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Heavy Metals Bioremediation from Soil and Water using Microorganisms

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A vast array of microorganisms, especially bacteria, algae, yeasts, fungi and periphytons have received increasing attention for heavy metal removal and recovery due to their good performance, low cost and large available quantities. They are unlike mono functional ion exchange resins, contains variety of functional sites including carboxyl, imidazole, sulphydryl, amino, phosphate, sulfate, thioether, phenol, carbonyl, amide and hydroxyl moieties. They are cheaper, more effective alternatives for the removal of metallic elements, especially heavy metals from soil and aqueous solution. In this study, the application of microorganisms for removing heavy metal from soil and water, is introduced and described based on mechanisms such as assimilation, adsorption, and biodegradation. The advantages regarding the use of microorganisms to remove pollutants are discussed.

Key words: Bioremediation, Heavy metals, Microorganisms.

Throughout the world there is growing concern that the heavy metal content of soils are increasing as the result of industrial, mining, agricultural and domestic activities[1,2]. Three kinds of heavy metals are of concern, including toxic metals (such as Hg, Cr, Pb, Zn, Cu, Ni, Cd, As,Co, Sn, etc.), precious metals (such as Pd, Pt, Ag, Au, Ru etc.) and radionuclides (such as U, Th, Ra, Am, etc.)[3]. Unlike many other pollutants, heavy metals are difficult to remove from the environment. These metals cannot be chemically or biologically degraded, and are ultimately indestructible. The toxic effects of heavy metals result mainly from the interaction of metals with proteins (enzymes) and inhibition of metabolic processes. When accumulated in soils, heavy metals such as copper, cadmium, lead, zinc, nickel, mercury and chromium can be present in concentrations toxic to plants, animals, humans and aquatic life[4]. To date, the amount of heavy metals discharged into the environment keeps on increasing. Heavy metals like copper, mercury, chromium, cadmium, lead, nickel and zinc cause serious threat to environment, animals and human for their extreme toxicity[5].

Heavy metals enter the environment from natural and anthropogenic sources. The most

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significant natural sources are weathering of minerals, erosion and volcanic activity while anthropogenic sources include mining, smelting, electroplating, use of pesticides and (phosphate) fertilizers as well as biosolids in agriculture, sludge dumping, industrial discharge, atmospheric deposition, etc.[6]. Table 1 gives anthropogenic sources of selected heavy metals in the environment.

Health authorities in many parts of the world are becoming increasingly concerned about the effects of heavy metal (loid)s on environmental and human health and their potential implications to international trade. Heavy metals have adverse effects on human health and therefore heavy metal contamination of food chain deserves special attention. Many heavy metals and metalloids are toxic and can cause undesirable effects and severe problems even at very low concentrations[7]. Heavy metals cause oxidative stress (Mudipalli, 2008) by formation of free radicals. Oxidative stress refers to enhanced generation of reactive oxygen species (ROS), which can overwhelm cell's intrinsic antioxidant defenses and can lead to cell damage or death[8]. Furthermore, they can replace essential metals in pigments or enzymes disrupting their function[9]. Regarding their toxicities, the most problematic heavy metals are Hg, Cd, Pb, As, Cu, Zn, Sn, and Cr [10]. Out of these, Hg, Cd, Pb, and As are non-essential heavy metals while Cu and Zn are essential heavy metals (trace elements). Toxic heavy metals can cause different health problems depending on the heavy metal concerned, its concentration and oxidation state, etc. Table 1 gives harmful effects of selected heavy metals on human health.

Methods for removing metal ions from aqueous solution mainly consist of physical, chemical and biological technologies. Conventional methods for removing metal ions from aqueous solution have been suggested, such as chemical precipitation, filtration, ion exchange, electrochemical treatment, membrane technologies, adsorption on activated carbon, evaporation etc. However, chemical precipitation and electrochemical treatment are ineffective, especially when metal ion concentration in aqueous solution is among 1 to 100 mg L^{-1}, and also produce large quantity of sludge required to treat with great difficulty. Ion exchange, membrane technologies and activated carbon adsorption process are extremely expensive when treating large amount of water and wastewater containing heavy metal in low concentration, they cannot be used at large scale. Volesky (2001) summarized the advantages and disadvantages of those conventional metal removal technologies[11]. Biological treatment, based on living or non-living microorganisms or plants, offers the advantages, such as low operating cost and high efficiency[11]. The aim of this work is to present the state of the art of bioremediation investigation of heavy metals and to compare results found in the literature.

Heavy Metal bioremediation

Metals are not degradable. Unlike hydrocarbons, biodegradation of metals into innocuous CO_2 and water is not possible. Irrespective of the available reactions, the same metal will still be present but bacterial strains have been found to have the capacity to concentrate or remediate them into forms that are precipitated or volatilized from solution and hence less toxic and easily disposable. In other words, microorganisms can only alter the speciation of metal contaminants and convert them into non-toxic form[13].

The interaction of heavy metals with has become an increasing global interest because of its potential as a biotechnological method in removing heavy metals from polluted aqueous systems. The possibility of removing heavy metals saturated from its environment by using biomass may provide an economic method for removing heavy metals from wastewater. The removal of heavy metals from industrial waste water or recovery of heavy metals from their solutions as part of their mining by leaching can be accomplished by biotechnological methods that make use of microorganisms as sorbent[14].

To date, the use of technologies based on microorganisms has provided a wide range of useful and promising strategies to clean up many types of pollutants, such as cadmium, copper, lead[15] and microcystins[16] environmentally benign technologies based on microorganism and microbial aggregates, such as periphyton and hybrid bioreactors are now used to remove pollutants from aquatic systems[17]. These technologies have been applied worldwide and are generating an explosion of data on the pollutant removal process and improvements in removal efficiency, some of which will be discussed in this review. Little information however, focuses on describing the mechanisms of the technologies based on the use of microorganism and/or microbial aggregates to remove pollutants from water and wastewater.

Environmentally friendly technologies based on microorganisms are usually used to remove pollutants from aquatic ecosystems. The cell wall constituents play a key role in metal sequestering[18]. Such compounds possess numerous functional groups, including carboxylate, hydroxide, amine, imidazole, sulfate and sulfhydryl, with various charge distributions and geometries, so they can selectively bind certain metal ions. The main pollutant removal mechanisms include to ion exchange, adsorption, complexation, microprecipitation and crystallization processes occurring on the cell wall[19]. The various ways by which metal uptake can occur are depicted in Fig. 1. The most common microbial materials used for micro-remediation include bacteria, algae, yeasts and fungi. Most of these microorganisms are environmentally benign and can be isolated from natural ecosystems. The use of technologies based on microorganisms offers benefits of bioremediation not only reduces pollutant

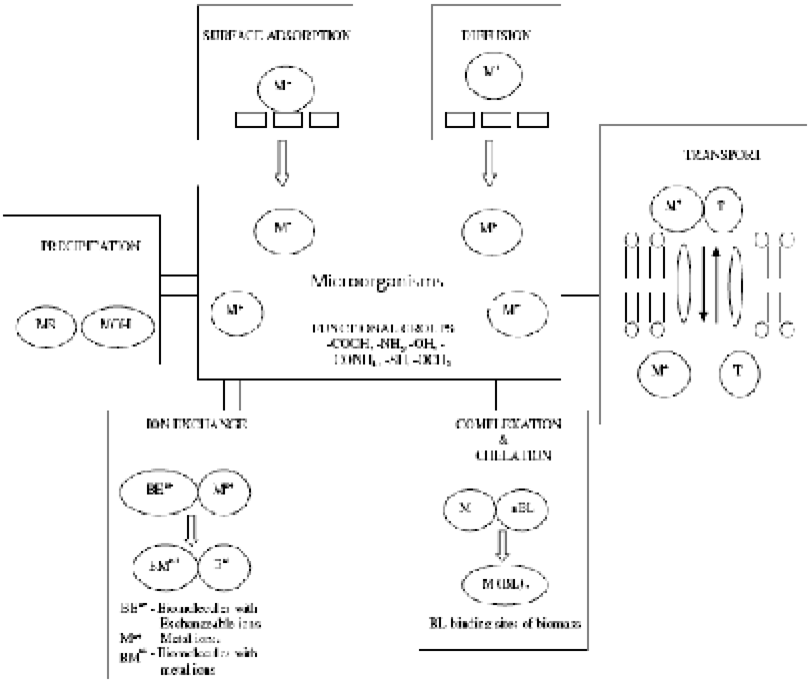

Fig. 1. Possible mechanism of bioremidation[21]

concentrations effectively but can also transform end-products into nontoxic, harmless and stable substances, e.g. carbon dioxide, water and nitrogen[20]. Compared with chemical processes, which often require high temperatures and pressures, biodegradation is an economical investment as evidenced by its low cost, low consumption, better results, more stable processes and simple operation.

Bacteria

Bacteria have a complex membrane containing abundant potentially active chemosorption sites in their walls. The cell surface hosts multiple functionally and structurally different proteins and they differ considerably from Gram-negative to Gram-positive bacteria[22]. This enables them to destroy the pollutants present in the contaminated sites[22, 23]. Amongst bacteria, *Bacillus*[24], *Pseudomonas*[25], and *Streptomyces*[26] acts as a potent metal biosorbents. The potential of Streptomyces strain to retain trace elements from polluted waters has recently been confirmed by many workers. Some

Table 1. Sources and toxic effects of heavy metals on human beings[12]

Metal	Source	Toxic effect
Lead	Electroplating, manufacturing of batteries, pigments, ammunition	Anaemia, brain damage, anorexia, malaise, loss of appetite, diminishing IQ
Cadmium	Electroplating, smelting, alloy manufacturing, pigments, plastic, mining, refining	Carcinogenic, renal disturbances, lung, insufficiency, bone lesions, cancer, hypertension, Itai–Itai disease, weight loss
Mercury	Weathering of mercuriferous areas, volcanic eruptions, naturally-caused, forest fires, biogenic emissions, battery production, fossil fuel burning, mining and metallurgical processes, paint and chloralkali industries	Neurological and renal disturbances, impairment of pulmonary function, corrosive, to skin, eyes, muscles, dermatitis, kidney damage
Chromium (VI)	Electroplating, leather tanning, textile, dyeing, electroplating, metal processing, wood preservatives, paints and pigments, steel fabrication and canning industry	Carcinogenic, mutagenic, teratogenic, epigastric pain nausea, vomiting, severe diarrhoea, producing lung tumors
Arsenic	Smelting, mining, energy production from fossil fuels, rock sediments	Gastrointestinal symptoms, disturbances of cardiovascular and nervous system functions, bone marrow depression, haemolysis, hepatomegaly, melanosis, polyneuropathy and encephalopathy, liver tumor
Copper	Printed circuit board manufacturing, electronics plating, plating, wire drawing, copper polishing, paint manufacturing, wood preservatives and printing operations	Reproductive and evelopmental toxicity, neurotoxicity, and acute toxicity, dizziness, diarrhoea
Zinc	Mining and manufacturing processes	Causes short term ‘‘metal-fume fever”, gastrointestinal distress, nausea and diarrhoea
Nickel	Non-ferrous metal, mineral processing, paint formulation, electroplating, porcelain enameling, copper sulphate manufacture and steam-electric power plants	Chronic bronchitis, reduced lung function, lung cancer

other common microorganisms used in the process of remediation (Table 2) are species of: *Achromobacter, Alcaligenes, Arthrobacter, Bacillus, Cinetobacter, Corynebacterium, Flavobacterium, Micrococcus, Mycobacterium, Norcardia, Pseudomonas, Vibrio, Rhodococcus* and *Sphingomonas*[27]. These microorganisms are used for the treatment of contaminated sites containing a wide variety of pollutants.

A study by Asku[28] demonstrated that *Chiarella vulgaris* and *Zoogloea ramigera* showed biosorption of copper through adsorption and formation of bonds between metals and amino or carboxyl groups of cell wall (polysaccharides). A study by Doyle *et al.*,[29] also indicates that heavy metal cations showed adsorption to the cells walls of Gram-positive bacteria. Many bacteria, such as Actinomycetes, Azotobacter and *Pseudomonas*, synthesizes different substances to capture Fe^{2+} which they require for their metabolic activity and biosorption[30]. A study by Jayashree *et al.*.[31] proved that the Pseudomonas acts as fuel eating bacteria which can degrade the hydrocarbons. *Pseudomonas syringae* also showed the formation of bond which play Important role in the accumulation of calcium, magnesium, cadmium, zinc, copper and mercury *Geobacter metallireducens* is a Fe (III) reducing organisms that can oxidize a variety of aromatic contaminants such as benzene and naphthalene and removes uranium (a radioactive waste) from drainage water in mining operations and from contaminated groundwater[32]. Soylak and co-workers developed a number of methods including bacteria as substrate for solid phase extraction procedures. *Bacillus thuringiensis* var. *israelensis* immobilized on Chromosorb 101 was used for the pre concentration and separation of Cd (II), Co (II) Cr (III), Mn (II), Ni (II) and Pb (II) in environmental samples[33].

Yeasts

Yeasts are readily available source of biomass which shows the ability to resist under unfavorable environment. The cell walls of yeast include a large number of complex organic compounds and their polymers, such as glucan (28%), mannan (31%), proteins (13%), lipids (8%), chitin and chitosan (2%) [45]. Different cell components allow different charge distributions and geometries, providing to yeast the possibility of binding different elements. The research over the last ten years involving the use of yeast for heavy metal biremidation is detailed in Table 3.

As observed in Table 3, the most representative microorganism employed for biosorption from the yeast group is *S. cerevisiae.* In our opinion it is because this substrate is easy to obtain and cheap. Other species have been employed like *Debaryomyces hansenii* and *Candida tropicalis*[46]. Within the *Saccharomyces* genus, other species besides cerevisiae have been employed like *S. carlsbergensis*[47]. For Cd^{2+} preconcentration, Menegário *et al.* immobilized *S. cerevisiae* in agarose gel as a binding agent for diffusive gradients[48]. Mapolelo *et al.*,[48] employed a simpler configuration with a commercial preparation of baker's yeast, strains, with 90% of cell viability[49]. *S. cerevisiae, D. hansenii* and *C. tropicalis* strains were tested for Cd^{2+}, Cr^{3+}, Cr^{6+}, Cu^{2+}, Pb^{2+}, and Zn^{2+} preconcentration[50]. A study by Kujan *et al.*,[51] also showed that *Candida utilis* biomass can conveniently be used for cadmium biosorption from aqueous solutions. There are some yeast like *Rhodotorula mucilaginosa* which is efficient in bioadsorption of lead and are also known to accumulate free and complexed silver ions by metabolism dependent and independent processes[52]. A comparative study was made by Ksheminska *et al*.[53] on the sensitivity of yeast *Pichia guilliermondii* to Cr (III) and Cr (VI) as well as on the uptake potential of Cr. The results indicated accumulation of Cr (III) and Cr (IV) by Pichia sp. and also showed increase in Cr tolerance by the addition of riboflavin.

Algae

The term "algae" refers to a large and

Table 2. Heavy metal bioremediation by Bacteria

Bacteria	Metal	Reference
Bacillus subtilis	Cu, Cd	[34]
Streptococus pyogenes	Hg CH_3 Hg+	[35]
Bacillus thurigesis israelensis	Cu, Fe, Zn	[36]
Bacillus sphaericus	Cr, Cr	[37]
Geobacillus	Cd, Ni	[38]
Bacillus sphaericus	Ni, Ag	[39]
Corynebacterium glutamicum	Arsenate	[40]
Escherichia coli	Cu, Zn, Fe, Ni and Cd	[41]
Escherichia coli	Fe, Co, Mn and Cr(III)	[42]
Agrobacterium tumefacients	Fe, Co, Mn and Cr	[43]
Bacillus thurigesis israelensis	Cd, Pb, Mn, Cr, Ni, Co	[33]
Bacillus subtilis	Cu, Cd	[34]
Streptococus pyogenes	Hg, CH_3 Hg+	[35]
Bacillus thurigesis israelensis	Cu, Fe, Zn	[36]
Bacillus sphaericus	Cr, Cr	[37]
Geobacillus	Cd, Ni	[38]
Bacillus sphaericus	Ni, Ag	[39]
Corynebacterium	Arsenate	[40]
Escherichia coli	Cu, Zn, Fe, Ni and Cd	[41]
Escherichia coli	Fe, Co, Mn and Cr	[42]
Agrobacterium tumefacients	Fe, Co, Mn and Cr	[43]
Bacillus thurigesis israelensis	Cd, Pb, Mn, Cr, Ni, Co	[33]

diverse assemblage of organisms that contain chlorophyll and carry out oxygenic photosynthesis. Algae present some characteristics turning them into interesting options to be employed as biosorbents like growing in large quantities with relative easiness and simple handling[71] along with low cost production[72]. The cell walls of brown algae contain alginic acid (10–40%), fucoidan (5–20%), and cellulose (2–20%), being the carboxylic groups the most abundant acidic functional group[73]. Red algae cell walls' contain agar, carrageenan, xylans, lectin, and cellulose, while the cell walls of green algae contain mainly pectic substances and cellulose[73]. Zoe *et al*. established that the functional groups responsible of metal retention by Chlorella vulgaris were hydroxyl and ether[57]. Uptake of metals by living microalgae occurs in two steps. The first step is independent of cell metabolism and involves "adsorption" onto the cell surface (physical adsorption) afterwards these ions are transported slowly into the cytoplasm known as chemisorption[74]. The second step is dependent on cell metabolism and involves absorption or intracellular uptake of heavy metals. Many studies have showed that various metals such as Pb, Cu, Cd, Co, Hg, Zn, Mg, Ni and Ti are sequestered in polyphosphate bodies of algae and perform two functions i.e. storage and detoxification of metals[75]. Due to its role in sequestration of heavy metals by algal cell wall, these are considered as an ideal source of multifunctional polymers[76]. Algae are also known for effective removal of nitrogen from soil or water through the process of absorption and store it as biomass. As the time passes, the biomass decomposes and releases the nitrogen back into the soil (ammonia or urea) or atmosphere (N_2O), where it may be recycled or lost[77].

Accumulation of Cd and Zn was recorded with alga *Scenedesmus obliquus*, it also showed enhanced absorption with increased concentration of phosphorus in the media, where Se accumulation was found to be inhibited. Metals such as Cu, Pb, Cd and Co are also accumulated by *Cladophora glomerata* and *Oedogonium*

Table 3. Heavy metal bioremediation by yeast

Yeast	Metal	Reference
Saccharomyces cerevisiae	Cr	[50]
Saccharomyces cerevisiae	Sb	[54]
Saccharomyces cerevisiae	Pd	[55]
Saccharomyces cerevisiae	Cd	[50]
Saccharomyces cerevisiae	Cd, Cr, Cu, Pb, Zn	[46]
Debaryomyces hansenii,	Cd, Cr, Cu, Pb, Zn	[48]
Saccharomyces cerevisiae	Cr	[56]
Saccharomyces cerevisiae	Cd	[57]
Saccharomyces cerevisiae	As	[58]
Saccharomyces cerevisiae	Mo	[59]
Saccharomyces cerevisiae	As	[60]
Saccharomyces cerevisiae	Cd and Cd-metallothionein	[61]
Saccharomyces cerevisiae	Sn	[62]
Saccharomyces carlsbergensis	Fe, Co, Cr	[63]
Saccharomyces carlsbergensis	Zn, Cu, Cd	[64]
Saccharomyces cerevisiae	Sb	[65]
Saccharomyces cerevisiae	Pt, Pd	[66]
Candida utilis	Cd	[51]
Hansenula anomala	Cd	[67]
Rhodotorula mucilaginosa	Zn and Cd	[68]
Rhodotorula rubra	Hg	[69]
Streptomyces sp.	Pb	[26]
Saccharomyces cerevisiae	Cu, Zn, Cd, Pb, Fe, Ni, Ag, Th, Ra, U and Hg	[70]

rivulare. *Spirogyra hatillensis* a fresh water filamentous alga showed continuous uptake of Ni, Cr, Fe and Mn from aqueous solution[75]. The algae are significantly efficient in treating more than one problem at a time, which is not possible by conventional process of chemical treatment. The phycoremediation shows advantage over other chemical methods as the removal of algal mass from the treated effluents is easy and economic.

Fungi

The importance of metallic ions to fungal metabolism has been known for a long time [86]. The presence of heavy metals affects the metabolic activities of fungal cultures, and can affect commercial fermentation processes, which created interest in relating the behavior of fungi to the presence of heavy metals. Most fungi have a cell wall consisting largely of chitin and other polysaccharides[87]. The fungal mycelia secrete various extracellular enzymes and acids that break down the lignin and cellulose. The key to mycoremediation is to determine the right fungal species to target a specific pollutant

Penicillium can remove a variety of heavy metal ions from aqueous solutions, such as Cu, Au, Zn, Cd, Mn, U and Th, see Table 5 *Penicillium italicum*[88], *Penicillium spinulosum*, *Penicillium oxalicum*[44] *Penicillium austurianum*[90], *Penicillium verrucosum*[91], *Penicillium purpurogenum*[92], *Penicillium canescens*[93], *Penicillium griseofulvum*[94], *P. austurianum*[95], *Penicillium chrysogenum*, etc. were reported to

Table 4. Heavy metal bioremediation by Algae

Algae	Metal	Reference
Ascophyllum nodosum	Pb, Cu and Cr	[24]
Anabaena inaequalis	Cr	[78]
Chlorella vulgaris	Cd, Ag, Cu, Th, Zn, Pb, Ni, Ra, Fe and U	[79]
Cladophora glomerata	Cu, Pb, Cd, Cr, Ni, Fe, Zn, Mn, Sr and Cs	[74]
Cyanobacteria	Pb, Hg and Cd	[80]
Nostoc sp.	Hg, Pb , Cd	[80]
Oedogonium rivulare	Cr, Ni, Zn, Fe, Mn Cu, Pb, Cd and Co	[81]
Oscillatoria spp.	Cu, Pb, Cd and Co	[82]
Sargassum spp.	Pb, U, Cd, Ni, Zn, Cu and Cr	[83]
Scenedesmus obliquus	Cd and Zn	[84]
Spirogyra spp.	Ni, Cr, Fe and Mn	[82,24]
Spirulina spp.	Pb and Cd	[85]

Table 5. Heavy metal bioremediation by fungi

Fungi	Metal	Reference
Aspergillus tereus	Cr	[100]
Aspergillus niger	Pb, Zn, Cd, Cr, Cu, Ni and	[101]
Funalia trogii	Hg, Cd and Zn	[102]
Ganoderma lucidumk	Cr and Cu	[103]
Penicillium chrysogenum	Pb, Fe, Ni, Ra, Th, U, Cu, Zn, Ag, and Cd	[26]
Rhizopus sp.	Cr	[104]
Aspergillus niger, Mucor rouxii, Rhizopus arrhizus (living cells)	Au	[105]
Penicillium spp. (living cells)	AgCu	[105]
Penicillium, Aspergillus, Trichoderma, Rhizopus, Mucor, Saccharomyces, Fusarium (living cells)	Cd	
	Pb Pb	[105]
Aspergillus, Penicillium, Rhizopus, Saccharomyces, Trichoderma, Mucor, Rhizopus (living cells	Cu Cd Zn Th	
Phanerochaete chryosporium (living cells)	U Sr Cs La Cd	[106]

adsorb various metals.

Aspergillus niger is an important microorganism in biotechnological applications[96]. Waste biomass of *A. niger* from fermentative industry, is used to remove hazardous heavy metal ions, such as cadmium, lead, chromium, and copper from aqueous solution. As it produces organic acids, *A. niger* can be used to bioleach metals from mining ores. Yakout 2014, review various metal ions could be removed by *A. niger*[97]. Fungus *A. niger* 405 showed a good affinity for binding Cu^{2+}, Zn^{2+} and Ni^{2+} ions in single composition system, while in multi-component solution it occurred only for copper and zinc[98].

A waste fungal biomass containing killed cells of *A. niger* was efficiently used for the removal of toxic metal ions such as nickel, calcium, iron and chromium from aqueous solution. The adsorption capacity for various metal ions could be arranged as Ca > Cr (III) > Ni > Fe > Cr (VI)[99]. Non-living waste biomass consisting of *A. niger* attached to wheat bran was used as a biosorbent for the removal of copper and zinc from aqueous solution

Periphytons

Metal accumulation by periphyton has been relatively well investigated[107]. Recently reported their studies of copper exposure and its effects on the periphyton community in fluvial ecosystems, albeit under controlled conditions. Bere *et al.* (2012) found that Cr (III) and Pb (II), under field conditions, influence accumulation and toxicity of Cd (II) in tropical periphyton communities[108]. Concentration and speciation were observed to vary dynamically in a small stream during rain events. Analysis revealed that the Cd (II) content in periphyton closely followed Cd (II) concentrations in water, despite being in the presence of higher concentrations of Zn (II) and Mn (II). Decrease of the Cd (II) content in periphyton after the rain events was slower than its decrease in water and is suggestive of metal accumulation[109]. Many photosynthetic species such as the green alga *Chlamydomonas reinhardtii* also known to possess the immense capacity to absorb metals, and so have great potential for removing metals from waste waters[110].

Periphyton accumulate heavy metals by three main mechanisms[111]: adsorption in extracellular polymeric substances, cell surface adsorption, and intracellular uptake (or absorption). Metal uptake in periphyton by adsorption and absorption have been evaluated by measuring total and intracellular metal content[107]. Further research has revealed that inactive/dead microbial biomass also passively binds metal ions via various physicochemical mechanisms[112].

CONCLUSION

Overall, the application of environmentally friendly microorganisms in the prevention and control of environmental pollution is a promising prospect. The research findings discussed herein should encourage us to further investigate the mechanisms of pollutant removal and develop more effective and cost-efficient microorganisms to control aqueous environmental pollution.

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