

Lead toxicity in cereal crops

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Abstract

Lead has been redistributed from earth's surface to land and atmosphere as a consequence of industrialization, urbanization, mining and a variety of other anthropogenic actions. Lead is a non-essential trace element. Its higher levels of exposure disrupt plant water and nutritional relationships, as well as causing oxidative damage. Pb association with enzymatic activities leads to membrane disruption and stomatal closure, reduced seed germination and plant growth in stressed plants. The current study focused on lead toxicity in cereals and management techniques to reduce the amount of it absorbed by plants. Growing of low lead accumulating varieties could lower the risk of Pb accumulation in plants and humans through the food chain. Plant growth regulators, micro-organisms both organic and inorganic amendments may all be effective methods that have capability to minimize Pb levels even more in grain and the shoot.

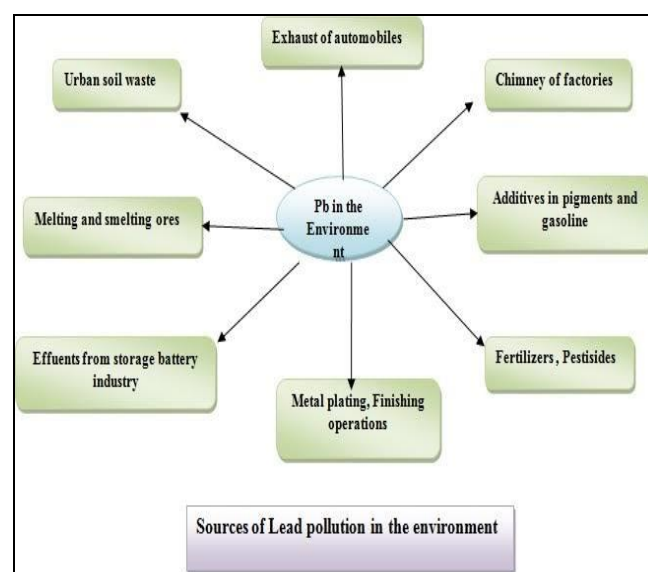
Keywords: lead toxicity, organic amendments, oxidative damage, phytoremediation

Introduction

Lead is a dangerous and non-disintegrative heavy metal comprising 0.002% of earth crust (ATSDR, 2015). The colour of lead is bluish or silver grey with atomic number 82, atomic weight-207.19, melting point of 327.5°C (WHO, 2001). It exists in stable state, typically compact, moldable and very soft with a low EC and resistant to corrosion. Lead is a non-essential trace element. Pb is present, predominantly in the climate but due to its distinctive function of cognition with soil fractions, its accessibility to plants is minimal. It has gained environmental interest and has been thoroughly investigated in the near times as a strong ecological pollutant that is difficult to extract from polluted environments (Kohli *et al.*, 2019). In addition, due to its adverse effects on living organisms, lead is the second most toxic metal after Arsenic. In the environment, various anthropogenic activities such as electroplating, mining, smelting, fossil fuel burning, steel industry, paints, gasoline, municipal sewage-sludges, atmospheric deposition, inorganic fertilizer and pesticide usage have increased their concentration. Natural causes that contribute only a small proportion of environmental pollution are mobilization, erosion and volcanic activity (Zulfiqar, 2019) [37]. In biological systems, it has no beneficial function and is toxic to living organisms like plants, animals and human beings for health. Approximately 25 µg/kg of human body weight is the acceptable limit weekly consumption of Pb in human food. In about all the food crops, it is present at low levels. The appropriate limits of Pb are 50-300 mg/kg of soil among distinct nations. In the soil, increased Pb has negative consequences on microbial population activity and vegetation (Wuana and Okieimen, 2011) [36]. Lead adversely affects the plant growth at physiological, morphological and molecular levels. In humans Pb toxicity has become a major problem, possibly with the use of lead contaminated cereals worldwide. Cereal absorption of Pb normally depends on the bio-accessibility of Pb levels in soils, duration of exposure, genotypes and crop translocation capacity within the plant (Lai *et al.*, 2018) [18]. Soil physical and chemical properties such as pH, CEC, organic material, ferric oxide, as well as distribution and organization of particle size primarily affect lead translocation from soil to plant. In environment trace elements, they are long lasting because they are not degraded by bacteria or other chemical

processes. As a result, decreasing lead bioavailability through certain adaptations and selecting low Pb accumulating resistant varieties could be a promising strategy for growth of cereals in soils polluted with Pb. By raising absorption, precipitation and by boosting properties of the soil and fertility, the application of soil improvers can decrease the bio-accessibility of Pb. To decrease Pb accumulation in cereals, various organic and synthetic additions and agronomic practices are intensively researched. Pb is known to be a common cytoplasmic toxin that is aggregate, delicate and slow-acting. Various strategies for reacting to toxic metal exposures have been developed by plants. They have internal detoxifying pathways that involve preferential metal uptake, excretion, complex formation by particular ligands and compartmentalization to cope with metal toxicity (Jiang *et al.*, 2010) [13].

Sources of Lead Contamination



(Source: ENVIS centre on plants and pollution)

Fig 1: Sources of lead pollution

Toxic effects of Pb in cereal crops:

Pb stress mainly affects the growth of the root because it contacts the root directly during plant uptake, which can hinder cell proliferation at tips of the roots (Fahr *et al*, 2013)^[7]. Mineral nutrition in all plants is a part of proper growth and development. Plant mineral nutrient content and lead stress were found to have negative relationship in majority of plants. Overall, with the difference of plant species and growth circumstances, toxic effect of Pb in cereals varies greatly.

Wheat

There was decline in fresh weight, dry weight, shoot length and root length in wheat crop at 100µM of Pb concentrations (Tripathi *et al*, 2016). Different dosages of lead concentrations in *Triticum aestivum* decreases in growth, dry weight and fresh weight total chlorophyll, chlorophyll a and b were apparently reduced (Lamhamdi *et al*, 2013). wheat seedlings exposed to various lead concentrations for 6 days decreased the germination rate of seeds, biomass, shoot and root elongation than the control levels (Rizwan *et al*, 2018)^[24] When wheat was exposed to various Pb concentrations in growth medium, a raise in the amount of MDA and H₂O₂ in roots was found (Kaur *et al*, 2012)^[15]. Increased lead doses steadily enhanced the content of MDA and hydrogen peroxide and changed antioxidant enzymatic mechanism in wheat and maize roots. Increased levels of Pb accumulated soils dramatically decreased the biomass of wheat crops. (Muhammad *et al*, 2018). SOD, POD, CAT, APX and GST roles have been shown to be increased in wheat seedling roots and leaves than control subjected to Pb for 6 days (Rizwan *et al*, 2018)^[24]. Lead exposed wheat plants decreased macro and micro nutrient concentration in plants for 5-30 days old seedlings (Lamhamdi *et al*, 2013). Increased lead levels have induced

morphological and physiological changes in wheat roots. MDA and H₂O₂ levels were higher in the roots and shoots of wheat seedlings when exposed to 100µM of Pb concentration (Tripathi *et al*, 2016).

In wheat plants during Pb stress the level of MDA has risen significantly. There was a major increase in soluble protein content (Lamhamdi *et al*, 2010). When exposed to Pb in wheat plants, potassium and sodium content decreased in roots and shoots (Bhatti *et al*, 2013)^[3]. In wheat, seedling development and growth were drastically decreased when exposed to lead. Excessive Pd concentrations decreased relative water content and water use quality, while abscisic acid concentration and saturation water deficit increased (Zulfiqar *et al*, 2019)^[37]

Rice

When rice plants are exposed to 100µM concentrations of Pb reduced the root and shoot length, fresh weight, dry weight, and reduced gas exchange parameters and pigment quality (Chen *et al*, 2017). In *Oryza sativa* different doses of Pb concentrations significantly decreased the total chlorophyll and carotenoid content of chl a and b (Ashraf *et al*, 2017)^[2].

Pb levels in rice seeds have had a significant impact on endosperm starch solubilization and alpha- amylase activity (Gautam *et al*, 2010)^[9]. Hydrogen peroxide and MDA levels are increased when exposed to 400, 800 and 1200 ppm of Pb concentration in rice plants (Ashraf *et al*, 2017). SOD, APOX and GR activities were all enhanced. The levels of antioxidants, such as ascorbic acid and GSH were decreased when exposed to 100µM Pb concentration in rice plants (Chen *et al*, 2017)^[6]. The increased lipid peroxidation and superoxide anion levels in *Oryza sativa* when exposed to lead concentration (Verma *et al*, 2003).

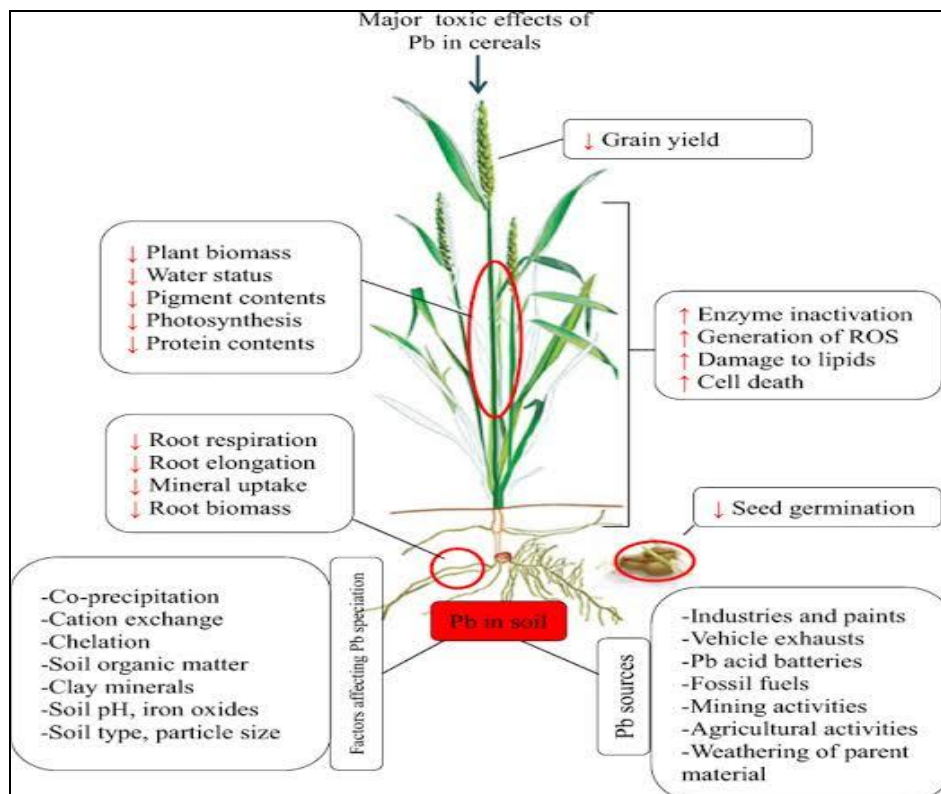


Fig 2: Toxic effects of lead in cereals (Source: Rizwan *et al*, 2018)

Maize

In lead stressed maize seedlings, antioxidant enzymes such as ASA, SOD and CAT were enhanced by the dose (0-200µM) and duration of exposure (1-7 days) under hydroponic conditions. (Gupta *et al*, 2010) [10]. Reduced concentrations of potassium and Copper in maize cultivars by means of elevated amounts of Pb contamination (Ahmad *et al*, 2011) [11]. Compared to control, decreased levels of calcium, magnesium and sodium and potassium in shoot and root of maize were reported under lead stress (Singh *et al*, 2015) [30]. When maize seedlings are exposed to different doses of lead concentrations, germination percentage and seedling growth were significantly reduced in terms of shoot and root length. Similar reduction in seedlings fresh and dry weight (Hussain *et al*, 2013) [11]. Several studies have shown that lead exposure causes Calcium, magnesium, zinc, manganese and iron to be replaced or depleted in *Zea mays* leaves (Seregin *et al*, 2004) [27].

In maize, changes in lipid composition and K⁺ ion leakage were observed as a result of being exposed to lead. Protein content was reduced in maize plants due to increase in oxidative stress and ROS production when exposed to

different levels of Pb concentration (Hussain *et al*, 2013). Root and shoot growth are drastically lowered, and total chlorophyll levels are sharply reduced in maize leaves (Ghani *et al*, 2010) [8].

Barley

When *Hordeum vulgare* exposed to (100 and 200µM) lead concentrations decreased the root and shoot length, fresh and dry weights (Arshad *et al*, 2017). Furthermore, it reduces the activity of carbohydrate metabolizing enzymes including α-amylase, β-amylase, acid phosphatase and acid invertase. In barley, rice and maize lead has been shown to inhibit germination (Tomulescu *et al*, 2004) [32]. This was emphasized that lead induced changes in respiration were due only to dark (mitochondrial) respiration, not photo respiration. Lead was found to stimulate dark respiration in *Hordeum vulgare* leaves or protoplasts (Romanowska *et al*, 2006) [25].

In barley leaves, Pb induced an increase in ATP content as well as increase in the ATP/ADP ratio.

Table 1: Effect of lead on metabolic process of cereal crops

Crop	Metabolic process	Enzyme associated	Effect	Reference
<i>Oryza sativa</i>	Antioxidant mechanism	Glutathione reductase	enhanced	Verma and Dubey (2003)
<i>Oryza sativa</i>	Sugar metabolism	α-amylase	inhibited	Mukherji and Maitra (1976)
<i>Oryza sativa</i>	Antioxidative metabolism	Ascorbate peroxidase	enhanced	Verma and Dubey (2003)
<i>Oryza sativa</i>	Antioxidant mechanism	Catalase, superoxide dismutase	increased	Verma and Dubey (2003)
<i>Zea mays</i>	Energy generation	ATP synthetase	interrupted	Tu Shu and Brouillette (1987)
<i>Zea mays</i>	CO ₂ fixation	Ribulose-1,5, bis phosphate, phosphoenol pyruvate carboxylase	inhibited	Vojtechova and leblova (1991)
<i>Avena sativa</i>	CO ₂ fixation	Ribulose-1,5, bis phosphate	interrupted	Moustakas <i>et al</i> , (1994)

(Source: M.L. Dotaniya *et al*, 2020)

Remediation for Lead Toxicity

Phytoremediation

Phytoremediation is a technique for cleaning up metal-polluted soils by growing hyper-accumulator plants that can absorb significant amounts of heavy metals. Phytoextraction, Rhizofiltration, phytovolatilization, phytodegradation, rhizosphere depletion, phytostabilization and phytorestoration are some of the methods used to remediate soil (Ali *et al*, 2013). Rhizofiltration, which involves plant life to rectify metal-polluted surface water, ground water or wastewater by precipitating metals within plant roots, comes after rhizosphere decomposition. Lead has been documented to be eliminated from soil and water by plants like Indian mustard, rye, sunflower, tobacco, maize and spinach (Camargo *et al*, 2003) [4].

Phytostabilization is actually in situ remediation by developing plants to limit the movement and bioaccumulation of heavy metals by lowering metal valence, sorption, complexation or precipitation (Kunito *et al*, 2001) [17]. Phytorestoration is the process of transforming degraded soils into fully productive soils by planting native plants native to the environment in order to restore the soil to its natural state (Zulfikar *et al*, 2019). The most famous method is phytoextraction, which is popular because of its precision over a wide area and low cost (Ali *et al*, 2013). Lead accumulating plants like black mustard (9400 mg/kg of lead accumulating efficiency), sunflower (5600 mg/kg), alfa-alfa (43300 mg/kg) (Koptsik, 2014) [16], brown mustard-10300 mg/kg (Shoeran *et al*, 2009), Canada thistle-1880 mg/kg (Lorestani *et al*, 2011) [20], Mexican marigold-381 (Salazar and pignata, 2014).

Application of plant growth regulators (PGRs)

Plant growth regulators are often used to increase plant resistance to stressful environments and can improve plant performance. PGRs effectively reduce the toxicity of heavy metals both directly and indirectly. Directly by immobilization and biotransformation, indirectly by several enzymes and metabolites (Zhuang *et al*, 2007) [38]. In wheat grown hydroponically under lead stress, application of nitric oxide reduced oxidative stress (Kaur *et al*, 2014). External application of indole-3-acetic acid increased for 5 days, lead content in maize roots and decreased its deposition in maize shoots raised in hydroponic conditions (Wang *et al*, 2007) [35].

Externally added phytohormones, both natural and synthetic are applied to plants during their development or before the sowing, i.e., phytohormone soaking. In barley 1.5g/kg seeds of soil inoculated *Azospirillum lipoferum* 137, *Agrobacterium radiobacter* 10 accumulated 500 mg/kg of lead and increased nutrient uptake, growth and inhibits lead accumulation (Belimov *et al*, 2004). In maize *Brevibacterium halotolerans* as soil inoculant accumulated 0.2 g/kg of lead and significantly enhanced accumulation of lead in shoots (Shanab *et al*, 2008).

Microbial remediation

Since micro-organisms bacteria, fungi and algae have the capacity to precipitate, sequester or change the oxidation state of lead they have shown to be very effective in restoration of lead contaminated soils. Strong metal remediation is also possible for agal isolates. Microalgae isolates *Phormidium ambiguum*, *Pseudochlorococum typicum*,

Scenedesmusquadricaudavar.quadrispina (chlorophyta) from fresh water were used to investigate lead resistance and elimination (Shanab *et al*, 2012) ^[26]. Sunflower biomass was improved and Pb toxicity was reduced by fungal isolates, *Funneli formismossease* and *Rhizophagusir regularis* (Hassain *et al*, 2013). With a sorption potential of 40 mg/g, *Pseudomonas aeruginosa* was used to treat lead polluted soil and significantly decreased the Pb concentration (Joshi *et al*, 2017).

Under lead stress, three bacterial strains of *Azospirillum*, *Azotobacter* and *Pseudomonas* significantly enhanced wheat Anatomical and yield related characteristics (Pazoki *et al*, 2014) ^[21]. The ability of a siderophore producing PGPRs starin, *Pseudomonas putida* KNP9, to improve the growth of mungbean plants during lead stress. (Tirupathi *et al*, 2005). *Pseudomonas monteilii* and *Wautersiametallidurans* enhanced wheat growth and lead absorption more than control, with the difference becoming stronger when the microbes were combined with EDTA (Rizwan *et al*, 2018) ^[24]. Microbes can minimize lead toxicity in cereals by improving plant growth, boosting the antioxidant system, and lowering Pb accumulation or mobilization from roots to shoots.

Application of chelates

Chelation is a mechanism in which individual metal ions from multiple coordinate bonds with macromolecular substances called as chelating agents. In the treatment of lead contaminated soils, ethylene diamine tetra acetic acid (EDTA) is being more widely used.

In barley application of 2.5 mmol EDTA/kg, increased biomass and 2400 mg/kg of soil metal is reduced (chen *et al*, 2004) ^[5]. Similarly, in maize application of 5mmol NTA/L reduced 1020mg/kg of soil metal. (Shilev *et al*, 2007). In wheat 8mmol EDTA/kg significantly enhanced the plant biomass and reduced soil metal up to 456 mg/kg (Saifullah *et al*, 2010).

The roots consume the majority of the lead absorbed by the plants, and only a small amount of Pb is transported to aerial plant sections. Nonetheless, this limited translocation may be attributable to lead precipitation as insoluble salts at root level (Pourrut *et al*, 2011). When brown mustard was treated with EDTA, it accumulated 1000-10000 times more lead (chen *et al*, 2004) ^[5].

Phytochelatin (PCs) are biomarkers for detecting metal toxicity in plants in the early stages. Furthermore, applying hydrogen sulphide to *Brassica napus* lead stress increased plant growth and reduced ROS yield by increasing enzymatic and non-enzymatic antioxidant activities (Ali *et al*, 2014). Under lead stress, the application of 5-aminolevulinic acid (ALA) enhanced growth by increasing macro and micronutrient absorption while also lowering ROS intake (Ali *et al*, 2014).

Application of Nano particles

Nanoparticles are the materials with a diameter of less than 100 nanometer on at least one side, and their high surface area gives them special characteristics. Depending on the soil form, nano zerovalent iron (nZVI) was good at immobilising lead and other metals (Gil Diaz *et al*, 2017).

Depending on the cultivar, foliar application of Si NPs improved biomass and yield traits thus lowering lead and other harmful trace elements in rice shoots and grains (Wang *et al*, 2016). All in all, NPs can mitigate lead toxicity

in plants by immobilising lead in the soil and by increasing plant growth and decreasing lead uptake by plants at the soil and plant levels, respectively.

Organic amendments

Press mud is by product of sugar industries. Since, press mud in synthetic water has a higher lead removal efficacy of up to 90%, as the ability to be used for heavy metal remediation (Ahmad *et al*, 2016).

Biochar is a black charred substance made from inadequate biomass combustion in an oxygen depleted environment. In pot and field trails, biochar significantly enhanced the growth and biomass yield of a variety of crop such as rice, wheat, corn, rapeseed and rye among others, growth in lead contaminated soils (Hussain *et al*, 2017). The use of rice straw biochar in the soil increased negative sites thus lowering acid-soluble lead and certain other metals in the soil.

Compost is an anaerobic produced, well decomposed organic material of plants and animals. It increases the soil composition as well as the soil fertility since it contains organic matter. At 40 ton/ha, application of Mexican sunflower and cassava peel composts in polluted soil with lead acid battery waste diminished bioavailable lead concentration in soil by 69 and 49% respectively, while application at 20 tons/ha reduced bioavailable lead concentration in soil by 58 and 34% (van Herwijnen *et al*, 2007). When organic manure was applied to lead contaminated soil, the phytoavailability of lead was decreased, and wheat growth was increased therefore less oxidative stress (Ahmad *et al*, 2011) ^[1]. In lead contaminated soil, fresh cow manure and compost used as organic material enhanced lead fixation and decreased its bioavailable fraction (Rehman *et al*, 2017) ^[23]. Depending on the soil texture and form of manure applied, farm manure decreased bioavailable Pb in the soil and its absorption by wheat (Rehman *et al*, 2017) ^[23].

Biochar may use a variety of mechanisms to immobilise lead in soil, including increased soil pH, absorption, precipitation, complex formation on the biochar surface, and cation exchange. The use of humic acids in soil contaminated with Cd and Pb raised the levels of Pb and Cd in wheat (Khan *et al*, 2006). Citric acid treatment improved wheat growth and lead concentrations more than regulation and other additives like EDTA.

Agronomic practices

Appropriate agronomic practices such as seed priming, irrigation management, plant density and inter cropping may be viable options for reducing lead levels in cereal crops. In contrast to maize monoculture, weeds co-cultivated with maize in pots decreased Pb levels in maize regardless of growth rate (Yang *et al*, 2012). Increased lead content in stem, kernel and husk with increased plant density was found in wheat grown in Pb toxic soil at five different densities (Zulfiqar *et al*, 2019) ^[37].

Wheat plants that were grown from spinach methanolic extract-treated seeds displayed enhanced lead tolerance due to lower MDA levels and increased antioxidant production (Lamhamdi *et al*, 2013). When rice was flooded, the concentration of Pb in the grain was significantly lower than when it was not flooded (Arshad *et al*, 2017).

Maize absorption of lead and other metals was significantly influenced by inter cropping with various legumes rather

than monocropping (Zulfiqar *et al.*, 2019) [37]. Seed priming with potassium nitrate improved maize photosynthesis and reduced lead concentration in shoots and roots compared to controls (Nawaz *et al.*, 2017) [12].

Conclusion

Lead has been redistributed from the earth's surface to the land and atmosphere as a consequence of industrialization, urbanization, mining and a variety of other anthropogenic actions. Lead toxicity affects many of the physiological functions of plants by disrupting enzymatic activity, causing oxidative disruption. The main cause of lead toxicity is the production of reactive oxygen species (ROS). These free radicals cause irreversible metabolic instability and cell death by disrupting cells redox status and inducing oxidative stress and DNA harm through oxidation. Furthermore, lead inhibits seed germination, root elongation, seedling formation, plant growth, transpiration, chlorophyll production as well as water and protein content. PGRs, fertilizer conservation, phytoremediation, microbes, inorganic and organic amendments and farming methods are among the most important strategies for remediation. The identification of precise metabolic pathways adapted by plants in the presence of lead toxicity at the molecular level in relation to plant nutrition is a crucial field for future study. Awareness of metabolomics, proteomics, transcriptomics and genomic approaches is important for a deeper understanding of the molecular processes underlying lead toxicity in crop plants. These techniques may be beneficial in the production of more resilient transgenic plants for environmental protection and soil quality regeneration.

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