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General Research Paper

Mycorrhizoremediation of Nickel and Cadmium: A Promising Technology

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ABSTRACT

Nickel (Ni) and cadmium (Cd) are two important environmental contaminants and have detrimental effects on ecosystems and are a risk to human health as they can enter the food chain via agricultural products or contaminated drinking water. Agricultural soils in many parts of the world are moderately contaminated by Ni and Cd due to mining and smelting of metalliferous ores, industrial waste, mineral fertilizers, pesticides, vehicle exhausts and municipal sewage sludge. The remediation of heavy metals contaminated environments is a challenging task because these elements are not degradable and once entering the soil, they can persist for a longer time. Traditional methods used for the removal of heavy metals from the environment are, in general, expensive and potentially risky due to the possibility of the generation of hazardous by-products. Phytoremediation, a sustainable and inexpensive technology based on the removal of pollutants including Ni and Cd from the environment by plants, is a burning issue in plant research. However, as phytoremediation is a slow process, improvement of efficiency and thus increased stabilization or removal of these heavy metals from soils is an important goal. Efficiency of removal can be improved by mediation through arbuscular mycorrhizal (AM) associations which are integral and functioning parts of plant roots, as enhancing plant growth on severely disturbed sites, including those contaminated with heavy metals in particular Ni and Cd. They are reported to be present on the roots of plants growing on heavy metal contaminated soils and play an important role in metal tolerance and accumulation. AM fungi act as a filtration barrier against transfer of heavy metals to plant shoots. The protection and enhanced capability of uptake of minerals result in greater biomass production, which is an important criteria for successful remediation. Isolation of the indigenous and presumably stress-adapted AM fungi can be a potential biotechnological tool for inoculation of plants in order to guarantee the effectiveness of AM symbiosis in the restoration of contaminated soils. Ni and Cd tolerant AM fungi like *Glomus mosseae, Glomus tenue* and *Gigaspora* spp. could protect plants against the harmful effects of metals.

INTRODUCTION

The excessive concentration of heavy metals adversely affects the plant growth and development. Soil microorganisms are also disturbed due to the presence of various pollutants in soil. Several physiological, biochemical and molecular processes are disturbed as a result of heavy metal stress in soil. Plant growth is inhibited and these heavy metals result in various defects like low seed germination, turgor loss, chlorosis, necrosis, senescence resulting plant death. Photosynthesis is also decreased as a result of heavy metal stress effects. The increased levels of antioxidant enzymes like catalase, superoxide dismutase and peroxide were observed under heavy metal stress conditions. Among heavy metals, cadmium and nickel cause the biological toxicity by affecting several physiological and biochemical processes of plants. There is an excessive accumulation of reactive oxygen species and methylglyoxyl due to which peroxidation of lipids, oxidation of proteins, inactivation of enzymes and DNA damage occurs, sometimes DNA reacts with other cell constituents. Pesticides, fertilizers and metal contaminated sewage are major causes of heavy metal deposition in soils. Burning of fossil fuels has increased these heavy metals in soils. The concentration of Cd and Ni has increased substantially in soil over the past two decades. Different plant species have evolved different mechanisms to tolerate heavy metal stress. These mechanisms involve inhibition of uptake and transport, immobilization, role of plasma membrane to expel these heavy metals, stress protein induction, salicylic acid synthesis, nitric oxide and pro and polyamine synthesis etc. Some plants can accumulate a large portion of heavy metals in their cell wall. It was indicated that metallothionins and ferritins protect plants against oxidative stress caused by excessive heavy metals in soil. Certain cadmium thiolate complexes are present in soil which is harmful to plants and microorganisms. These chelating metals accumulate in the cell walls of plants. These may also be deposited in the vacuoles of plants. There are also some reports of expression of genes in AM plants encoding proteins metallothionein, 90 kD heat shock protein, Glutathione-S-transferase in response to metallic stress. This indicates that proteins of these expressed genes may help in the immobilization of toxic heavy metals in plant rhizosphere.

Possible Strategies

Industrialization and extraction of natural resources have resulted in large scale soil contamination and pollution. Remediation of toxic metals in porous matrices requires a specific approach since metals cannot be mineralized and hence require appropriate methods like chemical, physical and biological methods, which are used to remove toxic metals from soils (Mc Eldowney et al. 1993). Remediation technologies available for reducing the harmful effects at heavy metal-contaminated sites include physical excavation, chemical stabilization of the metals in the soil on site and the use of growing plants or microorganisms to stop the spread of contamination.

Physical removal of contaminants from soil is perhaps the oldest remediation method for contaminated soil. It is still in use at many locations, including residential areas. Treatments make necessary metal extraction by solubilization or complexation to avoid their dissemination in the environment and/or the food chain contamination. Soil washing is a well known method that uses various chemical extractants. However, this method can only be applied to soils after their excavation, contrary to soil flushing, an *in situ* soil remediation. Though, both soil remediation methods are only suitable for point source contaminations. Non-point source contaminations, i.e., moderate metal concentrations, but wide surfaces contaminated, are less studied. Typically, many agricultural soils are concerned by these contaminations as a result of repeated applications of both fertilizers and pesticides containing trace metals at various concentrations, along with atmospheric deposits. Metal concentrations encountered are lower than those recorded in industrial sites, but are sometimes high enough to generate a risk for the environment and human beings, through the food chain.

Physical methods: Physical methods of soil reclamation are those that do not change the physico-chemical properties of the pollutants accumulated in the soil to be cleaned. Among these techniques are the simple engineering methods such as soil extraction and storage of polluted soil as well as complicated process technologies of electro migration. Physical methods of soil reclamation could be divided into:

Ex-situ methods: That requires the transportation of the polluted soil to the place of cleaning.

- Mechanical separation
- Extraction and storage

In-situ methods: That could be applied on-site, without the removal of the soil from the polluted site.

- Electro kinetic cleaning methods
- Cofferdam system
- Soil covering

Chemical methods: Chemical methods of soil reclamation aim to degrade the pollutants accumulated in the soil or make such changes to their physico-chemical properties so as to reduce their ecological hazard.

Chemical methods are developed on the basis of the following chemical processes:

- Oxidation and reduction
- Extraction
- Precipitation of sparingly soluble chemical compounds
- pH stabilization

Biological methods: Recently, biological methods of soil reclamation have received wide recognition. These methods are based on the biological activity of microorganisms and higher plants, which have the ability to degrade pollutants accumulated in the soil, including their mineralization, immobilization or removal. Two categories of biological reclamation methods can be distinguished:

- Bioremediation methods based on microorganisms' activity, which are commonly used for the reclamation of soils polluted by organic compounds. However, recently there have also been many investigations into applying microorganisms for detoxication and cleaning soil polluted by inorganic substances (e.g. heavy metals).
- Phytoremediation methods, in which the higher plants are used for the degradation and removal of different contaminants (both organic and inorganic) from the soil.

Among these methods, the most frequently used are:

- Phytostabilization
- Phytoextraction/phytodegradation

A better approach than these traditional methods is to completely destroy the pollutants. Bioremediation is defined as the process whereby organic wastes are biologically degraded under controlled conditions to an innocuous state, or to the levels below concentration limits established by regulatory authorities (Mueller et al. 1996).

Most bioremediation systems are run under aerobic conditions, but running a system under anaerobic conditions

(Colberg & Young 1995) may permit microbial organisms to degrade otherwise recalcitrant molecules.

This process is relied upon microbial enzymatic activities to transform or degrade the contaminants from the environment (Philip et al. 2005). It offers a cost effective remediation technique, compared to other remediation methods, because it is a natural process and does not usually produce toxic by-products. It also provides a permanent solution as a result of complete mineralization of the contaminants in the environment (Perelo 2010). Advantages of biological remediation compared to other treatment methods include (Okoh & Trejo-Hernandez 2006):

i. Destruction rather than transfer of the contaminants to another medium.
ii. Minimal exposure of workers to the contaminants.
iii. Long time protection of public health.
iv. Possible reduction in the duration of the remediation process.

Reclamation of these areas by traditional physical, chemical and biological methods is costly and often has a destructive impact on the soil environment (Mulligan et al. 2001, Padmavathiamma et al. 2007, Witters et al. 2009). Migration of contaminants into non-contaminated sites as dust or leach ate through the soil, and the spreading of sewage sludge are examples of events that contribute towards contamination of our ecosystems.

A promising alternative consists of optimizing the synergistic effect of plants and microorganisms by coupling phytoextraction with soil bioaugmentation, also called rhizoremediation. Among biological methods, mycorrhizal fungi can play a significant role in bioremediation of HMs pollution in soil (Rakshit & Ghosh 2008). Increased presymbiotic hyphal extension, sporulation, and spore germination were observed in *G. intraradices* under high Cd concentrations. AM fungi reduce HMs toxicity by metabolizing these metals. Metallothionines like polypeptides are known to cause Cd detoxification in AM fungal cells. Presymbiotic hyphal extension was also observed in an Al tolerant strain of *Gigaspora gigantia*. Mostly, ectomycorrhizal and ericoid mycorrhizal fungi enhance tolerance of host plants. Endomycorrhizal association is also involved in the bioaccumulation and immobilization of toxic heavy metals present in soil. Mycorrhiza is an association between fungi and the roots of higher plants. The fungus enters the plant roots and develops hyphae, arbuscules and vesicles. Transport of nutrients, especially phosphorus occurs as a result of this association. Some of the HMs like Cd and Ni interfere with many physiological and biochemical processes in plants like photosynthesis, respiration, nitrogen and protein metabolism etc. The effects of these toxic metals can be reduced by using suitable mycorrhizal fungi as inoculums in the heavy metal contaminated areas. Successful AM fungal association can be explained by finding fungal colonization (arbuscules, vesicles, hyphae) in plant roots. Plants inoculated with mycorrhizal fungi in a metal contaminated area are healthier as compared to uninoculated plants of metal polluted areas.

AM fungus is the most common symbiotic association between fungi and higher plants (Habte 2000, Pal 2011) and about 80% of plant species were reported to be colonized by AMF (Koltai 2010). AM is a versatile beneficial relationship, the most popular root-fungus association is vesicular arbuscular mycorrhizal association, also known as glomeromycotan association (Read 1997, Basta et al. 2005). Mycorrhiza term was used for plant-fungus relationship and is essential for survival of both and absorption of substance from soil takes place, through this relationship. Because of this intimate relationship, plant and fungal association is very different from other associations. AMF is known to improve plant growth on nutrient-poor soils and enhance their uptake of P, Cu, Ni, Pb and Zn (Khan et al. 2000, Zhu et al. 2001, Rai et al. 2013). AM fungi assist the host plant in P and N uptake and also some of the relatively immobile micronutrients and trace elements viz., Cu^{2+}, Fe^{2+}, Zn^{2+}, Ni^{2+}, Pb^{2+} and Cd^{2+} (Garg & Chandel 2010). Rhizospheric microbes play significant roles in recycling of plant nutrients, maintenance of soil structure, detoxification of noxious chemicals, and control of plant pests (Rajkumar 2010, Mackova et al. 2006). AMF were already reported to decrease or increase HMs uptake by plants (Leyval et al. 2002, Khan et al. 2000). They can provide a potential for decreasing health hazards of edible plant production and improve sustainable agriculture and phytoremediation technologies, including phytoextraction (Jurkiewicz et al. 2004). AMF are essential components of sustainable soil-plant systems (Schreiner et al. 2003) because of their role in increasing plant growth and nutrient uptake (Smith & Read 1997). Effective AM fungal associations have reduced the amount of phosphorus fertiliser needed to be applied to a plant or crop (Miyasaka et al. 2003). Alternative soil management techniques such as AM biofertilizer application is needed for resource poor farmers and has enormous potential for large scale agricultural systems and can be beneficial in sustainable production of main crops contributing to reduced input of expensive, environmentally harmful, low use efficient and limited phosphate fertilisers and chemical pesticides (Shrestha 2007). AMF was reported to play a significant role in efforts to restore cadmium-contaminated soils and the plants can be protected from heavy metals (Kapoor & Bhatnagar 2007). Isolating spores of AMF texa such as *Glomus* and *Gigaspora* associated with most of the plants

Table 1: Summary of AM fungi-Cd/Ni interactions and application in phytoremediation

Heavy metals	Tolerant fungi	Host used	References
Cd	*Glomus mosseae*	*Trifolium repens*	Vivas et al. 2003
	Glomus sp. and *Gigaspora* sp.	*Hordeum vulgare*	Tullio et al. 2003
	G. mosseae	*Trifolium subterraneum*	Joner et al. 1997
	G. mosseae	*Allium porrum*	Weissenhorn et al. 1993
Cd	*Glomus caledonium*	*Zea mays*	Liao et al. 2003
Cd	*G. mosseae*	*T. subterraneum*	Joner et al. 2000
Cd	AM fungi	*Biscutella laevigata, Plantago lanceolata*	Orlowska et al. 2002
Cd	*G. mosseae*	*T. subterraneum*	Joner et al. 2001
	G. mosseae	*Phaseolus vulgaris*	Guo et al. 1996
Cd, Ni	*G. caledonium*	*A. porrum, Sorghum bicolor*	**del Val et al. 1999**

growing in HMs polluted habitats (Chaudhry et al. 1980, Raman et al. 1993). Identified *Glomus* and *Gigaspora* spp. in the mycorrhizosphere of 14 plant species colonising a magnesite mine spoil in India. Glomalin and HM sequestration, HMs in the soil are associated with a number of soil components which determine their behaviour in the soil and influence their bioavailability (Benavides et al. 2005). The cell wall components such as free amino, hydroxyl, carboxyl and other groups of soil fungi can bind to potentially toxic elements such as Cu, Pb, Cd, etc. Many filamentous fungi can sorb these trace elements and are used in their commercial biosorbants. The proteins in the cell walls of AM fungi appear to have similar ability to sorb potentially toxic elements by sequestering them. There is evidence that AMF can withstand potentially toxic elements, AMF produce glomalin on hyphae can enhance HMs sequester.

AMF play a significant ecological role in the phytostabilization of potentially toxic trace element polluted soils by sequestration and, in turn, helps mycorrhizal plants survive in polluted soils. One of these components is Glomalin, a glycoprotein produced by the hyphae of AMF fungi , which is released into soil from AMF hyphae (Driver et al. 2005). The extra-radical mycelium of AMF, in addition to its crucial role in enhancing nutrition of host plant, also plays a role in soil particle aggregation and soil stability. The AMF can be screened for their ability to produce maximum levels of extra-radical mycelium in polluted soils (Joner et al. 2000) and to utilize adapted AMF to help accumulate HM both within the plant roots (phyto-accumulation) and the extra metrical fungal mycelium. Glomalin plays a vital part in sorption and sequestration of potentially toxic elements, reducing their bioavailability. It has been suggested that this sequestration could be important for heavy metal biostabilization in HMs polluted soils (Khan 2005). AM associations are important in natural and managed ecosystems due to their nutritional and non-nutritional benefits to their symbiotic partners. They can alter plant productivity, because AMF can act as biofertilizers, bioprotectants, or biodegraders. AMF are known to improve plant growth and health by improving mineral nutrition, or increasing resistance or tolerance to biotic and abiotic stressesChen et al. 2005, Pal et al. 2013). The potential advantages of the inoculation of plants with AM fungi in horticulture, agriculture, and forestry are not perceived by these industries as significant. This is partially due to inadequate methods for large-scale inoculums production. Monoxenic root-organ invitro culture methods for AMF inoculates production have also been attempted by various workers but these techniques, although useful in studying various physiological, biochemical, and genetic relationships, have limitations in producing inoculates of AM fungi for commercial purposes.

AMF enhances plant water relations through several mechanisms, potentially contributing to increased crop drought resistance (Auge 2001). Commonly, AM fungi occur in rhizosphere but some may also be present in the stem and thallus (Smith & Read 1997). As AMF increases the nutritional status of the host, in the same way fungal hyphae absorbs heavy metals, and transfer it to the plant. Therefore, on one hand mycorrhizal plant express increased heavy metal uptake and on other hand AM fungi helps in immobilization of heavy metals in soil. Therefore the cleanup of the ecosystem depends on the HMs plant mycorrhizal relationship through the removal of HMs from the soil system. During stress condition i.e., increased concentration of toxic heavy metals, plants employs various mechanisms to maintain the metal-ion equilibrium internally as well as in their surrounding environment. In this mechanism various types of genes are also involved, which help in accomplishing the mechanism of remediation of HMs toxicity which in turn helps in detoxification of heavy metals from contaminated soil.

CONCLUSIONS

In situ fixation is a process that creates new chemical compounds in which heavy metals are much less available to living things. This on-site reclamation is less disruptive to people's lives and to the environment compared to excavating and disposing contaminated soils elsewhere. Mycorrhizoremediation uses plants as well as microorganisms by several methods to contain or clean up heavy metals and has the edge benefit of being a relatively low-cost, natural solution to an environmental problem.

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