



DIMINUTION OF HEAVY METALS IN INDUSTRIAL SOLID WASTE BY AN AMALGAMATION OF MYCO AND VERMI REMEDIATION

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ABSTRACT

Due to the development of Industrialization and urbanization, a wide variety of industrial and consumer products, by products and solid waste has been produced. The solid waste generated constitutes the hazardous substance which possesses certain impacts on humans and their environment. In that heavy metal pollution from industries are the serious environmental problems. Rapid development in industries in the last few decades resulted in the strenuous task for finding to manage the waste generated. These hazardous solid wastes have been formulated into reusable end product by the process of bioremediation. Bioremediation is a natural process, which involves the use of organism to remove or neutralize the toxic pollutant from the contamination site. This review focus on the toxic effects of heavy metals on the environment and on the human health as well as the possible bioremediation method of these metals using fungus and earthworm. In order to conserve the environment and resources, the biological remediation by both fungus and earthworm for heavy metals and their efficiency have been summarised in detail.

Indexing terms/Keywords

Solid waste, heavy metals, fungus, earthworm, Bio-fertilizer.

Academic Discipline And Sub-Disciplines

Chemical Engineering; Environmental studies.

TYPE (METHOD/APPROACH)

Bioremediation; Mycoremediation; Vericomposting.

INTRODUCTION

The production of solid waste is one of the serious environmental issues in developing countries, which needs special attention. The industrial sectors are the major potential for generation of hazardous waste such as heavy metals, cyanides, pesticides, complex aromatic compounds (such as PCBs) and other toxic chemicals. The Central Pollution Control Board (CPCB) estimated that, hazardous waste generated in the India to be around 6.23 million tones. Out of this, 49.55 % is recyclable, 6.67 % incinerable and remaining 43.78 % is disposable in secured landfills (CPCB, 2009). Twelve states of the country (Maharashtra, Gujarat, Tamil Nadu, Orissa, Madhya Pradesh, Assam, Uttar Pradesh, West Bengal, Kerala, Andhra Pradesh, Karnataka and Rajasthan) account for about 97 % of the total waste generation. Among these hazardous waste, the presence of heavy metals in the solid waste plays a major role in the contamination of the environment. These heavy metals are toxic even at very low concentrations such as arsenic, cadmium, chromium, copper, lead, mercury, nickel, selenium, silver, zinc *etc.* are carcinogenic and mutagenic in nature (Salem et al., 2000). In that some of the heavy metals are essential for living beings growth and optimum performance. Due to the industrial revolution, the concentration of several heavy metals has been increased in soil and water. They created the alarming situation for human life and aquatic biota. The contaminated water bodies and land need to be rectified to make them free from heavy metals and trace elements, in order to make the environment healthier for human beings. There are numerous techniques to remove these heavy metals, including chemical precipitation, oxidation or reduction, filtration, ion-exchange, reverse osmosis, membrane technology, evaporation and electrochemical treatment. But most of these techniques become ineffective, when the concentrations of heavy metals are less than 100 mg/L (Ahluwalia and Goyal et al., 2007). Most heavy metal salts are water-soluble and get dissolved in wastewater, so it cannot be separated by physical separation methods (Hussein et al., 2004). When the concentration of heavy metals is very low, which means that utilization of physico chemical methods are very low or ineffective. The treatments technologies convert the waste into immobilize toxic components, or reduce the quantity of the waste. Use of microorganisms for remediation purposes is sustainable remediation technologies to rectify and re-establish the natural condition of heavy metal contaminated environment. The metabolic potential of fungus can be utilized for the reduction of heavy metals in the environment and make hazardous substance into less hazardous form (Asgher et al., 2007; Haritash and kaushik, 2009). Implementation of fungi in the process of remediation is called Mycoremediation. In recent days, microbes like fungus, bacteria and algae have been effectively used as adsorbing agents for removal of heavy metals (Terres et al., 1998; Munoz et al., 2006; Munoz and Guieysse, 2006). The high surface to volume ratio of microorganisms and their capability to detoxify metals are among the reasons that they are considered as potential alternative to synthetic resins for remediation of heavy metal contaminated liquid and solid wastes (Kapoor et al., 1999; Magyarosy et al., 2002). So the fungus can be use for the reduction of heavy metals from solid waste from the industries. Then the treated solid waste will be converted into bio-fertilizer by the process of vermicomposting. Vermicomposting is an aerobic composting process in which certain varieties of earthworms can be used, it have the ability to break down organic materials. Earthworms mechanically break down compostable and partially decomposed materials by eating them, and biochemical decomposition occurs by the use of bacteria and chemicals in the



worms' digestive system (Rajiv Singh and Das, 2005). The use of earthworms in solid waste management has been termed as Vermistabilization (Neuhauser et al., 1988). The various industrial wastes which have been Vermicomposted and converted into nutrient rich manure include paper waste (Elvira et al., 1998, Kaur et al., 2010), textile mill sludge (Garg and Kaushik, 2005), guar gum industrial waste (Suthar, 2006), sugar industry wastes (Sen and Chandra, 2007), distillery sludge (Suthar and Singh), leather industry (Ravindran et al., 2008) and beverage industry sludge (Singh et al., 2008), agroindustrial sludge (Suthar, 2010), primary sewage sludge (Hait and Tare, 2011), tannery industries (Ravindran and Sekaran, 2011). Earthworm plays an important role in the indication of an ecosystem health and many studies have been found for the response of earthworm to the heavy metals (Ndegwa and Thompson, 2001). Earthworms have the ability to survive in many kinds of chemical contaminants including heavy metal (Nahmani et al., 2007). The reduction of heavy metal by vermicomposting is an enhancement of the natural process, which integrates earthworm and microbes, they play an very important role in accumulating heavy metals in the earthworm tissues. In the process of vermicomposting, it supposes that earthworms are useful to clean up the environment from various pollutants and also heavy metals (Spugeon and Hopkin, 1999a). Different types of composting earthworms such as *Eisenia fetida*, *Perionyx excavates*, *Eudrilus eugenia*, *Eisenia Andrei* and *Lampito mauritii* these are the epigeic earthworm used for the heavy metal remediation process (Pereira and Arruda, 2003). In this review some of the efforts to be made to use fungi in the reduction of heavy metals from industrial solid waste and the earthworm to be inoculated in the partially reduced heavy metal contaminated solid waste. The composting worm can be used to reduce the remaining heavy metal concentration and convert the industrial solid waste into nutrient rich bio-fertilizers.

2. SOURCES OF HEAVY METALS

Heavy metals occur in the natural environment through the process of both geogenic and anthropogenic. Due to the pedogenetic processes of weathering of parent materials, the heavy metal levels that are regarded as *trace* (<1000mg kg⁻¹) and rarely toxic (Kabata-Pendias and Pendias, 2001; Pierzynski et al., 2000). Compare to the process of both pedogenic or lithogenic activity, heavy metals source from anthropogenic activity have more mobility in nature (Kuo et al., 1983; Kaasalainen and Yli-Halla, 2003). The major source of anthropogenic activity include metal mine tailings, disposal of high metal wastes in improperly protected landfills, lead based paints, fertilizer, sewage sludge, compost, pesticides, coal combustion residues, petrochemicals, application of p fertilizers, disposal of domestic waste materials (Khan et al., 2008; Zhang et al., 2010; Basta et al., 2005).

2.1 Fertilizers

The first influence of human to the soil is done by the activity of agriculture (Scragg, 2006). The major source of heavy metals from the fertilizers are p fertilizer, they especially contain cd. Phosphate compound contains the wide range of heavy metals (Loganathan et al., 2008; Bolan, 2003; Wuana and Okiyeimen, 2011). Contamination of agricultural soil by cadmium is the sever environmental concern, because it reaches the food chain by the regular use of Cd-containing P fertilizers. The growth of plants not only depend on the macro nutrients (N, P, K, S, Ca, and Mg), they also depend on the micro nutrients. Enormous amount of fertilizers are frequently added to the agricultural field to provide plenty of N, P, and K for crop growth. The heavy metals such as cd and pb have been presented as impurities in the compound, that providing the elements N, P, K (Jones and Jarvis, 1981).

2.2 Pesticides

In recent days, more amount of pesticides are widely used in agriculture and horticulture. But these contains the substantial concentrations of metals. In many formulation of cu containing fungicides such as copper oxychloride and 'Bordeaux' mixture, and as a growth promoter in piggery and poultry units (Bolan et al., 2003; Wightwick et al., 2013). As a result of continuous use of Cu fungicides leads to the Accumulation of Cu in agricultural soils (Chopin et al., 2008; Heemsbergen et al., 2010; Hildebrandt et al., 2008; Wightwick et al., 2010). Due to enormous accumulation of Cu in soils, it leads the toxicity to plants and microbial communities, for instance, formation of bare sterile patches in orchards (van Zwieten et al., 2007; Zhou et al., 2011).

2.3 Metal Mining and Industrial Wastes

Mining and milling of metal ores coupled with industries have been the backbone of the economy of the many countries, but it leads to the wide distribution of metal contaminants in soil. During mining the waste are directly discharged into natural depressions, including onsite wetlands resulting in elevated heavy metal concentrations (Devolder et al., 2003). Particularly in mining and smelting of Pb and Zn have resulted in contamination of soil that poses risk to environment. Waste from various industries such as textile, leather processing, dying, paper manufacturing, petrochemicals from accidental oil spills or utilization of petroleum-based products, pesticides, and pharmaceutical facilities and are highly discharging theirs both heavy metal contaminated effluents and solid waste (Sumner, 2000).

3. EFFECTS OF HEAVY METALS TO THE ENVIRONMENT

Heavy metal contamination in soil may create hazards to humans and the environment through: direct ingestion or through contaminated soil, the food chain, drinking of contaminated ground water, phyto toxicity, reduction in land usability for agricultural production causing food insecurity, and land tenure problems. Pb, Cr, as, Zn, Cd, Cu and Hg are the most common heavy metals in the contaminated sites (USEPA, 1996). These heavy metals have the ability to reduce the crop production due to the risk of bioaccumulation and biomagnifications in the food chain and also in the ground water. The hazardous effects of heavy metals depend significantly on the chemical form and speciation of the metal. Once heavy metals are entered into the soil, heavy metals are adsorbed by initial fast reactions (minutes, hours), followed by slow adsorption process (days, years) and redistributed into different chemical forms with varying bioavailability, mobility, and toxicity (Shiowatana et al., 2001; Buekers, 2007).

3.1 Lead



Lead is a metal belonging to group IV and period 6 of the periodic table with atomic number 82, atomic mass 207.2, density 11.4 g cm⁻³, melting point 327.4°C, and boiling point 1725°C. It is bluish gray metal, usually found as combined with other elements such as sulphur (i.e., PbS, PbSO₄), or oxygen (PbCO₃) (USDHHS, 1999). The most common forms of lead that are released into the environment are ionic lead, Pb(II), lead oxides and hydroxides, and lead metal oxyanion complexes. The most stable forms of lead are Pb(II) and lead-hydroxy complexes. Pb accumulates in the body organs (i.e., brain), which may lead to poisoning (plumbism) or even death. The presence of lead causes the affects to gastrointestinal tract, kidneys, and central nervous system. Children exposed to lead are at risk for impaired development, lower IQ, shortened attention span, hyperactivity, and mental deterioration, with children under the age of six being at a more substantial risk. Adults usually experience decreased reaction time, loss of memory, nausea, insomnia, anorexia, and weakness of the joints when exposed to lead (NSC, 2009). Lead is not an essential element and it leads toxic to the living beings. Lead can cause serious injury to the brain, red blood cells, nervous system and kidneys (Baldwin and Marshall, 1999). Depending upon the level and duration of exposure, it causes the wide range of biological effects.

3.2 Chromium

Chromium is a first-row *d*-block transition metal of group VI B in the periodic table with the following properties: atomic number 24, atomic mass 52, density 7.19 g cm⁻³, melting point 1875°C, and boiling point 2665°C. Chromium does not occur in elemental form, it can be only available in compound. Electroplating industry, Tanning industry and the disposal of Cr contamination waste are the major source of Cr contamination (Smith et al., 1995). The two available form of chromium such as Cr(III) and Cr(VI). Majority of the contaminated sites have the form of Chromium (VI). Chromium(VI) is the more toxic form of chromium and is also more mobile. Cr(VI) is always toxic to living organisms and has been listed as a priority pollutant and a human carcinogen by the US Environmental Protection Agency. In vivo studies have exposed that Cr(VI) is approximately 100 times more toxic (Beleza, 2001) and 1000 times more mutagenic than Cr(III) (Czako-Ver et al., 1999). Soluble and un-adsorbed chromium complexes can leach from soil into groundwater. If the pH of the soil increases the leachability of Cr(VI) also increases. Most of Cr released into natural water is particle associated, however, and is ultimately deposited into the sediment (Smith, 1995). The major cause of is allergic dermatitis to humans (Scragg, 2006).

3.3 Zinc

Zinc is a transition metal with the following characteristics: period 4, group IIB, atomic number 30, atomic mass 65.4, density 7.14 g cm⁻³, melting point 419.5°C, and boiling point 906°C. About 70 mg kg⁻¹ of zinc can be available in crustal rocks (Davies and Jones, 1988), due to the anthropogenic activity, the concentration of the zinc is rising enormously. The discharge from industrial sources may cause the concentrations of Zn in drinking water to reach levels that can cause health problems. Zinc is an essential for human health. But in the above the certain concentration it may increase the acidity of waters. Some fish can accumulate Zn in their bodies, when they live in Zn-contaminated waterways. Due to the presence of zinc in soil, they can lead contamination to groundwater. Moreover plants also uptake Zn to their system.

3.4 Cadmium

Cadmium is located at the end of the second row of transition elements with atomic number 48, atomic weight 112.4, density 8.65 g cm⁻³, melting point 320.9°C, and boiling point 765°C. In its compounds, Cd occurs as the divalent Cd(II) ion. Cadmium is also present as an impurity in several products, including phosphate fertilizers, detergents and refined petroleum products. In addition, acid rain and the resulting acidification of soils and surface waters have increased the geochemical mobility of Cd, and as a result its surface-water concentrations tend to increase as lake water pH decreases (Campbell, 2006). The application of agricultural inputs such as fertilizers, pesticides, and biosolids (sewage sludge), the disposal of industrial wastes or the deposition of contaminants increases the total cadmium concentration in soils, and the bioavailability of this Cd determines whether plant Cd uptake occurs to a significant degree (Wegglar et al., 2004). Cadmium is very biopersistent but has few toxicological properties and, once absorbed by an organism, remains resident for many years. Due to the presence of cadmium, which affects the several enzymes in the body. It is believed that the renal damage that results in proteinuria is the result of Cd adversely affecting enzymes responsible for reabsorption of proteins in kidney tubules. Cadmium also reduces the activity of delta-aminolevulinic acid synthetase, arylsulfatase, alcohol dehydrogenase, and lipoamide dehydrogenase (Manahan, 2003).

3.5 Copper

Copper is a transition metal which belongs to period 4 and group IB of the periodic table with atomic number 29, atomic weight 63.5, density 8.96 g cm⁻³, melting point 1083°C and boiling point 2595°C. Copper is a very versatile heavy metal, which can be available in two redox states, Cu(II) and Cu(I). In higher concentrations it can cause anaemia, liver and kidney damage, and stomach and intestinal irritation. If the blood copper level is excess of 7.9 mg/ml have the effects of jaundice, renal dysfunctions, and toxic shock. The contamination of copper in drinking water is due the reason of Cu pipes, as well as additives agents added for the control of algae growth. The tracer metal contamination in soil, may pose both direct and indirect threats: direct, through negative effects of metals on crop growth and yield, and indirect, by entering the human food chain with a potentially negative impact on human health. Even a reduction in yield of crops by a few percent could lead to a significant long-term loss in production and income.

4. MYCOREMEDIATION AS AN EMERGING TECHNOLOGY

Mycoremediation is the use of fungus and its processes for the reduction of pollution. The removal of heavy metals pollutant using fungal biomass is easier and cheaper than the conventional or traditional adsorbent techniques using



activated carbon, coal or ion exchange (Ashida, 1965). The end products from this remediation process are non-hazardous. Fungi are able to tolerate and detoxify metals by numerous mechanisms including valence transformation, extra and intracellular precipitation and active uptake (Gadd, 1993; Ross, 1975). By means of mechanism produced in direct response to metal species, the organism has been the ability to survive metal toxicity is termed as metal resistance. Biological mechanisms implicated in fungal survival include extracellular precipitation, complexation and crystallization, decreased transport or impermeability, transformation of metals, biosorption to cell wall and pigments, efflux, intracellular compartmentation and sequestration (Gadd, 1990; Gadd, 1992; Mehra, 1991; Bai and Abraham 2003). The studies have been conducted to screen the filamentous fungi from metal polluted sites for their diversity, metal tolerance and their biosorption potential. Filamentous fungi are well recognized for their superior capacities to produce a wide variety of extracellular enzymes, organic acids and other metabolites, and for their capabilities to adapt the environmental constraints (Elander and Neway, 1989). The members of Deuteromycetes such as *Aspergillus*, *Penicillium* and *Trichoderma* species are known to produce numerous extracellular enzymes, which are put to good use in biotechnology (Bumpus et al., 1985). Similarly, the *Basidiomycetes* white rot fungi such as *Phanerochaete chrysosporium* are significant for their abilities to produce nonspecific ligninases and peroxidases which can be used to degrade pollutants in liquid effluents and in soils (Qazilbash, 2004). The living and dead cell of the fungus have the capacity to eradicate the heavy metals. Fungi are used as an economical and nonstop supplying biomass for removing the metal ions in polluted area (Kapoor and Viraraghavan, 1998). Some of the isolated fungi such as *Trichoderma autoviride*, *T. harzianum*, and *T. virens* were found as the good biosorption agent of heavy metal ions (Siddiquee et al., 2013). The selected strains of fungi are potential utilized in the research area due to its presence in high polluted area (Lopez Errasquin and Vazquez, 2003). The study reported that *A. niger* is able to grow on culture plates amended with heavy metals and showed five times enhanced inhibition than the growth of yeast (Prince et al., 2001). The resistance level of different strains of filamentous fungi such as *T. aureoviride*, *T. harzianum*, and *T. virens* are diverse for different concentration of heavy metals. In that the fungal strain *T. virens* strain T128 gave the highest tolerance ability for Ni_{3+} and Pb_{2+} in a 1200 mg/L concentration (Siddiquee et al., 2013). The maximum removal of heavy metals have been mainly depend on the accumulation and uptake capacity of the strain *T. harzianum*. The strain *T. virens* showed the highest tolerance and uptake capacity towards the metal zinc. The mycelium of *Rhizopus* is an excellent biosorbent towards lead, cadmium, copper, zinc and uranium (Volesky, 1994). *Mucorales* species is found as the excellent biosorbents (Remacle, 1990) and *Fusarium flocciferum* is used to remove cadmium and nickel from industrial effluents (Delgado et al., 1998). However, the use of fungus in the bioremediation is not only the cleaning process, but also protect the environment and biodiversity as well as allowing for the subsequent reuse (Ashida, 1965; Gadd, 1986; Brierley and Beierley, 1993). Fungi have a versatile group, they can able to adapt and grow under varying conditions of pH, temperature and nutrient availability as well as at high metals concentration (Ashida, 1965; Yazdani et al., 2010; Anand et al., 2006). Fungi are one of the most appropriate organisms used for bioremediation. Because, they can abide varies environmental and toxic conditions such as a higher concentration of metals levels, and lower pH condition also. They have the ability and capacity to bind with heavy metals to their cell walls which are enhanced the intracellular accumulation of those toxins. The ability of selected fungi strains towards remediation of heavy metals ions are evaluated by the bioaccumulations characteristics of the fungus towards the heavy metals. Many researcher found that *Phanerochaete chrysosporium* ability to grow in both solid and liquid environment to degrade a wide range of xenobiotic effectively D. L. Huang (Huang et al., 2006). Researchers reported that the initial compost have 70.5 % of lead (pb) was bound to the residual fraction in reactor C (contain spore suspension of white-rot fungus) and 58.7 % of residual fraction of pb in reactor B (not contain spore suspension of white-rot fungus), after 80 days of composting the exchangeable lead content in reactor C and reactor B was reduced to 0 % and 2.86 % respectively. Result showed that potential hazards of compost in reactor C were lower than the Reactor B, which indicates that composting with white rot fungus could control the phytotoxicity of pb in contaminated solid waste. The white-rot fungi can have the capacity to chelate with pb by the carboxyl, hydroxyl or other active functional groups on cell wall surface (Zeng et al., 2007; Li et al., 2004; Yetis et al., 2000) and white-rot fungi could improve the composting process (Huang et al., 2003). The fungus strains such as *Aspergillus* and *Penicillium* have shown potential for metal bioremediation (Izadpanah et al., 2009). The adaptation of fungi exposed to heavy metal ions has been examined to increase the tolerance of fungi (yang et al., 2009). Among the fungus strains researcher concluded that, *Penicillium simplicissimum* was the most tolerant species and showed high growth, even at high concentration (8000 ppm) of Zinc. The researchers revealed that *P. chrysosporium* are capable of accumulating metal ions in their cells by intracellular uptake, as many researchers validated, and can also be chelated with metal ions by the carboxyl, hydroxyl or other active functional groups on cell (including the dead cell) wall surface. After 60 days of incubation, the concentration of soluble-exchangeable Pb in B soil (contaminated soil with inoculums of *P. chrysosporium*) even dropped to 0mg kg^{-1} , while that in A soil (contaminated soil without inoculums of *P. chrysosporium*) and C (soil without inoculums of *P. chrysosporium*) soil remained 100.5 and 77.0 mg kg^{-1} , respectively. The result of this study showed the least toxicity of Pb to living organisms, the least stress from Pb on environment for the significant reduction of Pb in B soil by co-incubating the soil with *P. chrysosporium* and the added straws, compared with those in the control soils. All these results might be because the Pb ion was absorbed by the mycelia of *P. chrysosporium* and chelated by the humus formed in the incubation process (Baldrian, 2003). Researchers reported that the Fungal strains such as *Aspergillus niger*, *Aspergillus sp.*, *Fusarium sp.*, and *Penicillium sp.*, were tested for their tolerance against different concentrations of heavy metals ($NiSO_4$, $ZnSO_4$, $CdSO_4$, $Pd NO_3$). The concentrations (1, 5, 10, 15, 20, 25, 30, 35, 40 ppm) of heavy metals ($NiSO_4$, $ZnSO_4$, $CdSO_4$, $Pd NO_3$) were used for the selection of fungi. Moreover fungi have been widely used in bioremediation of industrially polluted soils and waters, specifically in the removal of hydrocarbons and heavy metals (Akhtar and Mohan, 1995; Khan, 2001; Potin et al., 2004). Several researchers have reported the use of *Aspergillus niger*, *Aspergillus sp*, *Penicillium sp* and *Fusarium sp* to remove heavy metals Cr, Zn, Ni, Pd and Cd and checked their tolerance ability to see their tolerance towards $CdSO_4$, $ZnSO_4$, $PdSO_4$ and $NiSO_4$ in the Soil (Gadd, 1990; Fourest et al., 1994; Bai and Abraham, 2001; Teskova and Petrov, 2004). Similar study showed that *Aspergillus niger* was better to grow or tolerance heavy metals as



compared to other fungi (Price et al., 2001). Result shows that *Aspergillus niger* and *Aspergillus sp.*, were more tolerant as compared to *Penicillium sp.*, and *Fusarium sp* (Zn > Ni > Pd > Cd). Tolerance of toxic metals is based on ionic species associating with the cell surface or extra cellular polysaccharides, proteins and chitins (Volesky, 1990). Our preliminary findings indicate that fungus such as *P. chrysosporium*, *Aspergillus niger*, *Aspergillus sp.*, *Fusarium sp.*, and *Penicillium sp* have heavy metal tolerance capacity and also it reduces the heavy metal concentration in the contaminated solid waste.

Table 1. Potential of fungus in heavy metal bioremediation

S. NO	Fungus	Heavy metals	References
1	<i>A. flavus</i>	Pb	(Seema Dwivedi et al., 2012)
2	<i>A. sydoni</i> ,	Cr(VI)	(Bishnoi et al., 2007)
3	<i>Aspergillus</i> <i>Luchuensis</i>	Cu, Cd	(El-Gendy et al., 2011)
4	<i>Aspergillus</i> <i>Nidulans</i>	As	(Maheswari and Murugesan, 2009)
5	<i>Aspergillus</i> <i>niger</i>	Cd, Pb, Zn, Cu, Ni, Cr, As	(Pal et al., 2010; Junior et al., 2003; Amini et al., 2009; Ahmad et al., 2005a; Kumar et al., 2011; Thippeswamy et al., 2012a; Faryal et al., 2007; Shoaib et al., 2012; Rao et al., 1993; Venkobacher, 1990; Joshi et al., 2011; Adeyemi, 2009)
6	<i>Aspergillus</i> <i>ochraceous</i>	Cr	(seshikala and Charya, 2012)
7	<i>Aspergillus</i> <i>Oryzae</i>	Cr	(Nasseri et al., 2002)
8	<i>Aspergillus</i> <i>terrus</i>	Pb, Cu, Ni, Cr	(Joshi et al., 2011; Shoaib et al., 2012; Varshney et al., 2010; Seshikalai and Charya, 2012)
9	<i>Aspergillus</i> <i>Tubingensis</i>	Cu, Cd	(El-Gendy et al., 2011)
10	<i>Aspergillus</i> <i>Ustus</i>	Zn, Cu	(Chandrakar et al., 2012)
11	<i>Aspergillus flavus</i>	Pb(II), Cu(II)	(Akar and Tunali, 2006)
12	<i>Aspergillus flavus</i>	Zn, Cu, Ni, Pb	(Chandrakar et al., 2012; Thippeswamy et al., 2012a; Shoaib et al., 2012)
13	<i>Aspergillus foetidus</i>	Cr(VI)	(Shankar Congeevaram et al., 2007)
14	<i>Aspergillus foetidus</i>	Cr	(Prasenjit and Sumathi, 2005)
15	<i>Aspergillus fumigatus</i>	Cu, Cd, Co, Ni, Pb	(Rao et al., 1993; Ramasamy et al., 2011)
16	<i>Aspergillus niger</i> ,	Cr(VI)	(Bishnoi et al., 2007)
17	<i>Aspergillus oryzae</i>	Cr(III)	(Shankar Congeevaram et al., 2007)
18	<i>Aspergillus parasiticus</i>	Pb(II)	(Akar et al., 2007)
19	<i>Aspergillus sp.</i>	Cd, Cr, Pb, Zn, Cu	(Kumar et al., 2011; (Khan et al., 1998; Zafar et al., 2007; Congeevaram et al., 2007; Sen and Dastidar, 2007; Sen and Dastidar, 2010; Tahir, 2012)



20	<i>Aspergillus species</i>	Cr, Ni, Fe, Zn, Pb	(Ramesh et al., 2014)
21	<i>Aspergillus niger</i>	Cd, Zn, Zn, Ag, Th, U	(Congeevaram et al., 2007; Gunasekaran et al., 2003; Ashok Kumar et al., 2011)
22	<i>Botrytis cinerea</i>	Zn(II), Pb(II)	(Tunali and Akar, 2006)
23	<i>brown-rot fungus lentinus edodes</i>	Cd	(Chen et al., 2008)
24	<i>Candida tropicalis</i>	Zn	(Akhtar et al., 2008)
25	<i>Candida sp.</i>	Cu, Zn, Fe	(Anaemene, 2012)
26	<i>Candida utilis</i>	Cr	(Pattanapitpaisal et al., 2001)
27	<i>Cephalosporium aphidicola</i>	Pb(II)	(Tunali et al., 2006)
28	<i>cerevisae</i>	Cd, Ni, Pb, Cr, Zn, Cu	(Damodaran et al., 2011; Huang et al., 1990; Thippeswamy et al., 2012b; Prakasham et al., 1998; Volesky, 1992)
29	<i>Cladosporium Resinae</i>	Cu	(Gadd and De Rome, 1988)
30	<i>Cladosporium sp.</i>	Zn, Cu	(Chandrakar et al., 2012; Khan et al., 1998)
31	<i>Clavispora</i>	Cd	(El-Gendy et al., 2011)
32	<i>Curvularia Lunata</i>	Cu, Cd, Cr	(El-Gendy et al., 2011; Seshikala and Charya, 2012)
33	<i>Dactylosporium sp.</i>	Cr	(Seshikala and Charya, 2012)
34	<i>Drechslera hawaiiensis</i>	Cu, Cd,	(El-Gendy et al., 2011)
35	<i>Drechslera Rostrata</i>	Cr	(Seshikala, and Charya, 2012)
36	<i>Fungicola</i>	Cd	(El-Gendy et al., 2011)
37	<i>Fusarium sp.</i>	Co, Cr, Cd, Ni	(Shaheen Zafar et al., 2007)
38	<i>Ganoderma carnosum</i>	Pb(II)	(Akar et al., 2007)
39	<i>Geotrichum sp.</i>	Co, Cr, Cd, Ni	(Shaheen Zafar et al., 2007)
40	<i>Gliocladium sp.</i>	Cu	(Tahir, 2012)
41	<i>Harzianum</i>	Ni, Cr	(Shoaib, 2012) (Sarkar et al., 2010)
42	<i>Hymenoscyphus ericae,</i>	Hg (II)	(Kelly et al., 2006)
43	<i>Metarrhizium Anisopliae</i>	Pb	(Ismail et al., 2005)
44	<i>Monacrosporium elegans</i>	Cu, Cd	(El-Gendy et al., 2011)
45	<i>Monilia sp.</i>	Co, Cr, Cd, Ni	(Shaheen Zafar et al., 2007)



46	<i>Neocosmospora vasinfecta</i> ,	Hg (II)	(Kelly et al., 2006)
47	<i>Neurospora crassa</i>	Pb, Cu	(Ismail et al., 2005)
48	<i>Neurospora crassa</i>	Pb(II), Cu(II)	(Kiran et al., 2005)
49	<i>Penicillium canescens</i>	Cd, Pb, As	(Say et al., 2003)
50	<i>Penicillium chrysogenum</i>	Cr, Ni, Cu,U, Th, Zn, Pb, Cd	(Tan and Cheng, 2003; Skowronski, 2001; Tsezos and Volesky, 1981; Niu et al., 1993)
51	<i>Penicillium Cyclopium</i>	Cu	(Ianis et al., 2006)
52	<i>Penicillium decumbens</i>	Cd, Ni, Cr	(Levinskaite, 2001)
53	<i>Penicillium digitatum</i>	Cd, Cu, Pb	(Galun et al., 1987)
54	<i>Penicillium duclauxi</i>	Cu, Cd	(El-Gendy et al., 2011)
55	<i>Penicillium lilacium</i>	Cu, Cd	(El-Gendy et al., 2011)
56	<i>Penicillium Notatum</i>	Cr	(Seshikala and Charya, 2012)
57	<i>Penicillium Pupurogenum</i>	Cr	(Say et al., 2003)
58	<i>Penicillium Simplicissimum</i>	Cd, Zn, Pb	(Fan et al., 2008)
59	<i>Penicillium Spinulosum</i>	Zn	(Townsley and Ross, 1985)
60	<i>Penicillium janthinellum</i>	Cr(VI)	(Bishnoi et al., 2007)
61	<i>Penicillium sp.</i>	Co, Cr, Cd, Ni	(Shaheen Zafar et al., 2007)
62	<i>Penicillium sp.</i>	Cr, Cd, Ni, Zn, Cu, Pb	(Ahmad et al., 2005a; Khan et al.1998; Dugal and Gangawane, 2012; Velmurugan et al., 2010; Tahir, 2012)
63	<i>Pestalotiopsis</i>	Cd	(El-Gendy et al., 2011)
64	<i>Pyrenocheta Cajani</i>	Cr	(Seshikala and Charya 2012)
65	<i>Rhizopus sp.</i>	Co, Cr, Cd, Ni	(Seshikala and Charya 2012)
66	<i>Saccharomyces</i>	Cd	(Thippeswamy et al., 2012b)
67	<i>Sarcinella sp.</i>	Zn, Cu	(Chandrakar et al., 2012)

68	<i>Talaromyces helicus</i>	Cu	(Romero et al., 2006)
69	<i>Trichoderma Atroviride</i>	Zn	(Yazdani et al., 2010)
70	<i>Trichoderma</i>	Cu	(Shoaib et al., 2012)
71	<i>Trichoderma Longbrachiatum</i>	Cr	(Joshi et al., 2011)
72	<i>Trichoderma Virde</i>	Pb, Ni, Cd, Cr	(Prasad et al., 2013; Levinskaite 2001; Joshi et al., 2011; Seshikala and Charya, 2012; Hala and Eman, 2009)
73	<i>Trichoderma sp.</i>	Co, Cr, Cd, Ni	(Shaheen Zafar et al., 2007)
74	<i>Trichoderma sp.</i>	Cu Pb Cr	(Khan et al., 1998; Vankar and Bajpai, 2007)
75	<i>Trichoderma viride,</i>	Cr(VI)	(Bajgai et al., 2012)
76	<i>Trichosporon Cutaneum</i>	Cr	(Bishnoi et al., 2007)
77	<i>Verticillium</i>	Cu	(El-Gendy et al., 2011)
78	<i>Verticillium Terrestre</i>	Hg (II)	(Kelly et al., 2006)
79	<i>white-rot basidiomycete, Phanerochaete chrysosporium</i>	Pb	(Huang et al., 2008)
80	<i>white-rot fungus</i>	Pb	(Guang Ming Zeng et al., 2007)

4.1 Mechanism Involved In Fungus

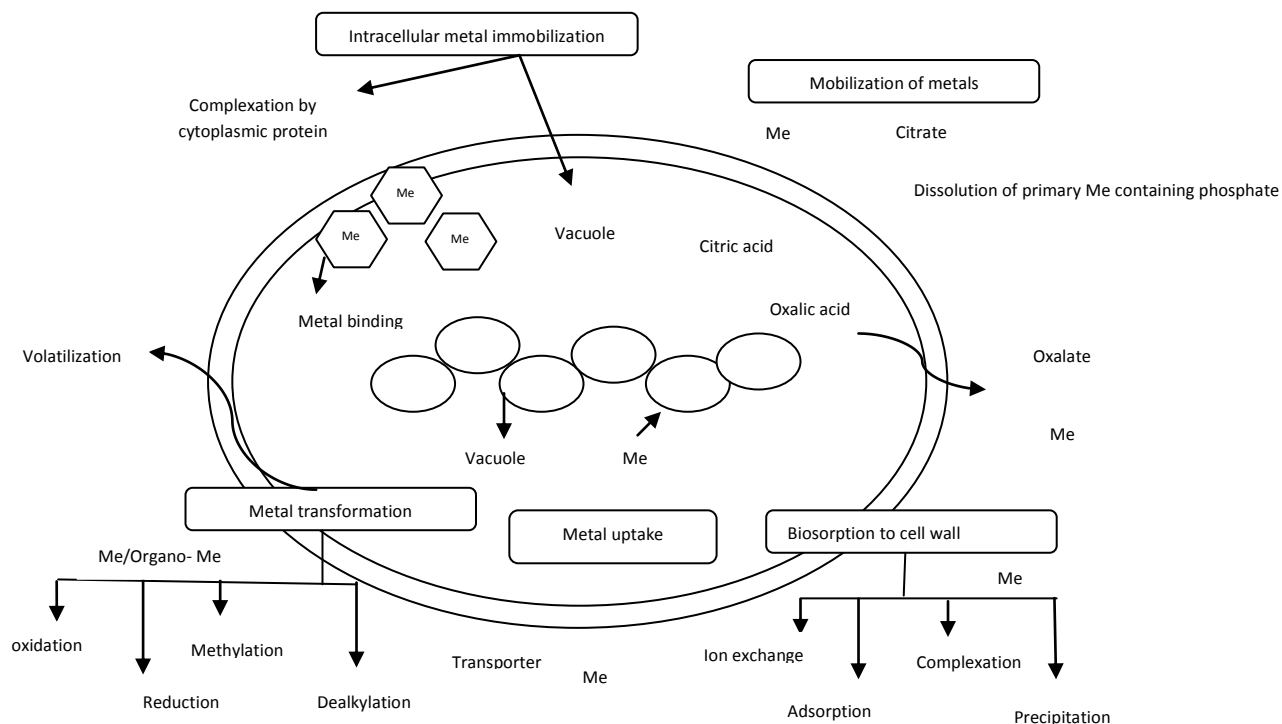


Fig 1: fungi-metal interactions (Gadd, 1993; Harms et al., 2011; Valls and de Lorenzo, 2002)



- The metals are mobilized due to the production and excretion of fungal products such as citric acid and oxalic acid. The citric acid acts as an efficient metal ions chelator and the oxalic acid interact with metal ions to produce insoluble oxalate, resulted from the dissolution of primary metals containing phosphate. The metal solubility may be increased by these organic acids by means of mycosphere acidification and production of metal-complex structure.
 - In the process of Bio-sorption, the metals are first interact with cell wall of the fungus, this cellular component play an important roles as a protective layer and barrier that control the uptake of toxic metals into the cell. A complex mechanism is involved in the Interaction of metal with fungal cell wall which includes several processes such as ion exchange, complexation, crystallization, adsorption and precipitation and also influenced by the biomass concentration and chemical behaviour of the metals.
 - Specific transporters are responsible for the uptake of metals. These transporters react with other type of metals. Carriers may consist of all the metabolically-coupled and H⁺- gradient driven transport system.
 - Two processes are involved in Intracellular metal immobilization, they are
 - Metallothioneins - vacuoles compartmentation and complexation by cytoplasmic protein
 - phytochelatins - a rich SH peptide .
- In the process of Metallothioneins, the metal-binding protein that can modulate the intracellular concentrations and bind both the essential metal such as Cu and Zn and inessential metal such as Cd. Fungal vacuole plays a major roles in molecular degradation, storage of metabolites, regulation of cytosolic concentrations of metal ions and detoxifies potentially toxic metal ions.
- Biotransformation of the metals in fungus is make possible through various chemical reactions such as oxidation, reduction, methylation and dealkylation. These are the major reactions which may lead to metal volatilization and reduce the metal toxicity. Metals may also transfer to the other parts of the fungi mycelium and plant symbionts by cytoplasmic vesicles and vacuoles.

5. ENHANCEMENT OF MYCOREMEDIATION BY VERMICOMPOSTING

Vermicomposting is one of the most effective biotechnology process, for the solid waste treatment. This process converting the solid waste into useful and environmental friendly end product. After the treatment of Mycoremediation, the remediated products can be undergone for vermicomposting. In the process of vermicomposting, the earthworm has been used for the conversion of urban, industrial and agro-industrial wastes to Biofertilizer. The earthworm acts as the blenders and they modify its biological, physical and chemical properties of the feed mixtures. Gradually decreasing the C: N ratios, increasing the surface exposed to microorganisms and further decomposition. Many researchers report that the earthworm could not tolerate the fresh industrial solid waste, so it can be spiked with different types of substrate to reduce the toxicity of industrial solid waste to the earthworm. Additional of at least 30% cow dung (on dry weight basis) was essential for the survival of the earthworm in the solid textile mill sludge (STMS) (Priya Kaushik and Garg, 2003). The study established that the potential of earthworm in devour of metals available in the sandy soil (Zorn et al., 2005). The mobility and solubility of the metals in soil may be affected by the microbial activity (Gadd, 1996). Microbes play an very important role in the immobilization of metals. It is well known that the earthworm activities increases the population of microbes and that the microbial communities are native to the earthworm species (Parle, 1963; Toyota and Kirmura, 2000). That the bacterial cells have the high ratio of surface area to volume, which provides strong capacity of adsorbing and immobilizing heavy metals (Beveridge and Schultze-Lam, 1995). It is well established fact that the earthworm activities increases the nitrogen in the cast through the microorganisms present in their gut (Ndegwa and Thompson, 2001). Many studies found that the final Vermicomposted product have the higher P and K concentration (Beveridge and Schultze-Lam, 1995). The final NPK content in the vermicompost is mainly dependent on the initial Nitrogen present in the feed material and the degree of decomposition (Benitez et al., 1999). The studies showed that textile mill sludge spiked with poultry droppings in different ratios using epigeic worm *Eisenia fetida*, shows the heavy metals (Fe, Zn, Pb and Cd) contents were lower in the final feed mixture than compared to the initial feed mixture (Crawford., 1983). During vermicomposting, earthworms maintain aerobic conditions in waste through burrowing, inverting and biochemical processes, which are improved by microbial decomposition of the substrate in the intestine of the earthworm (Garg and Priya Kaushik, 2005). Several reports are existing on the use of earthworms in composting sewage sludge and it has been recognized that epigeic forms of earthworm hasten the composting process with production of better quality of vermicompost as compared to traditional composting method (Majumdar et al., 2006). The report revealed the efficiency of *Eisenia andrei* (Bouche) in bioconverting paper-pulp mill sludge mixed with primary sewage sludge. The mixture at a ratio of 3:1 was a suitable medium for optimum growth and reproduction of the earthworms. The earthworms accelerated the mineralization of organic matter, favored the breakdown of structural polysaccharides and increased the humification rate (Ghosh et al., 1999). Solid paper-mill sludge mixed with sewage sludge in 3:2 ratio resulted in the highest growth rate and the lowest mortality of *Eisenia andrei*, whereas paper mill sludge mixed with pig slurry exhibited a high mortality (Elvira et al., 1996). The growth and reproduction of *Eisenia andrei* in mixed paper-pulp mill sludge and cattle waste found that, the number of earthworms increased between 22- and 36-fold and total biomass increased between 2.2- and 3.9-fold in different feed mixtures (Elvira et al., 1998). In the vermicomposting of activated sludge observed that approximately 1.0g worm could ingest 4.0g of activated sludge in 5 days (Hartenstein and Hartenstein, 1981). The textile mill sludge can be potentially useful as raw substrate in vermicomposting if mixed up to 30% with cow dung (Harms et al., 2011). *E. foetida* is an epigeic



earthworm species which lives in organic wastes and requires high moisture content, adequate amounts of suitable organic material and dark conditions for proper growth and development (Hartenstein and Hartenstein, 1981; Gunadi and Edwards, 2003; Gunadi et al., 2002; Chaudhari and Bhattacharjee, 2002). In order to utilize this species successfully in vermicomposting, its survival, growth and fecundity in different wastes should be known. Vermicomposting is the usual method that managed by earthworms and in addition to decomposing of organic waste, the availability of heavy metals decreases due to bioaccumulation of these metals and organo-complex formation during this process (Ghyasvand et al., 2008).

TABLE 2. HEAVY METAL REDUCTION EFFICIENCY OF VARIOUS EARTHWORM SPECIES

Species	Solid waste and Substrate	Duration of Experiment	Different Mixtures of substrate and solid waste	Reduction level of copper	Reduction level of Zinc	Reduction level of cadmium	Reduction level of chromium	Reduction level of Lead	Reference
<i>Eisenia fetida</i>	Solid Textile mill sludge (STMS) and cow dung (CD)	90 days	CD + STMS (g) 1000+0 900+100 800+200 700+300 600+400 500+500 400+300 300+700	(mg/kg) 19-24 19-19 18-19 22-25 23-25 19-19 26-27 30-31	(mg/kg) 196-150 186-115 191-169 183-158 186-118 190-164 201-169 190-119	DN	(mg/kg) 4-4 17-13 16-12 17-13 16-12 16-12 16-12 15-12	DN	(Priya Kaushik and Garg, 2003)
<i>Iranian Eisenia fetida</i>	Sewage Sludge (SS) and saw dust (SD)	60 days	SS + SD (kg) 7 + 1	(mg/kg) 380.1-215.2	(mg/kg) 390.7-206.2	(mg/kg) 17.4 – 8.6	(mg/kg) 108 - 38	(mg/kg) 184.4-75.6	(Shahmansouri et al., 2005)
<i>Australian Eisenia fetida</i>	Sewage Sludge (SS) and saw dust (SD)	60 days	SS + SD (kg) 7 + 1	(mg/kg) 380.1-225.8	(mg/kg) 390.7-219.9	(mg/kg) 17.4 – 7.1	(mg/kg) 108-35	(mg/kg) 184.4 – 70.4	(Shahmansouri et al., 2005)
<i>Lampito mauritii (Kinberg)</i>	Synthetic Contaminated soil (SCS) and farm	30 days	SCS + FYM (300 g) 1 : 9	DN	(mg/kg) 75 – 34.26 150 – 72.86 300 –	DN	DN	(mg/kg) 75 - 40.78 150 - 81.44 300 - 158.15	(Sulata Maity et al., 2010)



	yard manure (FYM)				152.15				
<i>Eisenia fetida</i> ,	paper mill waste water sludge (PMS) and cow dung (CD)	60 days	CD : PMS (400 g) 1:0 3:1 2:1 1:1 0:1 1:3 2:1	(mg/kg) 110.5 - 23.75 115.5-36.06 114.5-25.95 129.5-26.12 144.4-16.79 142.3-23.50 123.5-18.64	DN	(mg/kg) 9.29-7.91 8.77-7.89 8.75-7.90 8.71-7.89 8.75-7.90 8.67-7.91 8.64-7.89	(mg/kg) 124.4-65.6 121.4-23.2 114.2-25.6 128.3-32.3 187.3-43.6 175.3-39.3 158.7-32.0	(mg/kg) 59.6-2.0 45.6-2.0 42.3-1.41 49.7-1.53 62.5-1.54 42.8-2.0 35.4-1.66	(Surindra Suthar et al., 2014)
<i>Lumbricus rubellus</i>	Cow dung (CD), Kitchen waste (KW) and coffee ground (CG)	30 weeks	CD : KW 30:70 CD: CG 30:70 CD : KW : CG 30:35:35	0.006 % - 0.015% 0.003 % - 0.02% 0.007%-0.002% 0.016% -0.01% 0.005% to 0.012% 0.002% - 0.01%	DN	DN	DN	DN	(Adi ainurzman Jamaludin and Noor Zalina Mahmood, 2010)
<i>Californian earthworm Eisenia fetida</i> ,	Sewage sludge	70 days	Sewage sludge 60 kg	(mg/kg) 181.44-120.8	DN	DN	(mg/kg) 284.48-92.62	DN	(Ausra Zigmontiene and Indre Liberyte, 2014)
<i>Eudrilus eugeniae</i>	Municipal Solid Waste (MSW), Market Waste (MW) and Flower Waste (FW)	60 days	1:3(c:MSW) 1:3(c:MW) 1:3(c:FW)	89.2 % 95.1% 95.3%	94% 87.9% 94.7%	89.5% 88.7% 84.1%	DN	94.4% 96.1% 95.6%	(Swathi Pattnaik and Vikram Reddy, 2009)
<i>Eisenia fetida</i>	Municipal Solid	60 days	1:3(c:MSW) 1:3(c:MW)	86.6% 87.3%	91.0% 85.2%	85.9% 87.8%		92.3% 94.8%	



	Waste (MSW), Market Waste (MW) and Flower Waste (FW)		1:3(c:FW)	87.5%	80.9%	85.5%	DN	90.4%	(Swathi Pattnaik and Vikram Reddy, 2009)
<i>Perionyx excavatus</i>	Municipal Solid Waste (MSW), Market Waste (MW) and Flower Waste (FW)	60 days	1:3(c:MSW) 1:3(c:MW) 1:3(c:FW)	85.4% 78.1% 80.9%	84.4% 82.1% 77.6%	81.8% 81.5% 78.7%	DN	90.7% 92.9% 85.9%	(Swathi Pattnaik and Vikram Reddy, 2009)
<i>E. fetida Savignyi (Lumbricidae)</i>	Sewage Sludge (SS) and Cow Dung (CD)	12 weeks	SS - 10 kg SS+CD - 10+5 kg	DN	DN	19.2% 57.70%	DN	19.2% 55.4%	(Rajiv et al., 2009)
<i>Eisenia fetida</i>	Sewage Sludge (SS), cow dung (CD), sheep waste (SW) and Garden soil (GS)	90 days	(2:1) SS SS+CD SS+ SD SS+GS	DN	(mg/kg) 242 - 183 182.3-96 171.7-150.7 204.3-124.7	DN	DN	DN	(Hossein Azarpira et al., 2014)
<i>Eudrilus eugeniae</i>	Sewage Sludge (SS), cow dung (CD), sheep	90 days	(2:1) SS SS+CD SS+ SD SS+GS	DN	(mg/kg) 242-175 182.3-107.7 171.7-	DN	DN	DN	(Hossein Azarpira et al., 2014)



	waste (SW) and Garden soil (GS)				143.0 204.3 - 128.7				
<i>Eisenia fetida</i>	Cow Dung (CD), poultry dropping (PD) and solid Textile mill Sludge (STMS)	77 days	CD+PD+STMS (g) 0+105+45 0+90+60 0+75+75 0+60+90 0+45+105 105+45+0 45+105+0 37.5+37.5+75 150+0+0	DN	(mg/kg) 280-200 260-150 320-200 280-170 390-180 140-79 289-190 390-196 283-176	(mg/kg) 0.61-0.32 0.69-0.27 0.58-0.27 0.63-0.36 0.42-0.28 0.40-0.26 0.54-0.31 0.47-0.29	DN	(mg/kg) 0.82-0.62 0.95-0.71 0.83-0.60 1.20-0.65 0.83-0.52 0.76-0.64 0.98-0.73 0.94-0.69 1.21-0.73	(Hossein Azarpira et al., 2014)

6. CONCLUSIONS

This review focuses on both the population of fungus and earthworm can be able to survive and even flourish in environments contaminated with heavy metals. It concluded that, by the knowledge of the mentioned studies, several species of fungus have been utilized as the effective tool in Mycoremediation. Studies have exposed that fungal species can bioaccumulate heavy metals from metal contaminants. During vermicomposting process, fungus can be utilized in the pre-composting process, it reduces the pre-composting period and toxicity to the earthworm. By the combination of Mycoremediation and vermiremediation, we will get the final bio-fertilizer as the end product in short period of time. Fungus and earthworm show capabilities for accumulating heavy metals and discharge the non-hazardous bio-fertilizer. The end product comes from the vermicompost contains the enriched amount of NPK. This review seems to be highly promising in terms of the creation of platforms that encourage the development of bioremediation processes using fungus and earthworm.

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