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The Bioremediation of Heavy Metals and Activation of Antioxidant System in Plants through Arbuscular Mycorrhizal Fungi (AMF)

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Abstract

Contamination by heavy metals, specifically lead (Pb), cadmium (Cd), zinc (Zn), arsenic (As), chromium (Cr), and mercury (Hg), presents a considerable risk to both agricultural productivity and food safety. Exposure to these metals can lead to the induction of oxidative stress in plants, characterized by the excessive production of reactive oxygen species (ROS), resulting in damage to cellular constituents, including lipids, proteins, and DNA. This occurs when plants under stress cannot nullify ROS overproduction using their antioxidant defence system. This stress impairs plant growth, photosynthesis, and overall productivity. The present scientific report explores remediation technologies that control plant oxidative stress generated through metal accumulation. Several advanced and eco-friendly technologies have been developed to prevent plant heavy metal toxicity, including phytoremediation, Nanotechnology, Chelating agents, Genetic engineering, CRISPR-Cas9, and Biochar amendment. Microbial remediation involves using plant growth-promoting rhizobacteria (PGPR), fungi like Trichoderma, and Mycorrhizae to detoxify ROS under metal stress. Therefore, addressing heavy metal contamination requires practical, eco-friendly remediation approaches that restore soil health and maintain the best agricultural practices.

Keywords: Heavy metals, oxidative stress, ROS, antioxidant.

Highlights

- Agricultural productivity under metal stress
- Oxidative stress damages lipids, proteins, and DNA
- remediation technologies to build an antioxidant system of the plants
- Mycorrhizae as a bioremediator to detoxify ROS

1. Introduction

Heavy metals, including lead (Pb), cadmium (Cd), zinc (Zn), arsenic (As), chromium (Cr), and mercury (Hg), are prominent environmental pollutants owing to their persistent and bioaccumulative properties. These elements are non-degradable and enter ecosystems through various anthropogenic sources, such as mining activities, industrial effluents, agricultural runoff, and atmospheric deposition. Upon accumulation in soil, they adversely impact microbial activity and fertility, impede organic matter decomposition, and disrupt soil chemistry (Chaturvedi et al., 2015; Verma et al., 2016).

Low concentrations of essential metals like copper, manganese, nickel, and zinc can support biological functions. Still, elevated levels lead to toxicity and oxidative stress in plants and impact their nutritional status. This stress forms reactive oxygen species (ROS), damaging lipids, proteins, and DNA (Waszczak et al., 2018; Panda et al., 2005). In particular, toxic metals disrupt photosynthesis and enzymatic functions, reduce root and shoot growth, and accumulate in edible plant parts, posing a threat to human and animal health (Ayangbenro & Babalola, 2017; Verma et al., 2016). Therefore, addressing heavy metal contamination requires practical remediation approaches that restore soil health and maintain agricultural productivity. Assessing plants' physiological and biochemical indices under metal stress requires advanced analytical techniques. Electrical conductivity (EC) and pH are key indicators of soil health, influencing nutrient mobility and microbial activity. High EC values often correlate with the accumulation of toxic ions, impairing plant growth and biomass production (Ahmed et al., 2023; Salimi et al., 2012). Measuring EC helps to evaluate soil salinity and nutrient availability, particularly nitrate and potassium levels in non-saline soils (Minzan & Zhuo, 2010).

Furthermore, various spectroscopic and chromatographic techniques are employed to quantify metal concentrations and assess oxidative stress markers in plant tissues. These include Atomic Absorption Spectroscopy (AAS), Inductively Coupled Plasma Mass Spectrometry (ICP-MS), and High-Performance Liquid Chromatography (HPLC) for evaluating antioxidant compounds like chlorogenic acid and tocopherols (Limsuwan et al.2025)

1.1. Generation of reactive oxygen species

Oxidative stress is often a byproduct of normal cellular respiration (especially in the mitochondria) or can be introduced through external factors like pollution (waste organic matters), radiation, and toxins (metals). Commonly, it is generated due to heavy metal accumulation. Reactive oxygen species include i) Superoxide anion ($O_2^{-}\cdot$), ii) Hydroxyl radical (^{-}OH), and iii) Hydrogen peroxide (H_2O_2). ROS are naturally generated in plants during various metabolic processes, especially under stress conditions (e.g., drought, high light intensity, salinity, heavy metals, pathogen attack). While ROS at low levels are signaling molecules, excessive ROS accumulation causes oxidative stress and damages cellular structures. ROS attack on unsaturated fatty acids in membrane lipids, leading to lipid peroxidation, loss of cell membrane integrity, enhanced permeability, cellular leakage, and inhibiting nutrient transport and energy conversion. It attacks on amino acids, especially sulfur-containing ones like cysteine and methionine, and causes protein misfolding, inactivation, or degradation, affecting enzyme activities and signaling pathways. ROS generated in chloroplasts under high light or stress conditions damage the photosystems (particularly PS II), reducing photosynthetic efficiency and energy production. It also damages DNA and causes strand breaks, base modifications (e.g., 8-oxoguanine), cross-linking, leading to mutations, impaired replication, and disrupted gene expression.

The ROS production reaction occurred during cellular respiration in mitochondria (Hasanuzzaman et al, 2020).

$$O_2 + e^- \rightarrow O_2^{\bullet-} \quad (1)$$

Equation (1) shows the formation of superoxide anion, a ROS, from molecular oxygen, which further reacts to give the following ROS species

$$O_2^{\bullet-} + H^+ \rightleftarrows HO_2^{\bullet-} \quad (2)$$

$$H_2O_2 + HO\bullet \rightleftarrows HO_2^{\bullet-} + H_2O \quad (3)$$

$$^{\bullet}OH + {}^{\bullet}OH \rightleftarrows O^{\bullet} + H_2O \quad (4)$$

Then, the superoxide can be converted into hydrogen peroxide in the presence of superoxide dismutase and form Hydrogen peroxide

$$2O_2^- + 2H^+ + e^- \text{ (superoxide dismutase)} \rightarrow H_2O_2 + O_2 \quad (5)$$

$$O_2^{\bullet-} + HO_2^{\bullet-} + H_2O \rightarrow H_2O_2 + O_2 + {}^{\bullet}OH \quad (6)$$

H_2O_2 may dissociate into $^{\bullet}OH$ radical, which has a high oxidation potential

$$HOOH \rightarrow HO^{\bullet} + {}^{\bullet}OH \quad (7)$$

$$ROOH \rightarrow RH^{\bullet} + {}^{\bullet}OH \quad (8)$$

Hydrogen peroxide can form the highly reactive hydroxyl radical via the Fenton reaction:

$$Fe^{3+}/Cu^{2+}/Mn^{3+} + H_2O_2 \rightarrow Fe^{2+}/Cu^{+}/Mn^{2+}\ OH + {}^{\bullet}OH \quad (9)$$

$$Fe^{2+}/Cu^{+}/Mn^{2+} + H_2O_2 \rightarrow Fe^{3+}/Cu^{2+}/Mn^{3+} + HO_2^{\bullet-} + H^+ \quad (10)$$

A highly toxic oxidative species.

1.2. Technologies for Improvement in the Antioxidant Defense System

Oxidative stress is caused by an imbalance between the production of reactive oxygen species (ROS) and the body's ability to detoxify them using antioxidants. Several technologies nowadays are used to control the toxicity of the soil for normal growth of the plants, viz., oparticles, liposomes, hydrogels, electrochemical sensors, fluorescent probes, wearable biosensors, CRISPR-Cas9, RNA interference (RNAi), Low-level laser therapy (LLLT), red/near-infrared light, High-throughput screening, AI-based drug discovery, and biological or ecological technologies. All these technologies capture antioxidants like vitamin C, E, glutathione, or polyphenols in carriers that enhance bioavailability and targeted delivery. Protect antioxidants from degradation and improve their cellular uptake (Alam et al.,2019; Fariduddin et al, 2014; Chaturvedi et al, 2015).

2. Bioremediation Technologies

2.1. Mycorrhizal Remediation

Arbuscular mycorrhizal fungi (AMF) form symbiotic associations with the roots of most plant species, enhancing nutrient uptake and increasing tolerance to abiotic stress, including heavy metal toxicity (Bothe et al, 2010). Mycorrhizal hyphae provide an extended surface area for metal sequestration and can immobilize metals in the rhizosphere, preventing translocation to aerial plant parts (Gao et al., 2010). This method is auspicious due to its biological compatibility and sustainability. AMF spores can persist in the soil for years, making this method suitable for long-term ecological restoration. Additionally, AMF improves soil structure and helps establish vegetation in contaminated and degraded landscapes (Fig.1).

2.2. The Detoxification of Oxidative Stress through Arbuscular Mycorrhizal Fungi (AMF)

Arbuscular Mycorrhizal Fungi (AMF) play a crucial role in helping plants survive with metal toxicity in soil and control reactive oxygen species (ROS) through a combination of physical, biochemical, and molecular mechanisms (Ahmed et al.2023; Panda et al.,2005).

2.2.1 Physical and Structural Mechanisms

AMF help in metal immobilization in the rhizosphere by extending AMF hyphae into the soil, binding heavy metals (like Cd^{2+}, Pb^{2+}, Zn^{2+}) onto their surfaces via cell wall components such as chitin and glomalin, due to which metal immobilize consequently reduced its bioavailability to the plants and inhibit the metal accumulation in plants (Fig.1). It enhances growth, increases plant biomass, dilutes the metal concentration per tissue volume, and reduces toxicity (Ayangbenro & Babalola, 2023)

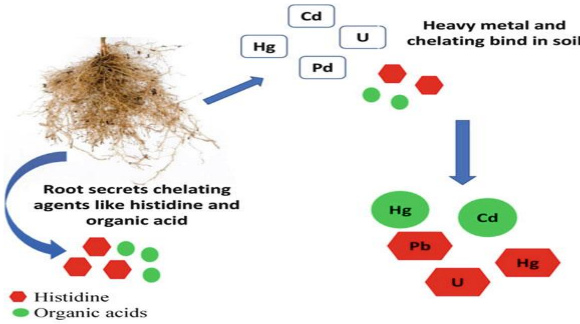

Figure 1: Process of Detoxification of metals in soil

2.2.2. Biochemical Mechanisms of ROS Control

AMF-colonized plants show upregulated activity of key antioxidant enzymes by binding heavy metals in the rhizosphere, lowering their uptake and encouraging metal sequestration in root cells (in vacuoles or bound to phytochelatins). It also prevents Fenton reactions by limiting free Fe^{2+} and Cu^{+} that catalyze hydroxyl radical (OH•) production (Equations 9 & 10). These reduce oxidative damage to lipids, proteins, and DNA caused by heavy metal-induced ROS. The following key enzymes are involved in the detoxification of ROS (Fig.2). AMF boosts glutathione (GSH) levels and the ascorbate-glutathione cycle, enhancing ROS detoxification. GSH also binds metals directly, forming metal-glutathione complexes that are less toxic. AMF stimulates the production of phytochelatins (PCs) and metallothioneins (MTs). These enzymes bind metal ions and sequester them in vacuoles, keeping them away from sensitive cellular machinery. AMF also induced systemic tolerance (IST) to trigger general signaling in the plant that enhances its defense capacity, including stress-related transcription factors, metal transporters, regulation, ROS-scavenging gene expression, and Hormonal Modulation (Fig. 2). It also modulates hormones like Abscisic acid (ABA) (improves stress signaling), Jasmonic acid (JA), and Salicylic acid (SA) (immunity and oxidative balance)(Limsuwan et al, 2025; Mehla et al., 2017; Suzuki et al.,2012)

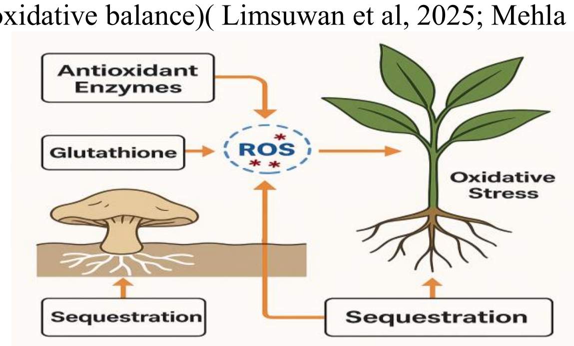

Figure 2: Fungal Control of Oxidative Stress in Plants. The Figure shows how fungi, mycorrhizal species, help plants manage oxidative stress through enhanced antioxidant enzyme production, glutathione regulation, chelation of heavy metals, and metal sequestration in the rhizosphere.

2.2.3. Enzymes Involved in Controlling ROS

- Superoxide Dismutase (SOD) Converts superoxide radicals into hydrogen peroxide and oxygen.

$$2O_2^{\bullet -} + 2H^+ \rightarrow H_2O_2 + O_2 \quad (11)$$

- Hydrogen peroxide is converted into water and oxygen in the presence of Catalase (CAT)

$$2H_2O_2 \rightarrow 2H_2O + O_2 \quad (12)$$

- Ascorbate Peroxidase (APX) uses ascorbate (vitamin C) to reduce hydrogen peroxide.

$$H_2O_2 + \text{Ascorbate} \rightarrow 2H_2O + \text{Dehydroascorbate} \quad (13)$$

- Glutathione Peroxidase (GPX) uses glutathione to detoxify hydrogen peroxide.

$$H_2O_2 + 2GSH \rightarrow 2H_2O + GSSG \quad (14)$$

- Glutathione Reductase (GR) uses glutathione to detoxify hydrogen peroxide.

$$GSSG + NADPH + H^+ \rightarrow 2\,GSH + NADP^+ \quad (15)$$

- Fenton Reaction (Uncontrolled ROS Generation). It produces highly reactive hydroxyl radicals but can be limited by metal chelation. Regenerates reduced glutathione (GSH) from GSSG.

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\bullet} \quad (16)$$

AMF modulates plant hormonal and stress signaling pathways (e.g., ABA, SA, JA) to enhance ROS detox gene expression, prime the plant for quicker oxidative responses, and balance ROS production and elimination (Kasote et al, 2015).

Conclusion

This scientific report on the significance of bioremediation technologies, with a particular emphasis on mycorrhizal symbiosis, in alleviating the detrimental effects of heavy metal-induced oxidative stress on plant cultivation.. The findings presented herein conclusively demonstrate that heavy metal contamination significantly threatens agricultural productivity and food safety by inducing oxidative stress in plants, which can damage cellular components and disrupt plant growth and development. The excessive production of reactive oxygen species (ROS) can overwhelm the plant's antioxidant defense system, resulting in oxidative damage to lipids, proteins, and DNA. However, various advanced and eco-friendly remediation technologies have been developed to mitigate the effects of heavy metal toxicity in plants. These include phytoremediation, nanotechnology, chelating agents, genetic engineering, CRISPR-Cas9, biochar amendment, and microbial remediation. Notably, arbuscular mycorrhizal fungi (AMF) have emerged as a promising bioremediation tool, capable of immobilizing heavy metals in the rhizosphere, enhancing antioxidant enzyme activity, and promoting plant growth. Understanding the mechanisms underlying AMF-mediated ROS detoxification, we can develop effective strategies to restore soil health and maintain agricultural productivity. Addressing heavy metal contamination requires a multidisciplinary approach integrating practical, eco-friendly remediation technologies with sustainable agricultural practices to ensure food safety and environmental sustainability. Further investigation into the mechanistic pathways underlying stress mitigation and the development of scalable remediation applications will be essential for advancing supportable agricultural technologies.

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Conflict of Interest

NA

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