A review on heavy metal toxicity on the growth & development with DPPH-scavenging activity in different food crops

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***Abstract*: Heavy metal toxicity is an everlasting environmental hazard affecting our ecosystem including plants and animals respectively. Assimilation of heavy metal has increased drastically with the expansion of world-wide population and environmental pollution. The high level of contamination by these metals such as lead (Pb), nickel (Ni), cadmium (Cd), chromium (Cr), arsenic (Ar) & mercury (Hg) is detrimental. Their uptake by plants and subsequent accumulation in food crops is exceedingly harmful as it alters biochemical, physiological, and metabolic processes of the plants. This comprehensive review focuses on the negative effects of heavy metal in growth and development of different food crops. In-depth discussion is further conducted on morphological changes, antioxidant enzyme activities, oxidative damages, & DPPH- scavenging activity.**

***Keywords*: Heavy metal, seed germination, oxidative stress, enzyme activity, DPPH-scavenging activity**

Introduction:

Heavy metals are ubiquitous components of the Earth's crust and it is considered as trace elements because they are present in trace densities in the environment. Over the last few decades, the emission of pollutants including heavy metal into the environment has increased enormously due to rapid industrialization, urbanization and overuse of agricultural amendments. Heavy metal contamination is now a prime environmental concern that threatens the plants, animals, and humans' health. These metallic elements are considered as systemic toxicants which are known to be harmful even at lower levels of exposure. [2] Among earth's many resources soil is one of the foremost. Hence soil's importance in our lives is vast. Both natural and anthropogenic sources are responsible for the accumulation of heavy metals in the soils. Natural sources include breakdown of parent rock materials, volcanic eruptions etc. Anthropogenic inputs like extensive use of inorganic and organic fertilizers, pesticides, wastewater irrigation, sewage sludge supplementation, higher atmospheric depositions by industrial units and combustion of fossil fuels have led to elevated levels of inorganic pollutants in the soils. Essential and non-essential heavy metals generally produce common toxic effects on plants, such as inhibition of growth, low biomass accumulation, chlorosis, altered water balance and accumulation of nutrients, which ultimately cause death of plants.

Heavy metal accumulation in the environment:

Heavy metals are among the most investigated environmental pollutants as well as potentially hazardous because of bioaccumulation through the food chain. [35] Cadmium (Cd) is considered as one of the most toxic metals for plant growth and development which gets naturally released by volcanoes or by weathering rocks & contaminated with soil. It is an unessential trace element in plants, due to its acute toxicity, high water solubility, non-degradability, and persistence inside most living organisms. Anthropogenic input of cadmium to soils occurs by aerial deposition and sewage sludge, manure and phosphate fertilizer application and disposal of products containing cadmium. The accumulation of Cd severely restricted photosynthesis and its non-stomatal limitation in regulating the photosynthetic performance of Hybrid Pennisetum. At 10 and 20 mg kg^{-1} Cd concentrations the leaf chloroplasts showed no noticeable changes, but the chlorophyll content at 50–100 mg kg^{-1} Cd concentration significantly decreased by 9.0–20.4%. [9] .

Nickel (Ni) is a necessary micronutrient for plant growth and development. Global input of Ni to the human environment is approximately 150,000 and 180,000 metric tons per year from natural and anthropogenic sources. [41] The primary sources of Ni emissions into the environment are industries of combustion of coal and oil for heat or power generation, Ni mining, steel manufacture, and other miscellaneous sources, such as cement manufacture. Active transport system & passive diffusion are the main mechanisms through which Ni is taken up by plants. Soluble Ni compounds are absorbed by plants passively, through a cation transport system whereas chelated Ni compounds are taken up through secondary, active-transport-mediated systems [20]. Once absorbed by roots, Ni is easily transported to shoots via the xylem through the transpiration stream and accumulates in buds, fruits, and seeds. Exposure to 1 mM NiSO4 solution decreases the mesophyll thickness, the size of vascular bundles, the vessel diameter and lateral vascular bundles, and the width of epidermal cells in *Triticum aestivum* leaves.[36]

Chromium (Cr) stress is one of the most adverse factors that affects sustainable crop production. Acidification of soil influences chromium uptake by the crops. [21] Cr exists in several oxidation states but the most stable and common forms are Cr (0), Cr (III) and Cr (VI). Its toxicity in plants depends on its valence state as Cr (VI) is highly toxic, while Cr (III) is less toxic. Chromium is taken up by plants through carriers of essential ions such as sulphate.[42] This metal is widely used in industry as plating, alloying, tanning of animal hides, textile dyes and mordants, pigments, ceramic etc [43]. Cr also causes deleterious effects on plant physiological processes such as photosynthesis & metabolic alterations [39]. In a study where barley seedlings grown in 100 µM

Cr showed 40% inhibition of growth [38].
Lead (Pb) is unessential heavy metal, well-known for its ecotoxicology and non-biodegradable in nature [22]. High concentrations of lead (Pb) in the soil negatively affect biochemical and physiological processes of food crops [40]. Lead is one out of four metals that have the most damaging effects on human health. [44] Sources of lead pollution in India may be divided into two major categories: Industrial and domestic. The industrial lead exposures are mainly generated by coal burning and roasting of minerals like iron pyrites, dolomite, alumina etc. and the domestic lead exposures come mainly from cooking by use of the solid fuels like coal, biomass, agricultural waste, paints, ceramic glazes, cosmetic, etc. Increasing the amount of lead creates measures for environmental health problems in India.
Mercury (Hg) is a heavy metal belonging to the transition element series in the periodic table. It is exceptional because it exists in nature in three forms which are elemental, inorganic, and organic, but all these forms are toxic even at low quantities. At room temperature mercury exists in a liquid form with a high vapor pressure and released into the environment as mercury vapor. The main sources of Hg are from the industries of Alkali and metal processing, incineration of coal, medical wastes, and mining of gold. Atmospheric deposition is the dominant source of mercury accounting to its wide-spread distribution. Natural sources of mercury include volcanoes, geological deposition, and volatilization from the ocean. Although all rocks, sediments, water, and soils naturally contain small but varying amounts of Hg.[46] This metal is a widespread environmental toxicant and pollutant which induces severe alterations in the plant tissues and causes a wide range of adverse effects in both plants and animals.
Cobalt (Co) is biologically important for living organisms at lower doses but they can be phytotoxic at higher concentration. The tolerance limit of Co in soil for the growth of plants was found to be 0.2–0.5 ppm.[46] It exists in the form of minerals like cobaltite, erythrite, and heterogenite. Total 15% of cobalt produced worldwide from manufacturing industries of hard metals [47]. Cement industries and power tool grinder industries are also responsible for Co accumulation [48,49]. Industries related to e-waste processing have also been found to release Co at a high level[50,51] ,diamond polishing disc that is made up of fine cobalt is also a source of generating Co dust[52,53] ,pigment and paint industries are respectively responsible for Co deposition [54], incinerators produce bottom ash which contains Co that leaches to the soil and ground water [55], mobile batteries, televisions, liquid crystal display TVs, and computer monitors also contain Co and become potential sources of Co contamination [57,58,59] . Several cosmetic products are also a source of Co as impurities [60].

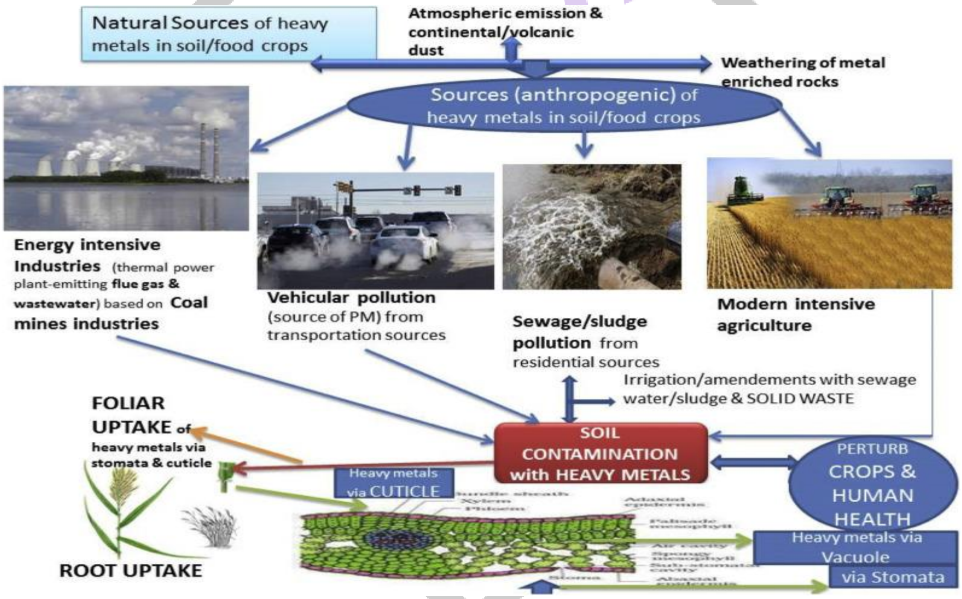

Fig 1: Heavy metal sources in environment [37]

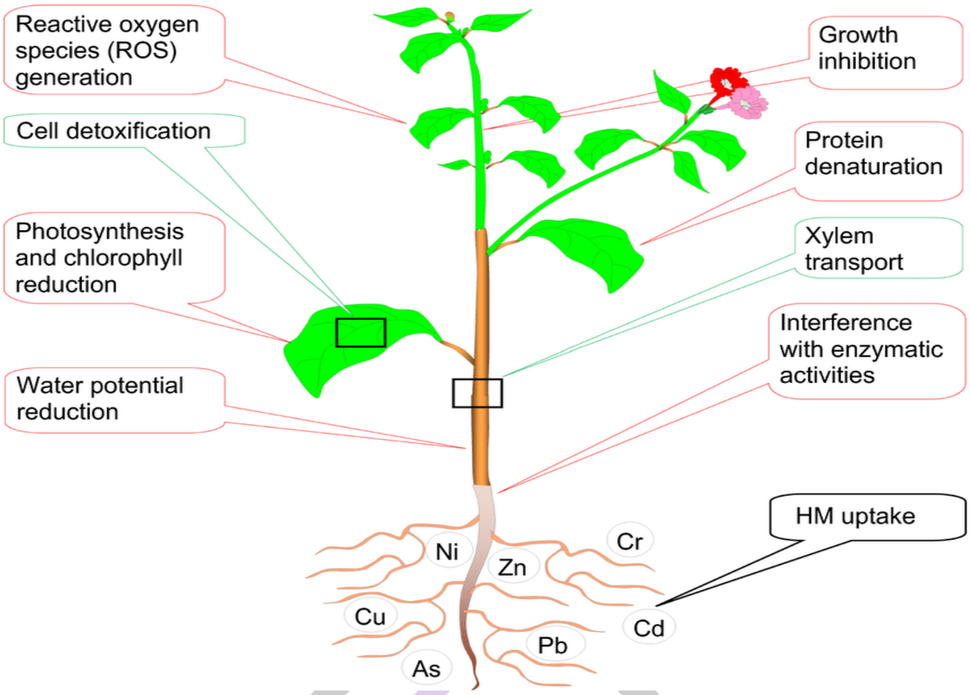

Fig 2: Heavy metal uptake & side effects in plants. [15]

Heavy metal effects on plant morphology:

Increased concentrations of heavy metals exhibited the highest detrimental effect on morphological characteristics in almost all seedlings. At higher concentrations of Pb, seed germination has stopped with abnormalities. The interactive effect between different concentrations of heavy metal showed that Pb with 200 mg L^{-1} had the lowest germination percentage and the control had the highest germination rate. With escalating concentrations of Pb in the medium the highest daily mean germination was obtained (5.58) at 200 mg L^{-1}. The lowest coefficient of velocity of germination was observed in 200 mg L^{-1} (138.9), On the other hand the lowest average value of germination (1.88) was achieved by the application of lead at 200 mg L^{-1} concentration [27]. Another study shows that arsenic (Ar) exposure in tomato (*Lycopersicon esculentum* L.) reduces the fruit yield, decreases the fresh weight of leaf [1]. Whereas, in canola (*Brassica napus* L.) arsenic causes stunted growth, chlorosis and wilting [5]. Further, arsenic in rice (*Oryza sativa* L.) reduces the seed germination, decreases in seedling height, reduces leaf area and dry matter of the plants. [6,7,12]

Effects on photosynthetic pigments:

Heavy metal reduces the quantity of **photosynthetic** pigments in plants. The concentration of chlorophyll, carotenoid and xanthophyll decreases with the increasing concentration of heavy metal. Chlorophyll content was decreased in various plant species exposed to Cd [24], Cu [18], Ni [20], Pb [17], and Zn [16]. Excess amount of Ni in soil causes various physiological modifications and toxicity symptoms such as chlorosis and necrosis in different plant species, including rice. In pigeon pea (*Cajanus cajan* L.) nickel concentration is responsible for decreasing chlorophyll content and stomatal conductance, growth reduction, and programmed cell death [19]. Carotenoid content in plants exposed to Cd do not exhibit a set pattern and may either increase or decrease.[8]

Effects on antioxidant enzymes: -

The important antioxidant enzymes include superoxide dismutase, peroxidase, catalase that get affected by the different heavy metals. Peroxidase (POD) includes physiological functions, such as participation in plant photosynthesis, respiration, formation of lignin, auxin metabolism, and virus resistance, etc. Peroxidase plays an important role in scavenging oxygen free radicals, resisting membrane lipid peroxidation and protecting cell membranes. A study suggests that Reaction of peroxidase observed in maize (*Zea mays* L.) based on the stress of Pb2+ was higher than that from clear environment.[61]

Catalase (CAT) is the first tetrameric enzyme to be discovered and characterized. It is the terminal of a series of antioxidant enzymes in biological oxidation. CAT's main function is to remove hydrogen peroxide produced by photorespiration in leaves. In the chloroplasts, CAT removes hydrogen peroxide by catalytic reaction. A study on CAT activity with the effect of Cadmium stress under three different Cd concentrations (0, 250, 500 umol/L), results that CAT activity of seedling within 100 hours with the extension of stress time at first increases and then declines, Root's CAT activity showed a downward and then slightly rebounded trend.[26]

Effects of heavy metal on stress marker: -

Increasing H2O2 level denotes low enzyme activities in heavy metal contaminated seedlings which lead to reduction in growth and metabolism in the seedlings. Moreover, elevated levels of H2O2 contents in the leaves in Cd stress environments may also affect photosynthetic chain performance [5,6]

Proline which is an imino acid, plays a vital role in plants. It protects the plants from several pressures and helps the plant recover from stress more quickly. When applied exogenously to plants exposed to stress, it enhanced the growth and other physiological characteristics of plants. Proline induces the formation of phytochelatins that chelate with heavy metals such as Cd thereby reducing their toxicity. In response to heavy metal stress plants accumulate a large quantity of proline. Many plants have been revealed to accumulate proline when contaminated with heavy metal. In addition to acting as an osmoprotectant and ROS quencher, the proline acts as a heavy metal chelator, thereby relieving the heavy metal pressure. Endogenous proline content was significantly higher in pigeon peas under different cadmium treatment. Proline accumulates in relative amounts under stress conditions. Arsenic treatments increase the proline content in rice seedlings indicating the effect of increased oxidative stress. [12,29].

Effects on DPPH- scavenging activity:
Among all antioxidant assays used for plant extract, DPPH- assay is most commonly used. The 2, 2-diphenyl-1-picrylhydrazyl (DPPH)-scavenging activity showed higher activity in control plants without heavy metals treatment. Additionally, the Cd contaminated plants showed decreased antioxidant activity. Cr and Pb were less toxic as compared to Cd. [10]. Selected heavy metals not only affect the plant development but also distressed plant metabolic pathways. Increasing arsenic toxicity affected the activities of different antioxidant scavenging enzymes in the test of rice seedlings was also observed [12]. Based on another study, steady and significant increase in total antioxidant activity was observed in 1.5 mM concentration , as compared to the control, and then slightly declined at the highest Cr (VI) concentration (1.8 mM). Though total antioxidant content increased with the stress, DPPH radical scavenging activity, i.e., the percentage of free radical inhibition, did not increase as compared to the control. Percentage of inhibition was 90.8% in control plant followed by 89.4, 84.3, 78.2 and 83.7% with Cr (VI) dose of 0.1 mM, 0.3 mM, 0.5 mM, and 1 mM, respectively. At the concentrations 1.5 mM and 1.8 mM, the inhibition decreased sharply to 56.8% and 68.1% respectively.

Table1: Effects of heavy metal in different plant species

Sl. no	Metal	Plant species	Effects
1.	**Cr**	*B. napus* L**.**	ultrastructural damage in leaf mesophyll and root tip cells.[30]
2.	**Pb**	*Brassica sp*. L.	Ultra-structural damage in plant tissues (leaf and root) was observed that led to the reduction in plant growth and biomass production chlorophyll contents, photosynthetic activity, reduction in the uptake of macro or micronutrients.[31]
3.	**Cd**	*Brassica juncia* L.	The root and shoot elongation in 7 days old seedlings were inhibited. Also, the dry and moist weight decreases gradually with the concentration increase.[32]
4.	**Ar**	tomato (*Lycopersicon esculentum* L.)	reduces fruit yield, decreases the leaf fresh weight.[1]
5.	**Ar**	rice (*Oryza sativa* L.)	reduces seed germination, decrease in seedling height, reduces leaf area and dry matter production.[7]
6.	**Ni**	pigeon pea (*Cajanus cajan* L.)	nickel decreases chlorophyll content and stomatal conductance; decrease enzyme activity. [34]
7.	Hg	rice (*Oryza sativa* L.)	decreases plant height, yield reduction and increase of its bioaccumulation respectively in shoot and root of the seedling.[33]

CONCLUSION
Heavy metals usually produce toxic effects in plants which is a serious concern for food safety, particularly in developing countries of the world. The high concentration of heavy metal in food crops retards biomass accumulation, causes chlorosis and inhibition of growth and photosynthesis, alters water balance and nutrient assimilation, which eventually cause plant death. Plants require certain heavy metals for their growth and upkeep and when they are exposed to high level metals toxicity, the crops' strength and yield gets limited. The study gave a clear vision about the metals such as Pb, Cd, Cr, Hg, and As which do not play any advantageous role in seeds germination & growth as unpleasant effects have been recorded even at a minimum concentrations of these metals in the growth medium. Moreover, the reduction in height of rice plants growing on the soil contaminated with Hg is also noticed. (33) For Cd toxicity, reduction occurs in the shoot and root growth in wheat plants when Cd is lower than 5 mg/L in the soil. (32) Most of the reduction is in growth parameters of plants which are growing on polluted soils and reduced photosynthetic activities, plant mineral nutrition, and reduced activity of some enzymes can also be observed. So, from all the research throughout the world to determine the effects of toxic heavy metals on plants we can conclude that the contamination of agricultural soil or water by heavy metals has become a life-threatening environmental concern due to their adverse ecological outcome. Such toxic elements are considered as soil pollutants due to their extensive existence and their severe and long-lasting toxic effect on plants grown on such soils. (29)

ACKNOWLEDGMENT

I humbly thank our director, prof (Dr.) Abhijit Sengupta and principal prof (Dr) Lopamudra Datta, for providing the opportunity to present my review. I am also very grateful to my mentor, Dr. Bhaskar Choudhury, for this assistance in data collection and more.

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