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Heavy metal accumulation in mulberry and its implications on silkworm

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Abstract

Mulberry trees are indeed remarkable for their versatility and environmental benefits. Their wide distribution across various continents and ability to thrive in different climates make them a significant plant species. The robust root system of the mulberry tree plays a crucial role in improving soil shear strength and enhancing the land's resistance to erosion. This not only helps in the conservation of water and prevention of soil erosion but also contributes to the restoration of degraded lands due to heavy metal contamination and the bioremediation of polluted sites. This review explores the role of mulberry in the remediation of soils contaminated with heavy metals such as Lead, Arsenic, Cadmium, Chromium and Cobalt, as well as its potential for improving soil quality in polluted environments.

Also highlights the different mechanism and response mulberry plant to heavy metals. Recent advances in mulberry research related to heavy metal response are highlighted, with a focus on its ecological functions. The discussion also covers the well-developed root system of mulberry roots which is non-hyper accumulator, having rapid growth rate, and resilience to specific environmental stressors. The paper also examines the potential implications of heavy metal accumulation in mulberry leaves on the health and productivity of the silkworm, an important economic insect that feeds exclusively on mulberry leaves.

Keywords: Environmental, important, economic

Introduction

Mulberry is widely cultivated across the world, primarily for its leaves which serve as the sole food source for silkworms, an economically important insect. The ability of mulberry to accumulate heavy metals raises concerns about the transfer of these contaminants to the silkworm, which can potentially impact its health and productivity.

Mulberry plants have been reported to accumulate significant amounts of heavy metals within their tissues, particularly in their roots. This ability to bioconcentrate heavy metals has made mulberry a promising candidate for phytoremediation of soils contaminated with toxic metals. (Ma *et al.*, 2016) [46] (Sharma *et al.*, 2023) [64].

The global accumulation of heavy metals in the environment is an escalating concern. The simultaneous presence of multiple heavy metals, rather than a single stress factor, often poses the greatest risk and complexity (Atkinson *et al.*, 2012) [9]. Even in trace amounts, heavy metals can be highly toxic to various organisms, including humans. Among these, lead (Pb) and cadmium (Cd) are of particular concern to the scientific community due to their high toxicity and non-biodegradable nature, which makes them especially harmful to entire ecosystems.

In addition, the ecotoxicity of these heavy metals is relatively large, and the impact on the environment and the health of the population is more worthy of attention. Therefore, reducing the concentrations of toxic metals to safe levels is an urgent priority. Soil contamination by heavy metals has proved to be a serious global environmental issue, so developing an innovative, effective and sound remediation technology is necessary (Zeng *et al.*, 2011; Zhong *et al.*, 2015) [84, 89].

The cultivation of mulberry (*Morus* spp.) is crucial to sericulture providing the primary food source for silkworm (*Bombyx mori* L.). However, heavy metal contamination from industrial activities poses a significant threat to the agro-ecosystem. Accumulation of heavy metals from sources like mining, electroplating and pesticide application affects mulberry plant

health and consequently silkworms consume contaminated leaves. While essential micronutrients contain heavy metals exceeding phytotoxicity thresholds damages plant growth (Bennett, 1993) ^[11]. Tolerant plants often sequester non-essential metals in cell walls and vacuoles to mitigate damage (Wang *et al.*, 2008) ^[79].

It has been documented that heavy metals with inorganic and water-soluble forms in plants have higher activities (Wang *et al.*, 2008) ^[79] that enable them to be easily transferred to the next trophic level. Compared to plants that are sensitive to heavy metals, tolerant plants usually accumulate more non-essential metals in the cell wall and vacuoles to prevent them from injuring the more sensitive cell metabolic sites. The non-essential metals in the cell wall often are integrated with pectates and protein, in addition to becoming water insoluble.

Silkworms sensitive to their diet, suffer from reduced growth, increased mortality and impaired silk production when exposed to heavy metals, threatening sericulture economic viability. Understanding heavy metal uptake in mulberry and its transfer to silkworms is crucial for developing mitigation strategies. Utilizing silkworm studies on excrement, pupae and cocoons can help reduce environmental health risks from heavy metal contamination. Silk larvae have been successfully used as a bioindicator and to identify environmental pollution with insecticides because of the insect's high sensitivity to them. Literary sources indicate as good bio indicators of environmental status of mulberry trees and silkworm larvae. The silk larva has been successfully used as a bioindicator and for detecting environmental pollution with insecticides due to the high sensitivity of the insect to them.

What is a Heavy Metal?

A heavy metal is a type of chemical element that typically has a high atomic weight and density, which is at least five times greater than water. Heavy metals have a high density (more than 5 g/cm³) and a high atomic number. Many heavy metals are toxic or poisonous at low concentrations. For

example, lead (Pb), mercury (Hg), cadmium (Cd), and arsenic (As) are well-known toxic heavy metals that can cause serious health issues if ingested, inhaled, or absorbed by the body. Heavy metals do not degrade or break down in the environment easily. Once they are released into the environment, they can persist for long periods and accumulate in the soil, water, and living organisms. Some heavy metals, such as iron (Fe), copper (Cu), and zinc (Zn), are essential for biological processes in trace amounts but can be harmful in excess. Others, like mercury and lead, have no biological role and are toxic. Heavy metals are widely used in various industries for manufacturing electronics, batteries, pigments, and other products. Their widespread use has led to environmental contamination and concerns about their impact on human health and ecosystems. Exhibit toxic properties and can have adverse effects on the environment and living organisms due to their persistence and potential for bioaccumulation. Concern in areas such as pollution, environmental chemistry, and industrial processes where their presence may pose risks to human health and ecosystems.

Sources of Heavy metals

Various heavy metals are essential in nature, playing critical roles in the normal growth and development of plants. Key heavy metals such as copper (Cu), zinc (Zn), iron (Fe), manganese (Mn), molybdenum (Mo), and nickel (Ni) are vital in plant biochemistry and physiology (Zhuang *et al.*, 2009) ^[92]. As essential micronutrients, Cu and Zn are particularly important for normal plant growth, serving as cofactors and activators in enzyme reactions, or acting as catalytic agents in metalloproteins, such as those with prosthetic groups. These metals are also involved in redox reactions, electron transfer processes, nucleic acid metabolism, and are integral components of several enzymes. While the presence of these metals in specific concentrations is crucial for healthy plant growth, their excess can lead to toxic effects (Nagajyoti *et al.*, 2010) ^[51].

Table 1: Different sources of heavy metals in the agriculture and environment.

Sources	Heavy metals	References
Mining activities/smeltering	Cr, Pb, Zn	Sumner (2000) ^[72]
Geological and anthropic activities	Cr, Co and Ni, Cr, Pb, Zn	Facchinelli <i>et al.</i> (2001) ^[16]
Sulfide mineral deposits	As	Nordstorm (2002) ^[56]
Earth's crust	Cd, Hg, Pb, As	Järup (2003) ^[26]
Phosphate fertilizers	Cd	Järup (2003) ^[26]
Fossil fuels combustion, chemical industries, electroplating	Ni, As, Cr	Khodadoust <i>et al.</i> (2004) ^[36]
Coal mining and steel processing industries	Zn	Greaney (2005) ^[22]
Sewage, manures, sludge, limes	Cd	Yanqun <i>et al.</i> (2005) ^[83]
Poultry waste, feed additives	As	Mukherjee <i>et al.</i> (2006) ^[48]
Surface runoff from rain/snow	Hg	Kowalski <i>et al.</i> (2007) ^[39]
Weathering of sedimentary rocks like sandstone and dolomite	Mn, Cr, As, Fe, Zn	Viers <i>et al.</i> (2007) ^[74]
Industrial wastes	As	Tripathi <i>et al.</i> (2007) ^[73]
Anthropogenic activities	Pb, Zn, Cu, Co, Ni, As	Jordanova <i>et al.</i> (2008) ^[32]
Waste dumping	Cd, Pb, Cu	Zhuang <i>et al.</i> (2009) ^[92]
Ores/mineral dissolution	Fe, Mn, Cr, Cd, Hg	Huffmeyer <i>et al.</i> (2009) ^[25]
Pesticides	Hg, As, Cu, Zn	Arao <i>et al.</i> (2010) ^[4]
Aerosols	As, Zn, Cd, Pb	Nagajyoti <i>et al.</i> (2010) ^[51]
Atmospheric aerosols, windblown dust	Pb, Hg, Ni	Kang <i>et al.</i> (2011) ^[35]
Industrial wastes and geological minerals	As	Murcott (2012) ^[50]
Industrial waste pipes/additives	Cu	Mohod and Dhote (2013) ^[47]
Volcanic emissions, forest fires, atmospheric deposition	Pb, Hg, Ni	Li <i>et al.</i> (2014) ^[44]
Geological minerals	As, Cr, Pb, Mn	Kibria (2014) ^[37]
Sewage irrigation	Cd, Pb, Cr, Hg	Su (2014) ^[71]

Industrial pollution	As	Gillispie <i>et al.</i> (2015) ^[21]
Geological processes and anthropogenic activities	Pb, As, Hg, Cd	Nkansah <i>et al.</i> (2016) ^[55]
Metallic mining	Cd, Zn, Pb	Romero-Baena <i>et al.</i> (2017) ^[61]
Chemical industries and geological minerals	Cd, Pb, Zn, As	Bech (2018) ^[10]
Anthropogenic activities and industrialization	Cu, Cr, Zn, Ni	Adimalla (2019) ^[1]
Smelting activities and agricultural industry, industrialization and urbanization	Ni, Cu, Cr, Mn, Zn, Fe	Doabi <i>et al.</i> (2019) ^[15]
Geological and anthropogenic activities	Al, Fe, Mn, Si, As Cr, Hg, Ni	Kambunga <i>et al.</i> (2019) ^[34]

These heavy metals are also known as trace elements (10mg/Kg or mg/L in soil/aquatic medium) or ultra-trace elements (1µg/Kg or µg/L in soil / aquatic medium) because of their presence in the soil environment.

In addition to these essential trace elements, another category of heavy metals, Class B metals, considered to be non-essential trace elements such as As, Cd, Hg, Cr, Pb, and Co etc., are in nature very toxic. The mechanism of heavy metal-induced toxicity in plants is summarized in Figure 1.

In terrestrial systems, plants are stationary, with roots serving as the primary contact points for trace metal ions. In aquatic systems, however, the entire plant is exposed, allowing metal ions to be absorbed directly through leaf surfaces due to particle deposition (Kibria, 2014) ^[37]. Thus, plants primarily absorb heavy metals through their roots, though leaf surfaces also play a role, especially when particles containing metals are deposited.

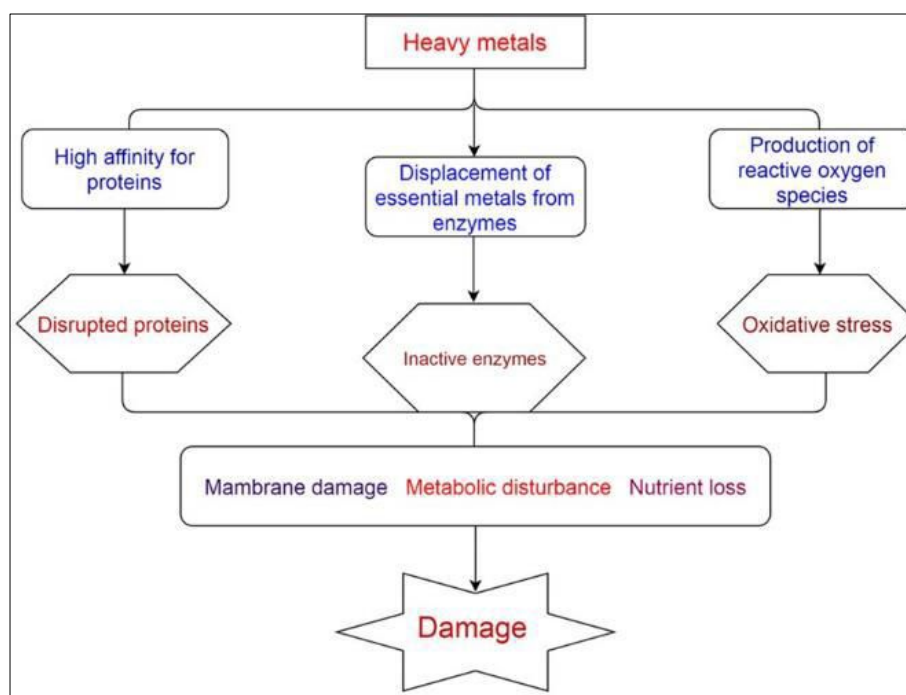


Fig 1: Summary of heavy metals induced toxicity mechanism in plants (Adopted from: Kumar *et al.*, 2016) ^[41].

Response of Mulberry plant to Lead (Pb) accumulation

Lead (Pb) is a non-essential and toxic trace element in the environment. The soils in some locations are contaminated by Pb due to anthropogenic activities (Stafilov *et al.*, 2010; Jin *et al.*, 2015) ^[70, 31]. Pb pollution in soils is covert, persistent and non-biodegradable. Soils polluted by Pb could be a source of contamination for water and air (Laidlaw *et al.*, 2012) ^[42].

Biomass allocation and photosynthetic characteristics of mulberry under different lead (Pb) concentrations are studied by combining exogenous biochar and partial root zone drought (PRD) treatments. The results revealed that mulberry could reduce the stem mass ratio (SMR), as well as increase the leaf mass ratio (LMR) and root mass ratio (RMR) in Pb-contaminated soil, which supported the functional balance theory of biomass allocation. (Wang *et al.* (2020) ^[76].

With higher soil Pb concentrations, the LMR of the control revealed an upward trend. Under Pb-free and mild soil Pb (50 mg kg⁻¹), both the PRD and biochar treatments tended to increase the LMR of mulberry seed lings, while under

moderate and severe soil Pb stress, they tended to decrease the LMR.

Nasir *et al.*, (2007) ^[52] reported the lead in soil results obtained clearly showed that the amount of Pb (II) deposited in soil by synthetic effluents was strongly dependent on effluent pH and effluent Pb (II) concentration. The highest deposited amount of lead was found to be 326.5-0.04 mg/kg at pH 5 and initial lead concentration of 400 mg/litre. Widely spread lead is leading toxic heavy metal to living organisms. Ozer *et al.* (1994) ^[57] found that the main lead contamination source were sugar finishing industries, automobile batteries, pigments for printing processes, etc. Results of this study clearly showed that lead concentration was much higher in soil irrigated using lead-containing effluent in comparison to the control soil sample. Lead precipitation occurred after pH 5. It is suggested that effluent pH should be increased to 5 to cause Pb (II) precipitation from the effluent used for irrigation.

Heavy metals especially lead (Pb) and cadmium (Cd) are pollutants of very high priority concern in the scientific community because apart from being toxic to the entire ecosystem, they are non-biodegradable. Si *et al.*, (2021) ^[68]

studied the effects of Pb and Cd on seed germination, seedling growth and silkworm growth under single stress and combined stress, attempting to explore accumulation in mulberry plants. The experiment was conducted on treatment with Pb concentrations of 0, 100, 300, 500, 700, 900, 1000, 1100, 1200 and 1300 mg L⁻¹ and analyzed the toxic effects of mulberry (*Morus alba*). Mulberry seeds survived when the concentration of Pb is lower than 1200 mg L⁻¹ indicating that mulberry seeds have a certain tolerance to Pb. Lanier concluded that the Pb-treated plants exhibited longer leaves and roots and less DNA damage than Cd treated ones. Therefore, when screening plant heavy metal species for soil restoration, whether the plant is resistant to various heavy metals and the law of reaction to heavy metal complex stress (Lanier *et al.*, 2019) [43]. It also found that Pb accumulated mainly in the roots of mulberry plants and therefore has a greater influence on growth and also found that mulberry is resistant to lead and cadmium ions and can be used for metal fixation.

Heavy metals have many effects on plant growth and metabolism, especially on photosynthesis. The results showed that the net photosynthetic rate of mulberry leaves was inhibited by Pb and Cd. The important reason may be that the chloroplast structure of plants under heavy metal stress is poisoned, which directly interferes with the biosynthesis of chlorophyll (Arriagada *et al.*, 2005) [5].

Jiang *et al.*, 2020 [30] have evaluated effects of planting density and mulberry varieties on heavy metal accumulation and also studied the Bioconcentration factors and Translational factors of Cd and Pb in mulberry.

The transportation of Pb in the soil mulberry-silkworm food chain was also evaluated by pot experiment. Plenty of Pb was stored in root, reducing its migration to stem and leaf. Over 92% of the Pb in the leaves was deposited in the cell wall, and 95.3-95.6% of the Pb in the mulberry leaves was integrated with oxalic acid, pectates and protein, which had low bioavailability and reduced the damage to organelle (Zhou *et al.*, 2015) [91].

Wang and Ji 2021 investigated the responses of mulberry (*Morus alba* L.) to lead (Pb) contamination in soil, focusing on glutathione (GSH) metabolism and calcium (Ca) secretion. The study reveals that mulberry demonstrates considerable adaptability to Pb stress, maintaining biomass across various Pb levels, which highlights its potential for phytoremediation efforts. The findings indicate that GSH plays a crucial role in detoxifying Pb by chelating heavy metals, thereby reducing their biological toxicity. The expression of GSH-related genes is sensitive to Pb presence, suggesting a biochemical response that aids in mitigating toxicity. Additionally, the research identifies calcium oxalate crystals as significant in the detoxification process, particularly under severe Pb stress. These crystals may facilitate the excretion of Pb-Ca oxalate complexes, enhancing the plant's ability to cope with heavy metal stress. Morphological adaptations, such as leaf thickening and increased vascular development, were also observed, which may improve Pb transport to aerial parts. Overall, this study underscores the importance of understanding the physiological and biochemical mechanisms in mulberry that contribute to its resilience against heavy metal stress, providing valuable insights for future environmental remediation strategies. Mulberry has a higher resistance to soil Pb stress, and it can be planted in moderate Pb-

contaminated soils for no loss of biomass and can safely harvest the branches and leaves.

Response of Mulberry plant to Cobalt (Co) accumulation

The transfer of Co from soil to plants appears to be a viable route to expose lower trophic levels to biologically significant concentrations and potentially accumulates in biomass and top soil (Gal *et al.*, 2008) [19]. Cobalt exposure has been linked to reduced growth and nutritional quality in mulberry leaves, with higher concentrations leading to declines in total protein and chlorophyll content.

In higher plants, the absorption of Co²⁺ by roots involves active transport, where the metal is taken up and then moved through the cortical cells via both passive diffusion and active processes. Once in the xylem, Co²⁺ is primarily transported by the transpirational flow, while its distribution through the sieve tubes is acropetal, facilitated by complexing with organic compounds (Palit *et al.* 1994) [58].

The soil, in which mulberry plants were planted, was irrigated with Co (II) synthetic effluents with pH ranging from 3 to 5 and with initial Co (II) concentrations ranging from 25 to 400 mg/L. The concentration of cobalt in soil before irrigation using wastewater was 8.45-0.02 mg/kg. After irrigating soil using wastewater, the concentration of Co (II) in soil increased significantly. The maximum amount of Co (II) residues in soil at pH 4 and 400 mg/L initial concentration in synthetic wastewater. It was found that the Co (II) contents in soil were increased with increase concentration of Co (II) effluents. It reached its maximum value of 273.5-0.04 mg/kg at the highest concentration of 400 mg/L after 75 days of irrigation (Ashfaq *et al.*, 2009) [8].

Increasing concentration effect was also observed by Perez-Espinosa *et al.* (2005) [59]. The lowest Co (II) contents were recorded to be 59.5-0.01 mg/kg at 25 mg/L dose rate after the same time period. The Co (II) contents in soil were also increased with the increase in pH and were maximum (206-0.04 mg/kg) at pH 4 and minimum (19.5-0.01 mg/kg) at pH 3 after 75 days. The accumulation of Co (II) in other plants was studied by previous researchers. They observed significant effect of Co (II) on plant growth.

Response of mulberry plant to cadmium (Cd) accumulation

Cd was reported to be the one of the most prominent soil HMs with substantial toxicity (Calao and Marrugo, 2015) [12]. Cd in the soil is easily transferred to phytophagous insects (Gall *et al.*, 2015) [20].

Combined stress of cadmium and lead could increase the transfer coefficient and enrichment coefficient of Pb and Cd in the mulberry, and promote the absorption of heavy metal ions from the soil. Further they have found that mulberry seeds can be used to fix heavy metal ions from the soil and mulberry roots was found to be key part in heavy metal-enrichment in root as strong ability to enrich cadmium and accumulated in the order root, stem and leaf. Thus, mulberry can be considered for restoration of contaminated soils. (Si *et al.*, 2021) [68].

When the Cd content in soil was 8.49mg/kg, there was no apparent harmful effect on mulberry growth. A yield reduction in the leaves became distinct at the soil Cd content of 75.8 mg/kg. Whereas at 145 mg/kg Cd in soil, the plants exhibited marginal growth (Wang *et al.*, 2004) [75]. In one of the studies found that 53.3-70.2% of Cd the mulberry root

accumulated from soil was stored in the root and only about 10% was in the leaf. Lots of the Cd was located in the cell wall of the mulberry root and in soluble fraction of the mulberry leaf (Huang *et al.*, 2018) ^[27].

Usually, toxic metal-tolerant plants utilize two of the most important detoxification strategies-cell wall deposition and vacuolar compartmentalization (Zhang *et al.* 2013) ^[88]. Most of the heavy metals deposited in cell wall can be fixed by pectin acid, polysaccharides, and proteins so that they lose their activity and do not injure more sensitive intracellular metabolic sites (Wojcik *et al.* 2005) ^[81]. The chemical forms of Cd obtained from different extraction solutions are closely related to their biological function and migration ability (Wang *et al.* 2008) ^[79].

In Cd treatment groups (0–8 mg kg⁻¹), the concentrations in the roots and stems were higher than those in the soil. These indicated that the mulberry survives by sequestering the majority of Cd in the roots to protect the leaves, whereas the high TFs from the soil to roots and from the roots to the stems favour phytoremediation. Subcellular distribution and chemical forms of Cd in the mulberry leaves indicated that mulberry plants use cell wall deposition and compartmentalization as detoxification mechanisms. (Zhou *et al.* 2015) ^[91]. Percentage of Cd bound to the cell wall declined for the 64 mg kg⁻¹ Cd group, the percentage of Cd in the organelles was stable and remained lower than control due to increased soluble percentage of Cd, that large amount of intracellular Cd was transferred into vacuoles and chelated with low molecular weight organic acids after the cell-wall binding sites reached saturation (Krämer 2000; Weng *et al.* 2012) ^[40, 80].

Mulberry trees could grow naturally in the soils strongly polluted by Cd and Pb. The concentrations of Cd and Pb in the mulberry root were higher than that in stem, branch or leaf. Thus, the mulberry trees could become candidate species for phytostabilization for heavy metals polluted soils (Jiang *et al.*, 2019) ^[29].

Basically, soil Cd pollution has no unfavourable effects on mulberry tree. The leaf biomass and number of branches showed initially a slight increase when the Cd concentration in soil was up to 20mg/kg, and decreased afterward (Prince *et al.*, 2002) ^[60].

Jiang *et al.*, 2018 ^[27] studied adsorption behaviour of Cd ions in rhizosphere soil (RS) and non-rhizosphere soil originated from mulberry field. The maximum adsorption of Cd²⁺ for RS (5.87 mg/g) was slightly bigger than that for NS (5.36 mg/g). Mulberry roots modified the physical and chemical properties of the RS under field conditions, which also affected the Cd sorption efficiency by soil components during experiment. Understanding Cd²⁺ sorption processes in the rhizosphere of mulberry trees are crucial if these trees are to be used for phytoremediation of Cd-contaminated soils.

Considering use of polluted paddy soils and the increase of farmers' income, Nongsang 14 and Qiangsang 1 could be recommended to plant in the Cd and Pb contaminated soils with the planting density 45,000 plants/ha. Once the mulberry garden is established, they could reduce the soil erosion and phytostabilize heavy metals by changing the physicochemical properties of rhizosphere soil (Jiang *et al.*, 2017) ^[28].

Cadmium significantly inhibited mulberry plant growth and primarily accumulated in mulberry roots. Antioxidant enzymes were induced by cadmium in all tissues of

mulberry. Subcellular fractionation analyses indicated, majority was compartmentalized in soluble fraction in roots while it mainly located in cell wall in leaves and stems. Cadmium was integrated with proteins and pectates in tissues and revealed various metabolic pathways involved in cadmium response. (Dai *et al.*, 2020) ^[14].

Biomass of *M. alba* L. was significantly increased with cultivation time in Cd-contaminated soil. Normal photosynthesis, antioxidant protection, and macronutrient regulation contribute to high tolerance. Catalase (CAT) activities in plant leaves and the Ca and Mg contents in plant roots were enhanced in response to Cd stress after 180 d of cultivation Cd (Zeng *et al.*, 2020) ^[87].

Response of Mulberry plant to Chromium (Cr) accumulation

Cr (VI) seems to inhibit growth by act principally on plant roots (Mukherji and Roy, 1978) ^[49]. Cr causes oxidative damage by destruction of membrane lipids and DNA damage or may even cause the death of plant species (Singh *et al.*, 2013) ^[69]. Chromium Cr (VI) is one of the most toxic, especially when compared to trivalent chromium (Korshoj *et al.*, 2015) ^[38].

Solubility of Cr (III) in soil was dependent on pH and decreased dramatically at pH greater than 4.5. At pH values less than 3, the predominant form of Cr in the Cr (III) system was Cr (III). The toxicity symptoms of Cr appeared as severe wilting and chlorosis of plants. Chlorosis appeared in the upper leaves of Cr-affected plants.

Cr (VI) bio accumulated in mulberry leaves was 51.43±0.001 mg/kg when initial concentration of chromium in wastewater is 300 mg/L. In Cr (VI) uptake pH of wastewater is one of most important parameters. When soil pH 4 then maximum bioaccumulation of Cr (VI) in mulberry leaves occurred were 89.76±.031 and at pH 8 minimum was 23.23±.02 And suggests that it could harmful for ecosystem (Ali *et al.*, 2021) ^[3].

The amount of Cr (III) accumulated into soil, in the *Morus alba* leaves increased with pH of the effluent. The maximum Cr (III) amount observed in soil and leaves was 99.89± 1.02, 95.67 ± 0.58, respectively at pH 5 and at 200 concentration (200 mg/L). Concentration of Cr (III) in leaves of mulberry was increased with increase in concentration and pH of synthetic effluents and suggested that bioavailability of Cr (III) can be reduced by decreasing the pH of effluents being used for irrigation at certain limit (Ashfaq *et al.*, 2012) ^[7].

Ali *et al.*, (2009) ^[2] reported that *B. mori* body length and body weights were significantly reduced by Cr (III) concentration in larval body and mortality rate increased. Control larvae showed more body length and body weight and less mortality rate in comparison to those larvae that accumulated Cr (III) into their bodies. Some other research workers also noted negative impact of heavy metal accumulation on larval growth of silkworm larvae.

Response of Mulberry to other heavy metals

Shukla *et al.* (2011) ^[67] examined the heavy metal(loid) uptake capacities in *Morus alba* L. plant and found in the order of Zn > Cd > Pb > As. Heavy metal(loid) was transported from underground parts to shoots via long-distance translocation in the xylem, complexation with cellulose in the cell wall, and storage in the cells' vacuoles. In this study, Cd, Pb and Zn were mainly deposited in the roots of *M. alba* L., and the maximum contents could reach

23.4, 7.40 and 615.5 mg/kg, respectively. The immobilisation or isolation of heavy metals in the cell walls of the plant's roots is one of the important detoxification processes for plant growth (Zeng *et al.* 2018b) ^[86].

Mercury contamination from human activities, especially industrial, increased significantly from the industrial era, with Asia's share rising to 56%. Aerial organs of Mulberry nigra have had significant difference for mercury accumulation in various concentrations and suggested that the absorption mercury in the root is more than it in leaf and stem. The mercury accumulation in plant tissues in cell surface is toxic and cause growth decrease (Hashemi *et al.*, 2018) ^[23].

Ashfaq *et al.* 2009 ^[2] found deposition of Zinc in soil and mulberry plants when irrigated with synthetic effluent, Zinc (II) deposited in soil and plant was increased when pH of the effluent was in the acidic range. The maximum Zn (II) amount detected in soil and leaves was 386.51 ± 0.03 , 142.85 ± 0.001 mg/kg. Further, concluded bioavailability of Zn (II) can be reduced by increasing the pH of effluents being used for irrigation.

Reports have shown that mulberry trees could grow in soils where the Pb, Zn and As concentrations were 734, 1194 and 53 mg kg^{-1} , respectively, without showing noticeable stress symptoms (Xu *et al.*, 2015) ^[82].

Nikolva, 2015 recorded the presence of heavy metals in green and yellow mulberry leaves, among heavy metals Zinc accumulated in higher concentration in the green 149 mg/kg and in the yellow leaves 112 mg/kg due to this element plays a role in the cellular defense system by affecting lipid, protein and chlorophyll. Pb also found in the mulberry leaves (112.0 mg/kg in the green and 85 mg/kg in the yellow) and this difference in accumulation is due to rapid growth, nutrition and development of plants in the growing season the concentration of heavy metals in the aerial parts of mulberry can be influenced by the intensity of transpiration. When the yellow leaves this process is delayed and the number of heavy metals in plant samples is less. The presence of copper (Cu) and cadmium (Cd) is low and there is no essential for the proper development and function of the plants, thus ease of breeding, great foliage and deep root system make it suitable for phytoremediation.

Implications of Heavy Metal Accumulation through Mulberry on Silkworms

Silkworms (*Bombyx mori*) are poisoned by heavy metals through a soil-mulberry-silkworm system, which inhibits larval growth and development and leads to a decrease in silk production. Silkworm is an important economic insect that suffers severely from heavy metals toxicity via the soil-mulberry-silkworm system (Jiang *et al.*, 2020) ^[30].

Cr (III) was found in larvae and faeces (61.32 ± 0.036 and $58.97 \pm 0.451 \text{ mg/kg}$) when plants irrigated with synthetic effluent in different concentrations. At pH 5 and at 200 mg/L concentration, Cr (III) present in *B. mori* body produced toxic effects on First instar of *B. mori*. Body length, body weight of *B. mori* decreased with increase in bio-accumulation Cr (III) amount in larval body (Ashfaq *et al.*, 2009) ^[6]. Similarly, effects on silkworm were observed in the silkworm body when deposited with Zinc (II) (Ashfaq *et al.*, 2010) ^[8].

Higher the accumulation of cobalt in mulberry leaves more the toxicity to silkworm population. Accumulation of Co in silkworm was maximum at pH 4.5 at an initial Co

concentration of 400 mg/L in the synthetic effluent clearly indicated that silkworm can be used as template to indicate local cobalt pollution as its body length, body weight and mortality rate was found to be strongly related to cobalt concentration (Ashfaq *et al.*, 2009) ^[6].

At 300 mg/L Cr (VI) concentration and pH 4 there was maximum deposition of Chromium (VI) in *B. mori* larvae, faeces and silk glands from the synthetic effluent. The maximum deposition $23.31 \pm 0.019 \text{ mg/kg}$, $41.32 \pm 0.069 \text{ mg/kg}$ and $35.67 \pm 0.04 \text{ mg/kg}$ observed respectively. Most of Cr (VI) remains in its body. Cr (VI) found in *B. mori* body was liable for toxic effects on its life cycle and the body growth and silk production was also inhibited under the effect of Cr (VI) accumulation. (Ali *et al.*, 2021) ^[3].

Zhou *et al.*, 2015 ^[91] found no adverse effects of lead on silkworm, which suggested that silkworms have a strong tolerance to Pb due to two detoxification mechanism "excrete" and "homeostasis". Metallothionines (MTs) played an important role in the tolerance mechanism of the animals against adverse environmental factors, especially heavy metal stress. MTs are a family of soluble, heat stable and low-molecular-weight proteins rich in cysteine, which exhibit a high affinity for metal ions and can detoxify non-essential metals to protect the cells and tissues (Kaegi and Schaeffer, 1988; Sauge-Merle *et al.*, 2012) ^[33, 62]. MTs1 found to be synthesized during low Pb stress, whereas MTs2 and MTs3 was induced strongly when excess Pb was transported.

In experiment, the weight, length and survival rate of silkworm were less affected by Pb and Cd, indicated that silkworm had higher tolerance to heavy metals (Si *et al.*, 2021) ^[68]. It may be that the detoxification mechanism of silkworm is influenced by different heavy metal types, the growth stage of silkworm and environmental temperature (Schaller *et al.*, 2011) ^[63].

Cr (VI) found in *B. mori* body was liable for toxic effects on its life cycle and the body growth and silk production was also inhibited under the effect of Cr (VI) accumulation. A considerable amount of Cr (VI) was also found deposited in the silk glands, cuticle and alimentary canal, and concentration of Cr (VI) in larval body increased *B. mori* death rate significantly when mulberry plants were irrigated with synthetic effluent of Cr (VI) (100 mg/L with pH 4.5).

The mortality rate of silkworms reared on mulberry leaves from fields with different contamination levels will vary, with higher contamination generally leading to higher mortality. This highlights the importance of monitoring and controlling heavy metal levels in agricultural fields used for growing mulberry, particularly in regions where sericulture is a significant economic activity. Mortality of silkworms might be due to the concentration of heavy metals in the leaf (Pb, negatively) and the concentrations of as and Pb in the leaf residual (both negatively) and the capacity of silkworms to excrete as and Pb (both negatively) (Feng *et al.*, 2019) ^[17].

Endocytosis, oxidative phosphorylation, and the MAPK pathways are involved in Cd toxicology in response of Cd exposure *B. mori* midgut. Novel metallothionein in silkworm, that improves heavy metal tolerance in *B. mori* cell lines was identified and transgenic overexpression of metallothionein in silkworm decreased larval death under Cd exposure, a new possibility improving silkworm breeding (Liu *et al.*, 2021) ^[45].

Gut microbiota of *B. mori* are reported to shape host health. Phyllosphere bacteria was investigated to evaluate endogenous Cd-polluted mulberry leaves at different concentrations and found change in the gut bacteria of silkworm and significant change in the abundance of dominant phyla of gut bacteria of *B. mori*. A significant variation in the specific bacterial community indicated the adaptation of *B. mori* gut for its role in heavy metal detoxification and immune function regulation (Chen *et al.*, 2023) ^[13].

Effect of Pb²⁺-polluted mulberry leaves on intestinal bacteria of silkworm exposure to Pb²⁺ increased the alpha diversity of gut bacteria and changed the composition of gut bacteria in *B. mori*. Silkworms increase in α -amylase and trypsin activity and a decrease in lipase activity. The results showed that the accumulation of peroxides induced by Pb²⁺-M exposure and the disorder of intestinal microbes led to physiological damage of the midgut of silkworm (Shi *et al.*, 2024) ^[65].

Conclusion

Mulberry's extensive ecological flexibility to light, temperature, water, soil, and other natural circumstances enable it to serve a variety of ecologically protective roles.

Understanding the plant response characteristics to heavy metals is important for the phytoremediation processes in contaminated soil. As for some mechanisms and morphological modifications in plants in the rhizosphere, which can improve plant growth, increase the availability and mobility of heavy metals (Loids) in contaminated soils, strengthen the resistance of plants to heavy metals and promote plant uptake of these metals.

The key parts of heavy metal enrichment are root and mulberry have a strong ability to enrich Cd, Co, Cr, Zn and Hg but a weak ability to enrich Pb. Most heavy metals are translocated to silkworms and most of them were excreted by silkworm faeces.

Mulberry can be planted in moderate Pb-contaminated soils since there is no loss of biomass and you can safely harvest the branches and leaves and find little effect on the silkworms. Maintaining the productive function of crops is a feasible option to mitigate the negative and toxic impacts of low and moderate Pb-contaminated soils.

Considering multiple factors influencing HM tolerance and accumulation, even if the heavy metal pollution in the soil is serious, the accumulation efficiency along the food chain is low, and the impact on the growth of silkworm is small. Therefore, the use of mulberry-rearing silkworm in heavy metal-contaminated areas can be considered for restoration, and the economic benefits can be obtained at the same time. The review provides valuable insights into the ecological and toxicological effects of heavy metal accumulation in mulberry and exposure to silkworm, provides a reasonable method for minimizing heavy metal pollution, and promotes sustainable development of sericulture. Thus, mulberry and silkworm can be a promising approach for the remediation of HM polluted soils.

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