Study on the effect mechanism of Arbuscular Mycorrhiza on the absorption of heavy metal elements in soil by plants

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Abstract. In recent years, human activities have produced lots of wastes, including a large number of heavy metals, which have entered the natural ecosystem, changing the activity and function of soil microorganisms. AM fungi can adapt to or resist metal stress, which means it has great utilization potentiality in promoting plant growth, enhancing nutrient acquisition and for phytoremediation in heavy metal contaminated soil. On the basis of previous research results, the leading mechanism of metal tolerance of AM fungi, the influencing factors of mycorrhizal effect and the application in promoting the process of phytoremediation are discussed in this paper, which provides theoretical bases for improving plant tolerance and enhancing mycorrhizal strengthening effect under heavy metal pollution.

1. Introduction

Nowadays, soil pollution has become increasingly severe, and human activities have discharged more and more waste gases, waste water and waste residue into the environment, among which heavy metals are the major ones that have the greatest impact on soil biology. Heavy metal pollution in our country (China) has certain particularity, which due to blindly believing that the soil is a kind of good processing system, nevertheless, ignoring the environmental carrying capacity of soil. And the excessive irrigation of farmland sewage and the application of sludge make the soil pollution more serious. The chemical or biological conversion of heavy metals includes oxido-reduction and methylation reaction, which is then retained in the soil by adsorption, precipitation and complexation, and finally removed by plant absorption and soil leaching. Heavy metals in soil can enter the food chain through different ways, producing heavy metal contaminated foods such as "cadmium rice", "poisonous vegetables" that endanger human health. Heavy metals can also cause root damage due to the aging of root tip cells in plants, making plants appear to be wilting, withered and yellow, whose biomass decreases or even dies. Remediation of heavy metals contaminated soil mainly includes physical-chemical process (such as soil rinsing, applying soil modifier, etc.) and bioremediation (such as microbioremediation, phytoremediation and mycorrhizal repair). Traditional physicochemical methods often fail to remove heavy metals from the environment directly, but rather transforming from one form to another, or only the concentration migration of soil pollutants, which results in the high cost by a large quantity of engineering in soil transfer project that cause the severe environmental disturbance, and even change the physicochemical and biological characteristics of the soil. That is to say, it can not fundamentally solve the problem. Due to these reasons, the physicochemical treatment should not be used in soil pollution caused by mining activities. However, it has difficulty repairing deep soil pollution through the long cycle of phytoremediation merely. And plant growth is restricted by edaphic conditions and climate, which reduce the efficiency of
phytoremediation[3], so there is not a wide range of application. The mycorrhizoremediation that combines effect of plant and microorganism is mainly by inoculating arbuscular mycorrhizal fungi to the soil, using a variety of organisms in soil—plant, mycorrhizal fungi and indigenous microorganisms that were used to fix, transfer and decompose the pollutants in soil[6] so that the concentration of heavy metals could be reduced to the level of soil safety, or converting toxic and harmful contaminants into harmless substances, which is now a main trend in the study of microbial agents. However, the safety and effectiveness of microbial agents, the mechanism of the immobilization and transfer of heavy metals in the rhizosphere, and the future reconstruction and application of contaminated environment still have problems to be solved urgently.

2. Mycorrhizoremediation overview

Mycorrhiza is the symbiotic body in which some fungi infect plant root formation [7]. The mycelium of mycorrhizal fungi extends from root of plant to the soil, which enlarges the absorption of soil moisture and mineral nutrients. Mycorrhizal fungi secretes enzymes, antibiotics, vitamins and other substances, which promote the growth of plant roots, while the plant supplies mycorrhizal fungi carbohydrates and oxygen, so that both plant and fungi lives a mutually beneficial symbiotic life [8]. It has already become the core research direction to improve the soil remediation efficiency and guarantee the clean production of agriculture by using the ternary remediation system composed of plant, arbuscular mycorrhizal fungi and indigenous microorganisms. In this paper, the mechanism of the mycorrhizal plant resisting heavy metal toxicity, in particular, the heavy metal tolerance of mycorrhizal fungi and the mechanism of how mycorrhiza promotes the migration and transformation of heavy metals in plants are reviewed, and the application trend of mycorrhizal technology in heavy metal contaminated soil is prospected, which provides a theoretical reference for the ecological safety, application prospect and long-term effectiveness of mycorrhizal bioremediation.

3. Effect of mycorrhizal effect on the enhancement of phytoremediation for heavy metal pollution

The combined action of arbuscular mycorrhizal fungi and hyperconcentration plants can improve the absorption of trace elements such as nickel, molybdenum, cobalt and iron by mycorrhiza activates insoluble phosphorus in the soil, which improves the resistance physiology of the host plants[9] and influence root microbial composition. There are many studies on the absorption mechanism of heavy metals by mycorrhizal plants, but there is not a consistent result. In some cases, arbuscular mycorrhizal fungi enhances the immobilization of heavy metals in plant roots, besides, mycorrhizal infection can also promote the transport of heavy metals from roots to aboveground parts [10]. These two different effects are determined by the types of metal elements, the role of fungi in plants and environmental conditions. It is inferred that the absorption mechanism of mycorrhizal plants to heavy metals in soil should include the direct and indirect effects of mycorrhizal fungi.

3.1. Direct effect

3.1.1. Immobilization of heavy metals on the external hyphae of arbuscular mycorrhizal fungi

The external mycelium of the root has important effect on the immobilization of metal in heavy metal contaminated soil, as the external mycelium has a larger surface area, which makes its ability of metal absorbing become stronger than that of plant root [11]. High metal adsorption capacity may be related to the structure of the fungal cell wall. Fungal cell wall, which mainly is composed of polysaccharides and chitin, can be used as a barrier of metal ions and other solutes to enter cells, controlling their absorption [12]. Free amino acids in the cell walls and functional groups such as hydroxyl and carboxyl groups can form a negatively charged structure that has the ability to adsorb the majority of the metal ions in the soil [6]. In addition, fungi can store metals in some structural parts (like spores) [9], while the storage of metallic elements in spores occurs in a single colony culture merely.
Fungal cell walls constitute the first barrier to obstruct the entry of heavy metals. However, when the metal concentrations are too heavy, some of the metal ions will also pass through the cell wall, being stored on the fungus cell membrane[13], or by transport across membrane to the fungal cytoplasm, reducing the utilizability of metals, thus decreasing the amount of removable metal in the plant.

3.1.2. Chelation of heavy metals by mycelium/hyphal secretion of arbuscular mycorrhizal fungi
The chelate mechanism of the hyphae secretion to heavy metals relies mainly on the extracellular glycoprotein. The chelate of glycoprotein and metal enters into the cell, reducing the content of the removable metal elements in the soil by promoting the immobilization of the chelate of the plants[14], increasing the agglomeration of particles, stabilizing the soil erosion to the wind and water and improving the soil structure, thereby reducing the utilizability of metals. The glomalin-related protein is a glycoprotein produced by the mycelium of AM fungus [15], and its structure is not yet fully established. Nichols[6] believes that the glomalin is composed by the monomer structure through the hydrophobic interaction, which is widespread in the soil. In soils with different levels of metal contamination, the content of the glomalin differs quite large, which indicates that its quantity depends on the degree of soil contamination. Besides promoting the agglomeration of soil particles and reducing the soil mobility, the toxic metals can also be isolated by the functional groups in the structure of glomalin, and its binding ability of the metal changes with the types of soils as well as the physicochemical parameters such as pH and redox potential. Furthermore, it is found that the quantity of glomalin is greatly correlated with the Pb concentration in the soil. Therefore, the stress on plants by heavy metals plays an positive role in isolating the potential toxic metals and in alleviating the degree of soil contamination. The application of arbuscular mycorrhizal fungi can help plants to resist environmental stress as well as promote the restoration and reconstruction of heavy metal contaminated soil [8].

3.2. Indirect effect

3.2.1. Promoting the absorption of nutrients by plants
The application of AM fungi is beneficial to plant growth, especially in unfavourable conditions such as in drought, nutrient-poor and heavy metal contaminated soils. AM fungi can promote the absorption of nutrients by plants, phosphorus mainly [16], as well as other nutrients such as N, Ca, S, K, Zn. The dilution effect of Glomus mosseae(GM) on plant growth may be the most significant protective mechanism against heavy metal stress in plants [17].

The absorption of metals in plants is related to their degree of dissolution in the soil, because the phosphatase secreted by arbuscular mycorrhizal fungi can hydrolyze insoluble phosphate in the soil to make it mineralized, increasing the soluble phosphate content [18]. On the one hand, it provides sufficient phosphorus nutrient for plants to accelerate the growth. On the other hand, the proportion of heavy metals in plants can be reduced, thus alleviating the stress effect of heavy metals. The organic acids secreted by arbuscular mycorrhizal fungi, such as oxalic acid, succinate, and propionic acid, can also activate insoluble phosphate, improving the absorption and transport of phosphorus by plants, as thus, the viability of plants under low phosphorus or poor nutrient soil conditions is enhanced[19].

3.2.2. Improving plant rhizosphere microenvironment
In all soil properties, pH value is the most important parameter affecting the solubility of metals in soil, besides, the change of soil pH can influence the degree of absorption of heavy metals by soil, changing the fixed effect of plant root on metal [20]. Because of the slight increase of pH value caused by the physiological action of fungus mycelium, the concentration of zinc in soil solution decreases drastically. Studies show that the pH of rhizosphere soil treated by microbial agent is lower than that of aseptic agent, which may be caused by the activity of Arbuscular mycorrhizal mycelium, by
changing the quantity and composition of mycelium secretion, and then changing the activity of arbucular mycorrhizal mycelium, so that give rise to the change of soil pH.

Soil microbes participate in different biochemical processes, such as soil formation, energy transfer and nutrient cycling, which accelerate revegetation, therefore, increasing the stability of the polluted ecosystems. Meanwhile, the species and quantity of soil microorganisms can also be affected by metals [21]. Continuous exposure to high concentrations of metal contaminated soil conditions can induce some specialized heavy metal resistant microbial populations, but the high concentration of metals are more toxic to soil microorganisms, resulting in reduced biomass, population and biodiversity. These changes of microbial community structure will affect the whole soil-plant-microbial ecosystem eventually. Experiments conducted by Robin et al showed that when G. Intraradices was applied to the soil, the quantity of Pseudomonas fluorescens increased markedly. Statistical analysis showed that plant growth was positively correlated with the metabolism of ketoglutaric acid, while negatively correlated with the metabolism of phenylalanine, which affected the activity of microorganisms indirectly.

3.2.3. Changing root morphology as well as physiological and biochemical traits
Generally, plant biomass is an important characteristic to measure plant growth, and high biomass is also associated with better growth and stronger metal tolerance of plants [23]. When the arbuscular mycorrhizal fungi and non-bacteria treated samples were applied, the length, weight and mean diameter of root were increased significantly, so that improving plant growth condition and promoting the tolerance to heavy metals[24]. Arbuscular mycorrhiza is produced symbiotically after fungal infection of plant roots, which has increased root vigor/activity, enhanced the absorptive capacity of soil nutrients, minerals and water and facilitated colonization in heavy metals contaminated soils [25].

3.2.4. Participating in the expression of regulatory genes
Up to now, four genes have been found in the AM fungus gene to resist metal stress and maintain homeostasis in the cells:(1) The GrosMT1 gene in Gigaspora rosea (2) Zinc transporter GinZnT1 in Glomus intraradices (3) That GmarMT1 gene in Gigaspora Margarita can encode MTs and regulate the oxidative reduction potential of fungi, which protects plants from oxidative stress caused by metals.(4) GintABC1 can encode a polypeptide of 434 amino acids and participate in the detoxification mechanism of copper and zinc actively[6][9]. The genes involved in fungal regulation are mainly induced or mediated by metals, and these gene-mediated transport protein play a vital role in the absorption, transport, and tolerance of metals.

3.2.5. Participating in the regulation and expression of enzymes
When the plant is subjected to metal stress, it will produce osmotic adjustment substances like antioxidant enzymes to remove active oxygen free radicals induced by metal, alleviating the oxygen damage caused by heavy metals and improving tolerance of plants. Antioxidant enzymes mainly include peroxidase (POD), superoxide dismutase (SOD), catalase (CAT), etc. Studies have showed that the content of antioxidant enzymes in plants decreased under metal stress, because SOD could remove excess active oxygen in plant to ensure the normal material and energy exchange of its membrane, which could be used as the index of stress resistance to heavy metal adversity, and the application of microbial agent promotes the production of SOD obviously[26].Therefore, the protective effect of arbuscular mycorrhiza is embodied in the regulation of the antioxidant enzyme system, reducing the accumulation of reactive oxygen free radicals in plant cells, alleviating the degree of membrane lipid peroxidation and ensuring material transport and energy transfer.

4. Application trend of mycorrhizal technology in heavy metal contaminated soil

4.1. Range of application
The main source of heavy metals in the environment is human activities, for example, mining industry, agricultural production and sewage discharge, while mining is the most important source of pollution of heavy metals [27], for the mining waste contains a large number of heavy metal particles, meanwhile, a large amount of waste water is produced in the metal extraction process, which results in serious heavy metal pollution in the mining area soil. Besides, the waste discharged from the process of urban infrastructure industrialization leads to the increase of metals through atmospheric deposition, entering into the soil and flowing water, which has harmful effects on ecosystems. High concentrations of metals in farmland can lead to irreversible degradation of soil properties, affecting their physical, chemical and biological properties, which limits the establishment of vegetation and their utilizability in agriculture. According to metal properties, soil conditions and cleanliness, mycorrhizal technology is applied to the process of phytoremediation in different ways.

4.1.1. AMF for plant immobilization

Due to its huge hyphae network, Arbuscular mycorrhiza can prevent the migration of metals by the absorption, immobilization and decomposition of the symbiotic mycorrhiza, reducing the bioavailability of pollutants. Mycorrhizal fungi and mycelium secretion can chelate metal ions and activate it [28], heavy metal segments are separated by different metabolic mechanisms of roots[29], directly or indirectly, remediating heavy metal contaminated soil. Plant immobilization can reduce the diffusion of metals in soil by means of alleviating metal leaching and subsequent metal pollution caused by erosion of wind and water in aquifer.

After the action of AMF and plants, a wide area--the rhizosphere, is formed, which can be used to immobilize metal ions by a mechanism that is similar to plant fixation. What the most significant is the immobilization of fungal cell wall and the chelation of different functional groups, as well as the intracellular immobilized mechanism, including the role of the chelating components in the cell wall of the fungi. Studies by Robin and others found that under the presence of Cd, mycorrhizal infection can greatly promote plant growth. Furthermore, Glomus mosseae(GM) has better effect on the immobilization of Cd on plant roots than the G. Intraradices(GI), and it has higher vitality under Cd stress. Therefore, the tolerance of GM to heavy metals in Cd contaminated soil is of great significance for phytoremediation of farmland soil.

4.1.2. AMF for phytoextraction

Plant extraction is now a low-cost but highly efficient, extremely attractive and eco-friendly technology for remediation of mining processes on the soil. Plant extraction can promote the transfer of metal ions to the upper part of the ground without any damage to the ecological environment of soil, burning the aboveground part of the plants after harvesting them[9], to remove the harmful metallic elements in the soil, which is much more advantageous than the plant fixation. The study of sunflowers in Cd-contaminated soil conducted by Saad et al[30] showed that their aboveground part inoculated by R.irregularis was increased significantly than in the non-microbial agent treatment in the concentration of CD and Zn under the low Cd condition. The model presented by Audet and Chareat[6] can be used to explain the contribution of AMF to phytoremediation: AM fungi can promote plant extraction, helping plants absorb more metal elements for growth when the metal concentration in the soil is low; While, when the concentration of metals in the soil is high, AM fungi can facilitate the fixation of metals by plants, accumulating the majority of harmful metal elements in plant roots to avoid their transportation to their aboveground part. However, phytoextraction is a slow process that takes several years to reduce soil metal concentration to harmless levels.

4.2. Applications

Nowadays, the most potential application of phytoremediation is superaccumulative plant, whereas, the majority of superaccumulative plants such as cruciferae, juncaceae do not produce arbuscular mycorrhiza, which have small biomass and wide biological diversity, being restricted by the geographical area greatly. The interaction between AM fungi and superaccumulative plants not only can promote the growth of plants, but enhance the absorption effect on metal and increase the
tolerance to heavy metals, ensuring its colonization in the soil with high concentration of metal, strengthening the effect of phytoremediation. In addition, chelating agents combined with mycorrhizal phytoremediation such as EDTA and CDTA can promote absorption effect and increase the utilisability of metals in the soil, but the chelating agent is usually not biodegradable and the absorption behavior is not selective, so its usage also increases the risk of metal leaching and groundwater pollution.

5. Prospect
There are lots of studies on AMF growth promotion and inhibition of heavy metal stress, however, the theoretical basis of AMF application in heavy metal contaminated soil is not mature enough, mainly manifesting in the controversy of AMF promoting mechanism on plant fixation or plant extraction. Nowadays in the third mechanism, it is believed that the plant extraction process will be promoted when the metal element content is low, to the contrary, plant immobilization is enhanced when the concentration of metallic elements is high. Therefore, the future study in this field should focus on the optimization of applied theory, including determining the mechanism of the absorption, immobilization, translocation and metabolism of metals by plants, perfecting the control mechanism of genes, and discussing the theoretical basis of the compatibility of AMF and superaccumulative plants. In a word, the application of mycorrhizal technology has more advantages than disadvantages, furthermore, how to apply it to practice, to increase the efficiency and utilisability of mycorrhizal remediation of metal contaminated soil are practical problems to be solved urgently.

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