 13 from wastewater [49,50]. *Arthrobacter viscosus*, both living and dead, can adsorb Cr(VI) and transform it into Cr(III) [92]. Biofilms of *Staphylococcus epidermis* eliminate Cr(VI) with high removal efficacy, followed by the Quindrich isotherm [93]. *Rhodobacter capsulatus* can adsorb Zn(II) with a maximum uptake capacity of nearly 164 mg/g, which follows Langmuir and Redlich–Peterson isotherms [94]. *Bacillus cereus* RC-1 absorbs Cd(II), with a biosorption capacity of about 31.95 mg/g and 24.01 mg/g for dead and living cells, respectively [95]. Extracellular polymeric substances (EPS) protect the microbes from toxic heavy metals by restricting their entry into the cell. EPS has anionic and cationic functional groups that help in accumulating heavy metal ions like cadmium, mercury, copper and cobalt

ions [96,97]. After adsorption, heavy metals can be converted into a different ionic state in the bacterial cell to reduce their toxicity. *Pseudomonas putida* SP1 is resistant to mercury and can absorb 100% of mercury in the marine environment and then reduce toxic Hg(II) to Hg⁰ by enzyme reductase, which envisages the bioremediation of mercury intoxication [98]. A study conducted by [99] reported a newly isolated strain (B9) of *Acinetobacter* sp. with the potential for detoxifying Cr released from the metal furnishing industry. The results further confirmed that the isolated strain was capable of tolerating up to 350 mg L⁻¹ of Cr (VI) and also showed a level of tolerance to Ni (II), Zn (II), Pb (II) and Cd (II). Furthermore, it was able to remove up to 67% of Cr (VI) (concentration, 7.0 mg L⁻¹) within 24 h [99]. In another study, almost 72 acidothermophilic autotrophic microbes were screened for their metal tolerance and biosorption potentiality. The results confirmed that the ATh-14 strain was efficient for solubilization of copper with 85.82% efficiency in the presence of 10⁻³ M multi-metal concentration within five days [100].

4.2. Fungi

Fungi have the ability to live in heavy metal contaminated sites and can adsorb heavy metal ions. The presence of chitin, polysaccharides, phosphate and glucuronic acid in/on cells of fungi play a vital role in the adsorption of heavy metals through coordination and ion exchange [101]. Different functional groups and various types of ionizable groups affect the ability to adsorb; furthermore, fungal strain specificity to the heavy metal ion also has an impact on the rate of adsorption. *Aspergillus niger* can eliminate Pb (II) with better biosorption ability [102]. *Termitomyces clypeatus* can detoxify Cr(VI) by adsorbing it onto the surface through phosphate, imidazole, hydroxyl, carboxyl and sulfhydryl groups [103]. *Saccharomyces cerevisiae* has been reported to eliminate Cu(II) from wastewater sources [104]. *Trichoderma* is known to detoxify Cd(II) [105]. Talukdar et al. [106] recently stated that in a liquid medium, more than 70% of Cr(VI) could be removed by *Aspergillus flavus* and *Aspergillus fumigatus*. *Aspergillus fumigates* was studied for its bioremedial potential in contaminated soils in the pre-urban area of Pakistan. The fungi showed good potential for the removal of lead (Pb), chromium (Cr), cadmium (Cd), nickel (Ni), copper (Cu) and zinc (Zn). Among these metals, the highest biosorption potential was shown for lead by *A. fumigatus* isolate K3 [107]. In another study, three fungal sp., namely *Penicillium citrinum*, *Trichoderma viride* and *Penicillium* sp., isolated from untreated tannery effluents were found to be highly tolerant against high concentrations, i.e., 100 mg/L of chromium IV and showed significant growth up to 250 mg/L indicating their good potential to tolerate and adapt to elevated chromium concentrations [108].

4.3. Algae

Studies have revealed that algae have a good ability to eliminate heavy metals from polluted sites. Algae produce various peptides that aid in heavy metal accumulation and defend against toxic heavy metal ions [109]. Studies on *Fucus vesiculosus* reported that it could adsorb Pb(II) with high potency [110]. *Cladophora fascicularis* is known to remediate Pb(II) from wastewater. The highest adsorption potential was estimated to be 198.5 mg/g and followed Langmuir and Freundlich isotherm models [111]. *Sargassum* marine algae has the potential to detoxify Cu(II) from aqueous solutions [112]. The adsorption properties of *Cystoseira crinitophylla* for copper were investigated, with a maximum adsorption ability of 160 mg/g [113]. *Saccharina fusiforme* and *Saccharina japonica* has detoxifying capacities for Zn(II), Cd(II) and Cu(II) [114]. *Desmodesmus* sp., a green microalgae, has been used for the remediation of Ni(II) and Cu(II) from wastewater [115]. A study conducted by Aslam et al. [116] revealed the role of microalgae in the accumulation of heavy metals from coal-fired flue gas in biomass and in medium. The results further communicated a higher accumulation of B, Mn, Cu and Zn under 3% CO₂ [116]. In a study carried out by Freitas et al. [117] on the biosorption of heavy metals by algal species in acid mine drainage (ADM) from coal mining in Brazil, it was found that algal biomass is able to accumulate heavy metals, more specifically Fe, which constitutes 6.3% of the biomass. Furthermore,

the study confirmed algal sp. chiefly, *Microspora*, *Mougeotia* and *Frustulia*, can survive in the AMD environment, with *Microspora* being the most dominant [117].

Table 1. Microbial remediation of heavy metal contaminated matrixes.

S. No	Type of Microorganism	Potential Microbial Species	Target Heavy Metals	References
1.	Bacteria	<i>Stenotrophomonas rhizophila</i> , <i>Variovorax boronicumulans</i> ,	Cadmium, Lead	[118]
		<i>Microbacterium oxydans</i>	Nickle, copper	[119]
		<i>Pseudomonas aeruginosa</i>	Mercury	[26]
		<i>Scopulariopsis brevicaulis</i>	Mercury	[120]
		<i>Pseudomonas putida</i> SP1	Mercury	[98]
		<i>Staphylococcus epidermis</i>	Lead	[121]
		<i>Pseudomonas</i> sp. 13	Lead	[50]
		<i>Penicillium chrysogenum</i> A15	Lead	[122]
		<i>Bacillus</i> sp., PZ-1	Lead	[49]
		<i>Bacillus cereus</i>	Chromium	[21]
		<i>Pseudomonas</i> sp., Cr13	Chromium	[123]
		<i>Staphylococcus epidermis</i>	Chromium	[121]
		<i>Staphylococcus epidermis</i>	Chromium	[93]
		<i>Arthrobacter viscosus</i>	Chromium	[92]
		<i>Bacillus</i> sp., <i>Pseudomonas</i> sp., <i>Serratia</i> sp., <i>Microbacterium</i> sp.	Chromium	[124]
		<i>Bacillus cereus</i> RC-1	Chromium	[95]
		<i>Stenotrophomonas rhizophila</i>	Zinc	[118]
		<i>Scopulariopsis brevicaulis</i>	Arsenic	[120]
		<i>Micrococcus luteus</i>	Copper, Lead	[125]
		<i>Bacillus firmus</i> TE7	Chromium, Arsenic	[126]
		<i>Bacillus cereus</i>	Mercury	[127]
		<i>Pseudochrobactrum saccharolyticum</i> LY10	Chromium	[128]
		<i>Ochrobactrum intermedium</i> LBr	Chromium, Copper	[129]
<i>Rhodococcus opacus</i>	Chromium, Copper, Lead	[130]		
<i>Bacillus methylotrophicus</i>	Chromium	[131]		
<i>Stenotrophomonas maltophilia</i> PD2	Copper	[132]		
2.	Fungi	<i>Penicillium chrysogenum</i> CS1	Chromium	[133]
		<i>Saccharina fusiforme</i> , <i>S. japonica</i>	Cadmium	[114]
		<i>Saccharomyces cerevisiae</i>	Lead	[104]
		<i>Penicillium chrysogenum</i> A15	Lead	[122]
		<i>Trichoderma</i> sp.,	Cadmium	[105]
		<i>Aspergillus clavatus</i> , <i>A.niger</i> , <i>Trichoderma viride</i> , <i>Penicillium glabrum</i>	Arsenic	[134]
		<i>Aspergillus flavus</i> CR500	Chromium	[135]

Table 1. Cont.

		<i>Termitomyces clypeatus</i>	Chromium	[103]
		<i>Clavulina humicola</i>	Cadmium	[136]
		<i>Rhizopus delemar</i>	Nickle, copper	[137]
		<i>Lentinus edodes</i>	Zinc, Cadmium, Mercury	[138]
		<i>Fusarium solani</i>	Chromium	[139]
		<i>Galerina vittiformis</i>	Chromium, Copper, Zinc, Cadmium, Lead	[140]
		<i>Fusarium sp. MMT1</i>	Chromium	[141]
		<i>Botrytis cinerea</i>	Copper, Cadmium	[142]
		<i>Neurospora crassa</i>	Copper, Lead	[143]
		<i>Lactarius scrobiculatus</i>	Cadmium, Lead	[144]
		<i>Amanita rubescens</i>	Cadmium, Lead	[145]
		<i>Rhizopus arrhizus</i>	Cadmium	[146]
		<i>Desmodesmus sp</i>	Copper, Nickle	[115]
		<i>Cystoseira crinitophylla</i>	Copper	[113]
		<i>Fucus vesiculosus</i>	Lead	[110]
		<i>Sargassum</i>	Lead	[112]
		<i>Cladophora fascicularis</i>	Lead	[111]
		<i>Ocillatoria angustissima</i>	Cobalt, Copper, Zinc	[147]
3.	Algae	<i>Spirogyra sp.</i>	Copper	[148]
		<i>Spirogyra sp.</i>	Lead	[149]
		<i>Caulerpa lentillifera</i>	Copper, Zinc, Cadmium, Lead	[150]
		<i>Fucus spiralis</i>	Nickle, Copper, Zinc, Cadmium, Lead	[151]
		<i>Oedogonium sp.</i>	Cadmium	[152]
		<i>Laminaria japonica</i>	Nickle, Copper, Zinc, Cadmium	[153]
		<i>Oedogonium hatei</i>	Chromium	[154]
		<i>Spirulina platensis</i>	Copper	[155]

5. Microbial Remediation for Pesticide Cleanup

Pesticides are extremely poisonous and can affect growth, reproduction, behavior, enzymes as well as DNA of the non-target individuals [14], so eliminating them from the environment is important. Bioremediation, an economically viable and efficient method of degrading them, employs living organisms to transform them into non-toxic forms and is typically dependent on the type of microorganism, environmental conditions and the type of pesticide [156]. Different microorganisms such as bacteria, fungi, etc., which are involved in the bioremediation of pesticide-contaminated sites are summarized in Table 2 and discussed below.

5.1. Bacteria

Bacteria are among the most explored microbial diversity studied for bioremediation processes. Scientists worldwide are harnessing indigenous bacterial strains, particularly those present at contaminated sites, for the remediation of organic pollutants. Bacteria possess a high capacity to degrade a variety of toxicants, chiefly because of the presence of catabolic genes and can withstand extreme environmental conditions. This strength of tolerance could be employed for the remediation of a variety of pesticides. For example,

Stenotrophomonas sp. G1 was screened from the industrial sludge and was capable of degrading methyl paraxon, methyl parathion (MP), phoxim and diazinon quite effectively, leaving no residue. Other pesticides, including chlorpyrifos, were degraded to 63%, profenofos about 38%, followed by triazophos about 34% at the concentration of 50 mg/L within 24 h of the evaluated period [111]. Studies have revealed that *Pseudomonas* sp. could effectively biodegrade chlorpyrifos generated from industrial effluents and agricultural soils [157]. Another investigation conducted by Pailan et al. [158] on the agricultural soils of India found that the bacterial species, mainly *Bacillus aryabhatai*, efficiently biodegrade parathion and chlorpyrifos at the concentration of 200 mg/L. From the agricultural soils of Cairo and Giza (Egypt), bacterial species, chiefly *Enterobacter* sp. and *Pseudomonas* sp., were able to break down chlorpyrifos and utilize the same primary sources of C and P. The same results were obtained for microbial strains, such as *Streptomyces olivochromogenes* and *S. chattanoogensis*, screened from the agro-soils of Southern Chile [159]. Fenitrothion is another pesticide effectively used in agricultural farms and golf courses. Several bacterial sp., such as *Arthrobacter* sp. and *Corynebacterium* sp., were found to be capable of degrading the same [160]. The typical example of OC pesticide mainly includes DDT and dieldrin [161]. Different species of *Bacillus*, *Arthrobacter*, *Pseudomonas* and *Micrococcus* have been confirmed to have potential for degrading these persistent pesticides [162]. One more investigation conducted by Jayashree and Vasudevan, [163] revealed that *Pseudomonas aeruginosa* was capable of degrading about 94% of endosulfan in the presence of Tween 80 surfactant at the optimal pH of 8.5. The end products of the degradation were endosulfan sulfate and endodiol, which were less toxic than their parent compound. Another study by Ozdal et al. [164] also found *P. aeruginosa* G1, *Stenotrophomonas maltophilia* G2, *B. atrophaeus* G3, *Citrobacter amolonaticus* G4 and *Acinetobacter lowffii* G5 strains capable of degrading endosulfan with the efficacy rate of 88.5%, 85.5%, 64.4%, 56.7% and 80.2%, respectively. Another study carried out by Fulekar [165] employed *Pseudomonas aeruginosa* in a scaled-up bioreactor for the bioremediation of fenvalerate. It was found that degradation of the contaminant was up to 62%, 86.9% and 100% in 50 mg/L, 25 mg/L and 10 mg/L in the presence of minimal salt medium (MSM). Likewise, in another study, bioremediation of chlorpyrifos was carried out by *Pseudomonas aeruginosa* (NCIM 2074) using a scaled-up technique. The study concluded that chlorpyrifos was completely degraded by *P. aeruginosa* at 10, 25 and 50 mg/L over a time duration of 1, 5 and 7 days, respectively. It was found that up to 50 mg/L concentrations of *P. aeruginosa* can bioremediate chlorpyrifos, but above 50 mg/L, it is inhibitory to organisms [166].

5.2. Fungi

Fungi are potential agents of remediation of organic contaminants and can stimulate bacterial activity by secreting exudates as an energy source, cooperating with them in the remediation process [167]. Pinto et al. [168] highlighted the capability of saprophytic fungi to respond and develop resistance, eventually metabolizing a wide variety of organic pollutants. A broad range of fungal sp., mainly belonging to *Aspergillus* sp., *Trichoderma* sp., *Cladosporium* sp., etc., have been studied for their role in pyrethroid degradation [169–172]. Wu et al. [173] studied the role of mycoremediation of manganese and phenanthrene and confirmed the potential of *Pleurotus eryngii* in the remediation process. In another study, the role of white rot fungi, typically *Pleurotus eryngii*, *Pleurotus ostreatus* and *Coprinus comatus*, were observed to be used in the degradation of Cd and endosulfan [174]. In another study, *Mortierella* sp. strains W8 and Cm1-45 resulted in 50–70% of endosulfan degradation in 28 days at 25 °C [175]. A co-metabolism of microbial consortium generally proves more effective in the degradation of pesticides, and the same results were observed during the breakdown of β -cypermethrin and 3-phenoxybenzoic acid by the mutual degradation of *B. licheniformis* B-1 strain and *Aspergillus oryzae* M-4 strain [176]. Another study reported that *Phanerochaete chrysosporium* sp. was capable of degrading isoproturon herbicide via solid-state fermentation [177]. Similarly, another experiment found that *A. niger* culture was able to tolerate a 400 mg/mL dose of technical grade endosulfan and completely degraded

the same within the incubation period of 12 days [178]. Investigations by Xiao et al. [179] revealed that *Phlebia lindtneri* GB-1027 and *Phlebia brevispora* TMIC34596, white rot fungi sp., were able to degrade seventy (70) and thirty (30) percent of DDT, respectively, within the twenty-one (21) days of incubation in a low-nitrogen medium. *Trichoderma harzianum* and *Trichoderma viride* was reported to degrade pirimicarb, and the efficacy rate was accelerated upon the addition of activated charcoal [180]. Hasan [181] also reported that *Aspergillus fumigates*, *A. flavus*, *A. terreus*, *A. sydowii*, *Penicillium chrysogenum* and *Fusarium oxysporum* fungal sp. were able to degrade organophosphates.

5.3. Algae

Algae, being the primary producers, have a tremendous ability to adapt and survive in any environment. Microalgae are utilized in the field of environmental bioremediation, where they mainly utilize organic pollutants as the source of nutrients, thus favoring biodegradation processes [182]. For instance, investigations by Ata et al. [183] reported that *Gracilaria verrucosa* was able to bioadsorb 2,4-dichlorophenoxyacetic (2,4-D) chiefly due to the presence of active hydroxyl, carboxyl and amine groups present on its cell wall. Several algal species can bioaccumulate a wide range of pesticides and subsequently degrade them [184,185]. Bioaccumulation of triadimefon has been reported by a green algal species, *Scenedesmus obliquus*. There was an increase in the production of triadimenol (its metabolite), indicating its simultaneous degradation [186]. An experiment carried out by Cáceres et al. [187] revealed that five Green algal sp., i.e., *Scenedesmus* sp., MM1, *Scenedesmus* sp., MM2, *Stichococcus* sp., *Chlamydomonas* sp. and *Chlorella* sp., were capable of degrading fenamiphos with the efficacy rate of 99% for the *Chlorella* sp. Microalgae cultivation for the purpose of contamination degradation is often accompanied by its co-cultivation with cyanobacteria. Numerous studies have supported the fact that this could enhance the biodegradation process [188]. The immobilization technique in the arena of bioremediation has received loads of interest. In this connection, the application of immobilized algae for the degradation of pesticides has shown promising results. For example, the immobilized algae, *Chlorella* sp., has been able to degrade butyltin chlorides with much higher efficacy [189]. Similar reports were furnished by Hussein et al. [190], revealing that *C. vulgaris* was able to remove 99% of carbofuran (20 µg/L) and 98% of pendimethalin (10 µg/L).

Table 2. Microbial remediation of pesticide-contaminated matrixes.

S. No	Type of Microorganism	Potential Microbial Species	Target Pesticides	References
1.	Bacteria	<i>Pseudomonas</i> sp.	Cypermethrin, Oxyfluorfen, Chlorpyrifos, Iprodione (fungicide), Atrazine	[191–194]
		<i>Stenotrophomonas</i> sp.	Tetrachlorvinphos, Chlorpyrifos	[195,196]
		<i>Micrococcus</i> sp.	Cypermethrin	[197]
		<i>Serratia</i> sp.	Tetrachlorvinphos	[198]
		<i>Sphingomonas</i> sp.	Oxyfluorfen	[199]
		<i>Enterobacter</i> sp.	Chlorpyrifos	[200]
		<i>Proteus</i> sp.	Tetrachlorvinphos	[198]
		<i>Synechocystis</i> sp.	Chlorpyrifos	[201]
		<i>Arthrobacter</i> sp.	Metamitron, Atrazine	[195,202]
<i>Yersinia</i> sp.	Tetrachlorvinphos	[198]		

Table 2. Cont.

		<i>Staphylococcus</i> sp.	Endosulfan	[203]
		<i>Bacillus</i> sp.	Lindane, Oxyfluorfen	[204,205]
		<i>Rhodococcus</i> sp.	Metamitron	[195]
		<i>Vibrio</i> sp.	Tetrachlorvinphos	[198]
		<i>Dyadobacter jiangsuensis</i> strain 12851	Chlorpyrifos	[206]
		<i>Acinetobacter</i> sp. and <i>Pseudomonas</i> sp.	Chlorpyrifos	[207]
		<i>Bacillus aryabhatai</i>	Chlorpyrifos	[158]
		<i>Streptomyces olivochromogenes</i> , <i>Streptomyces chattanoogensis</i>	Chlorpyrifos	[159]
		<i>Pseudomonas</i> sp.	Chlorpyrifos	[208]
		<i>Bacillus cereus</i>	Cypermethrin	[209]
		<i>Pseudomonas putida</i>	Organophosphate	[210]
		<i>Arthrobacter</i> sp. and <i>Corynebacterium</i> sp.	Fenitrothion	[160]
		<i>Bacillus subtilis</i> FZUL-33	Acephate	[211]
		<i>Trichoderma</i> sp.	Malathion	[212]
		<i>Pleurotus</i> sp.	Terbufos, Azinphosmethyl, Phosmet and Tribufos	[213]
		<i>Bjerkandera</i> sp.	Terbufos, Azinphosmethyl, Phosmet and Tribufos	[213]
		<i>Rhizopus nodosus</i> , <i>Aspergillus fumigatus</i> and <i>Penicillium citreonigum</i>	Diazinon	[214]
		<i>Aspergillus flavus</i>	Malathion	[215]
2.	Fungus	<i>Fomitopsis pinicola</i> and <i>Ralstonia picketti</i>	DDT	[216]
		<i>Phlebia lindtneri</i> , <i>Phlebia brevispora</i>	DDT	[179]
		<i>Pleurotus eryngii</i> , <i>P. Ostreatus</i> , <i>Coprinus comatus</i>	Endosulfan	[174]
		<i>Aspergillus niger</i>	Endosulfan	[178]
		<i>Trichoderma harzianum</i> , <i>T.viride</i>	Pirimicarb	[180]
		<i>Aspergillus</i> sp.	Carbofuran	[172]
		<i>Aspergillus</i> sp.	Carbofuran	[171]
		<i>Aspergillus</i> sp.	Carbofuran	[217]
		<i>Trichoderma</i> sp.	Carbofuran	[169]
		<i>Gracilaria verrucosa</i>	2,4-D (Herbicide)	[183]
		<i>Scenedesmus obliquus</i>	Triadimefon	[186]
3.	Algae	<i>Scenedesmus</i> sp., MM1, <i>Scenedesmus</i> sp., MM2, <i>Stichococcus</i> sp., <i>Chlamydomonas</i> sp., <i>Chlorella</i> sp.	Fenamiphos	[187]

6. Mechanism of Heavy Metal and Pesticide Remediation by Microorganisms

Utilization of indigenous microbes proficient in remediating heavy metals or GMOs (genetically modified organisms) to treat polluted environs by converting the toxic metals into non-toxic forms is a productive way of removing toxic contaminants from the environs and steadying the ecosystem [218]. Microorganisms are crucial in remediating heavy

metal-contaminated environs because they can withstand metal toxicity in several ways. Microorganisms have been exploited to precipitate, change the oxidation state or sequester a broad range of heavy metals [218,219]. Microbes employ various mechanisms for metal remediation: (a) The toxic metal sequestration by the components of the cell wall or by metal binding intracellular proteins and peptides such as metallothioneins and phytochelatins as well as bacterial siderophores. (b) Blocking metal uptake by altering biochemical pathways. (c) Enzymatic conversion of metals to harmless forms. (d) Usage of precise efflux systems to reduce the intracellular metal concentration [220]. Figure 1 depicts the mechanisms used in heavy metal remediation from polluted soils.

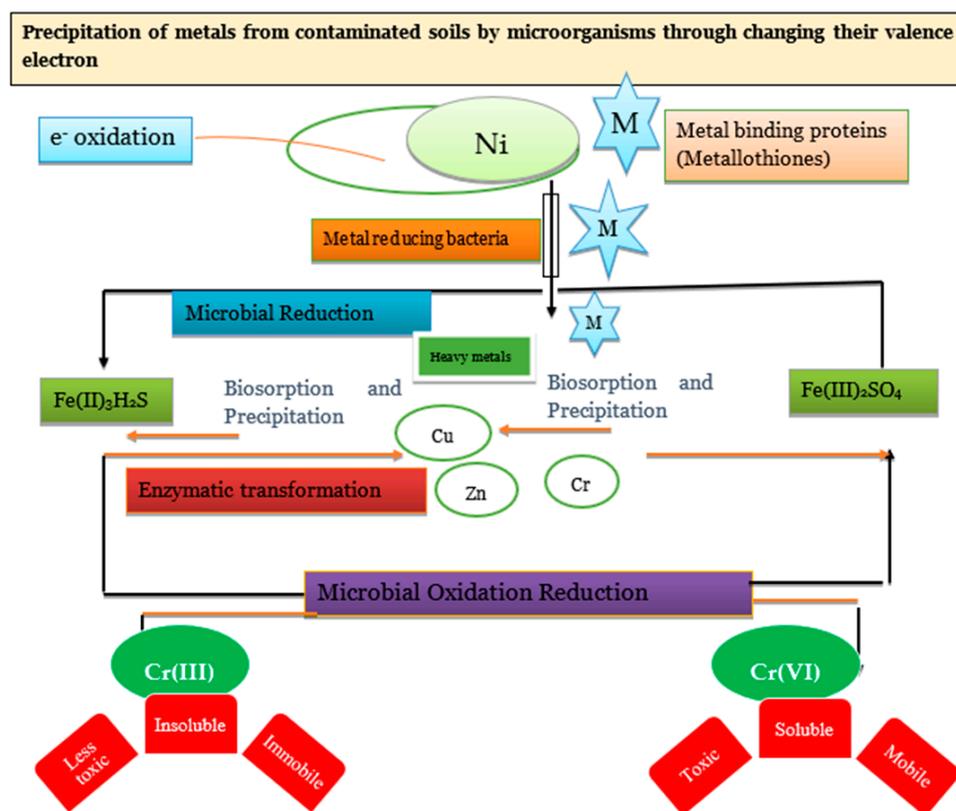


Figure 1. Mechanism of heavy metal removal from contaminated soils by microorganisms [220].

Microorganisms biodegrade pesticides or their by-products in the environs, or else they get accumulated in different environs and eventually become part of soil humus and enter the food chain [221]. The possibilities of environmental pesticide fate are depicted in Figure 2. The biodegradation mechanism and proposed pathway of degradation of some most important pesticides by microbes have been proposed. Chlorpyrifos, an OP pesticide extensively utilized to manage a variety of crop insects, is toxic to the environment and deadly to mammals. Hence, it is essential to eliminate it [222]. The degradation pathway for chlorpyrifos degradation by a bacterial strain, *Ochrobactrum* sp. JAS2, was proposed by Abraham and Silambarasan [223] (Figure 3). Initially, the parent pesticide, chlorpyrifos, was hydrolyzed to generate TCP (3,5,6-trichloro-2-pyridinol) and DETP (diethylthiophosphoric acid). TCP was then further converted by ring breakage, leading to its complete detoxification. However, in the case of DDT, an OC pesticide used extensively since the 1940s to eliminate malaria mosquitos [216], a consortium of brown-rot fungus *Fomitopsis pinicola* and the bacterium *Ralstonia pickettii* is capable of utilizing dichlorodiphenyltrichloroethane (DDT) as the main source of energy and carbon. DDT was first transformed to DDE and DDD by dichlorination at the trichloromethyl group, and then into DDMU (Figure 4). Similarly, in carbaryl pesticide, the carbaryl esterase enzyme broke an ester bond within the carbaryl pesticide at first, resulting in the formation of naphthol and methylamine. Then,

CYP450 and ligninolytic enzyme hydroxylate convert naphthol into 1,4 naphthoquinones in the presence of O₂. The quinones formed may be used as substrates for laccase and other peroxidases. Laccase and CYP450 break the ring of 1,4 naphthoquinone, forming benzoic acid, which may be further metabolized into CO₂ and water ([224] (Figure 5).

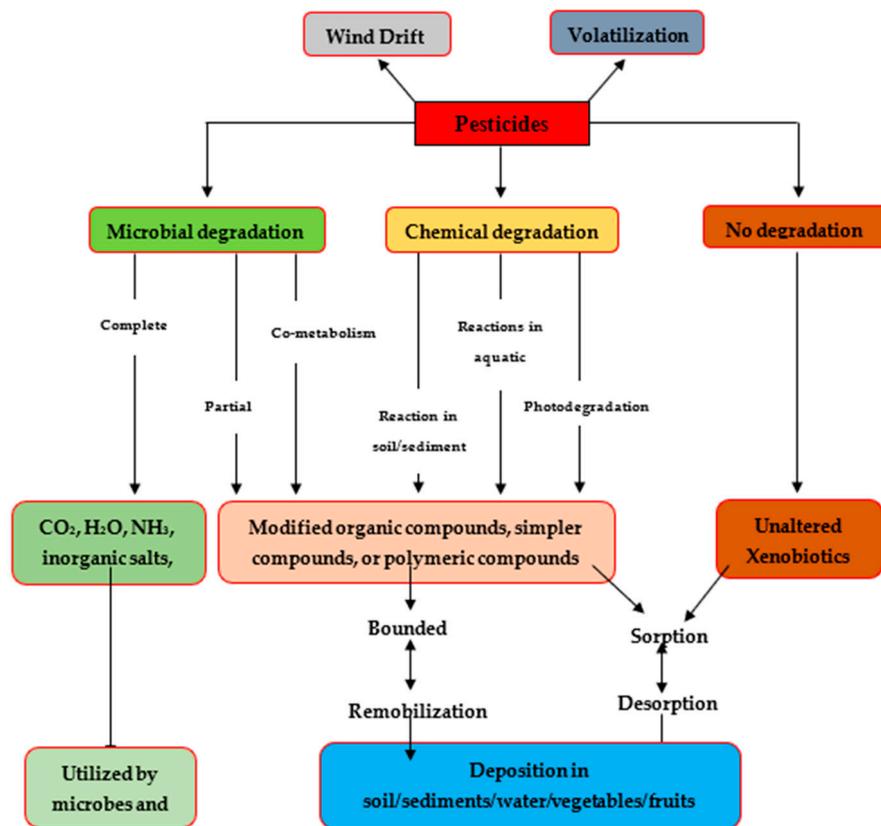


Figure 2. Possible fate of pesticides in the environment [78,225].

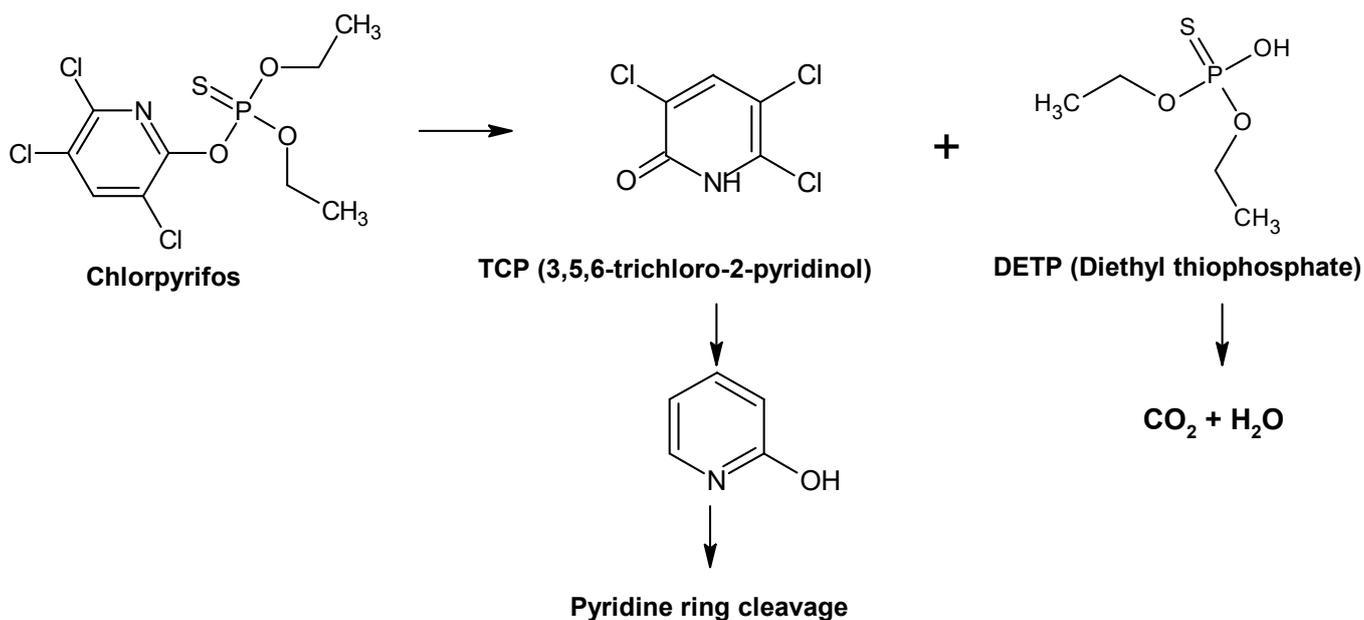


Figure 3. Proposed pathway for degradation of chlorpyrifos by *Ochrobactrum* sp. JAS2 [223].

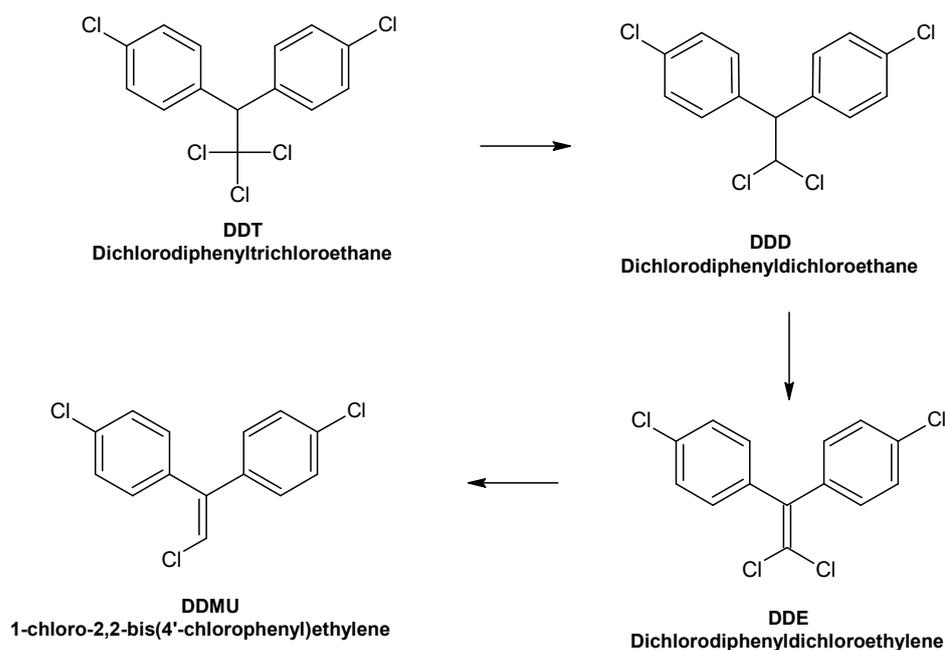


Figure 4. Proposed degradation pathway of DDT by the co-culture of brown-rot fungus *Fomitopsis pinicola* and the bacterium *Ralstonia pickettii* [216].

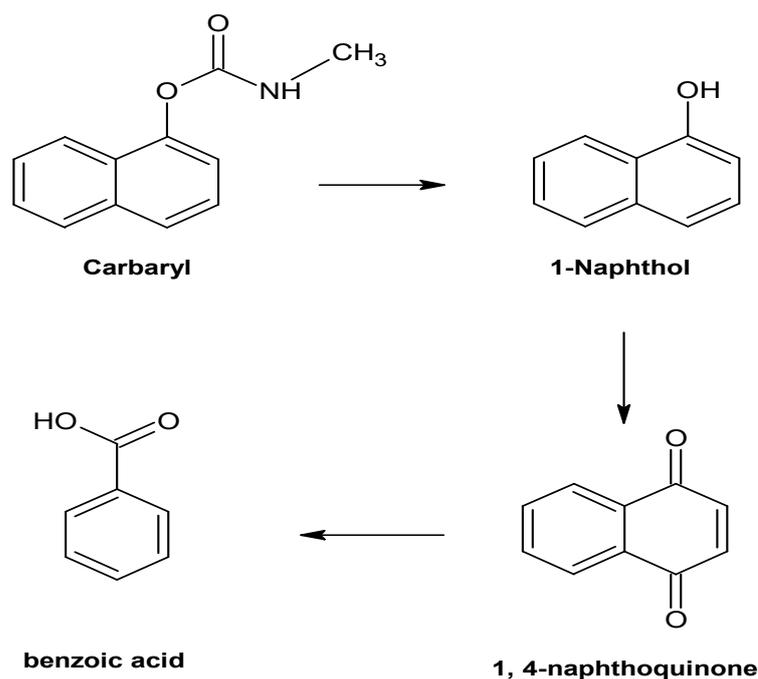


Figure 5. The proposed degradation pathway of carbaryl by *Xylaria* sp. [224].

7. Different Mechanisms of Heavy Metal Degradation

7.1. Bioremediation through Redox State Change

This approach involves the bioremediation of As, Hg and Cr (VI), consequently transforming them into other oxidative states for decontamination. Due to changed oxidative states, alteration in the mobility of heavy metals takes place [226]. Oxidation of toxic As to a less toxic form by the bacterial enzyme arsenite oxidase (aio), in several bacterial species, including *Achromobacter*, *Variovorax*, *Pseudomonas*, *Acaligenes*, *Agrobacterium*, *Sinorhizobium*, has potential for oxidation of As (III) [227]. Cr (VI), which is a toxic form of chromium, is transformed to nontoxic Cr(III) by Cr(VI)-reducing enzymes reported in

a wide variety of microorganisms, including *Bacillus*, *Pseudomonas*, *Serratia* and *Microbacterium* [124]. Cr(VI)-reducing enzymes, including oxidoreductases, lipoyl dehydrogenase and nitro reductase [228]. Various iron-reducing bacteria (IRB) and sulfur-reducing bacteria (SRB), e.g., *Acidithiobacillus* and *Desulfovibrio*, respectively, can oxidize Cr(VI) to Cr(III) [66]. Mercury, another heavy metal, can be both oxidized and reduced. Mercury oxide Hg(II), the more toxic form, is converted into Hg(0) (the less toxic form) by a number of different bacteria [229].

7.2. Biomineralization

In biomineralization, microorganisms activate mineral synthesis, and microbial cells are immobilized by coordination with the mineral phase. Microorganisms employ this bioremediation process under the influence of inorganic compounds and enzymes. Bacteria, such as *Staphylococcus epidermidis*, by carbonate mineralization, immobilize lead and chromium oxide Cr(VI) [121]. *Sporosarcina ginsengisoli* immobilized various elements by calcite, aragonite and vaterite biomineralization [230]. Heavy metals such as Pb and Cd have shown biomineralization abilities by utilizing various organic and inorganic metabolites [231]. Fungus, e.g., *Penicillium chrysogenum* CS1, was utilized for biomineralization of Pb and Cr (IV) isolated from cement sludge [133]. *Penicillium chrysogenum* A15 was able to biomineralize lead from contaminated sites [122]. *Bacillus subtilis* FZUL-33 aids in the biomineralization of Pb due to the formation of PO₄³⁻ released during the degradation of acephate [211]. Likewise, the degradation of organophosphate by *Pseudomonas putida* results in the formation of carbonated and phosphate minerals, which accelerates the precipitation of Cd [210].

7.3. Bio-Volatilization

In bio-volatilization, pollutants are converted into volatile compounds by the enzymatic activities of microbes. This treatment is suitable for Hg and As contamination along with all five toxic heavy metals. Other heavy metals, such as Sb, Tc and Bi, have been reported to be transformed into nontoxic compounds by bio-volatilization (Boriová et al. [120]. Bacterial enzymes such as arsenic methyltransferases transform As(V) into mono-, di- and tri-methylated species of As, which is then transferred to the atmosphere due to its volatile nature. Hg biovolatilization is executed by mercury(II) reductase (MerA) and mercurial lyase (MerB) present in Hg-resistant archaea and eubacteria [232]. *Scopulariopsis brevicaulis*, a filamentous fungus, was able to convert As(V) and Hg(II) to their nontoxic states [120]. Fungi, e.g., *Aspergillus clavatus*, *Aspergillus niger*, *Trichoderma viride* and *Penicillium glabrum*, were able to volatilize arsenic [134].

7.4. Biosorption

In biosorption processes, there is an attachment of pollutants to active components of the cell wall, which includes chitin, polysaccharides and cellulose derivatives and is achieved by chemical and physical binding with biofunctional groups. Biosorption involves Van der Waal's forces, electrostatic attraction, microprecipitation, covalent bonding and ion-exchange processes, which play a vital role in microbial–metal interactions [233]. Functional groups involve hydroxyl, carboxyl, amine, phosphonate and sulfhydryl on active cell components. For instance, Pb(II), Cr(IV) and Cu(II) can be accumulated by carboxyl and amine groups via proton displacement [234]. Some heavy metals have phosphoryl groups as their binding sites [235]. Co(II) and Cd(II) in *C. humicola* can be accumulated in polyphosphate, which has a vital role in the bioremediation of heavy metals [136]. Cultivated microalgae removed various pesticides, including atrazine, molinate, simazine, isoproturon, propanil, dimethoate, carbofuran, metolachlor, pyriproxin and pendimethalin, in the aqueous phase with the efficacy of 87–96% mainly through bioadsorption mechanisms [190].

7.5. Biodegradation

Biodegradation is a very important step in the bioremediation process. It involves the decomposition of heavy molecular weight pesticides into low molecular compounds. Effective microbial degradation involves various enzymes encoded by numerous genes. For example, the *lin* gene is present in various Gram-negative soil bacteria chiefly involved in the degradation of hexachlorocyclohexane [236]. Several other microbial genes, e.g., *atz*, *psb*, *ndo*, *tfd*, *puh*, *tri*, *trz*, etc., encode diverse groups of enzymes such as oxidoreductases (e.g., oxygenases, peroxidases, laccases) and hydrolytic enzymes (e.g., lipases, proteases, cellulases) involved in herbicide degradation [202,237]. The degradation of Atrazine was carried out by its transformation into cyanuric acid via hydrolytic or mixed oxidative-hydrolytic reactions [238]. It is pertinent to mention here that the co-contamination of heavy metals can affect pesticide degradation in many ways. Pesticide degradation can be accelerated in the presence of low metal concentrations, as metals may act as cofactors for various enzymes involved in the biodegradation of pesticides [239]. In some other cases, metals may antagonistically bind with some functional groups, thus hampering the degradation of pesticides.

8. Molecular Approaches for Bioremediation

With the advancement of techniques in the scientific world, the systemic biology and gene editing tools are employed in the remediation of heavy metals, acid drainage, xenobiotics, petroleum and persistent organic pollutants (POPs) [240–244]. Systemic biology provides details about the microbial organization [245]. Microbial systems respond differently under different conditions [246]. Interactions between microbes within a community are also examined with systemic biology approaches [247]. Systemic biology also helps in understanding the survival of microbes under different subsets of the environment like extreme pressure and temperature [248]. Omics, e.g., proteomics, transcriptomics, metabolomics and genomics, aid in the regulation of genes for bioremediation [249]). With the help of next-generation sequencing and high-throughput sequencing (HTS), genes responsible for bioremediation are resolved [250]. In gene editing, engineered nucleases or molecular scissors are used to manipulate DNA. The gene-editing process involves a guide sequence targeted against the sequence of the gene of interest. The guide sequence is a self-designed sequence that is complementary to the gene of interest. The gene-editing tool has a role in bioremediation, such as in xenobiotic elimination, transformation of more toxic to fewer toxic ones and the degradation of pesticides [240,251]. Among the gene-editing tools, ZFN, CRISPR-Cas and TALEN are of the foremost importance in meeting the demands of bioremediation [252,253]. These gene-editing tools have a role in the creation of double-stranded breaks in target gene sequences, repaired by non-homologous end joining (NHEJ) and homology-directed repair (HRD) [252,254]. TALEN and ZFNs utilize artificial restriction enzymes, which cleave target DNA sequences by the DNA binding domain of TAL effector-type and DNA binding domain of zinc finger-type, respectively [255,256]. These tools design microbes with complex genes and create microorganisms showing maximum traits [240,256]. Metaproteomics approaches to studying the adaptation of bacteria in contaminated sites like xenobiotics, heavy metals, persistent organic pollutants, etc. [257,258]. The whole genome of many bacterial strains has been sequenced, which are known for the bioremediation of pesticides, such as *Rhodococcus* sp. and *Pseudomonas putida*. Proteomics, metabolomics and transcriptomics study data led to the understanding of phenotypes and genotypes of particular microbes used for biodegradation. This helps in establishing a genome-scale model (GEM) that would display the best microorganisms for biodegradation and bioremediation of pesticides and various other xenobiotics. For example, in *Corynebacterium glutamicum* ATCC, 13,287 transcriptomic and metabolomic studies helped in understanding the metabolism of lysine [259]. Transcriptomic-proteomic analysis contributes to revelations of virulence networks of *Mycobacterium tuberculosis* [260]. In *R. jostii* RHA1, the capability of degrading intoxicants was promoted by molecular biology approaches. For example, in *R. jostii* RHA1, the degradation of phenol at the transcriptional level was analyzed by

real-time PCR [261]. There is no doubt that the contribution of molecular approaches in bioremediation will be of utmost importance.

9. Conclusions and Future Research

Nowadays, the remediation of pesticides and heavy metals by microbes is employed to detoxify pollutants for its remarkable advantages of low cost and high efficacy. However, there are certain constraints to its wide range of applications. As a molecular mechanism for detoxification, it requires further elucidation to amplify the accumulation of pesticides and heavy metals by microorganisms. Enzymatic detoxification, active export and intra/extracellular sequestration are the main resistance mechanisms of microorganisms to pollutants, which tends to diminish the toxicity of pesticides and heavy metals. There is an interconnection between microbial resistance systems to pollutants and their remediation capability. Better remediation may be attained by using microorganisms in combination with chemical and physical methods, which provides optimal surroundings for their activity. Usually, microorganisms can be resistant only to specific pollutants. Besides that, there are certain organisms that are pathogenic and cannot be extensively used. To overcome these challenges, the modification of genes is a preferred choice. With the help of genetic engineering, microorganisms displaying better remediation capability can be created as well as selected.

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