Research Article

Phytoremedial potential of maize (*Zea mays* L.) hybrids against cadmium (Cd) and lead (Pb) toxicity

Misbah Rasool¹, Muhammad Anwar-ul-Haq¹, Muhammad Jan^{2*}, Javaid Akhtar¹, Muhammad Ibrahim³ and Javed Iqbal⁴

1. Institute of Soil and Environmental Science, University of Agriculture, Faisalabad-Pakistan

2. Department of Soil and Environmental Science, Ghazi University, Dera Ghazi Khan-Pakistan

3. Department of Agronomy, Ghazi University, Dera Ghazi Khan, 32200-Pakistan

4. Soil and Water Testing Laboratory for Research, Dera Ghazi Khan-Pakistan

*Corresponding author's email: mjanleghari@gmail.com, mjan@gudgk.edu.pk

Citation

Misbah Rasool, Muhammad Anwar-ul-Haq, Muhammad Jan, Javaid Akhtar, Muhammad Ibrahim and Javed Iqbal. Phytoremedial potential of maize (*Zea mays* L.) hybrids against cadmium (Cd) and lead (Pb) toxicity. Pure and Applied Biology. Vol. 9, Issue 3, pp1932-1945. <http://dx.doi.org/10.19045/bspab.2020.90206>

Received: 05/02/2020

Revised: 25/04/2020

Accepted: 08/05/2020

Online First: 29/05/2020

Abstract

Heavy metals are contaminants of much environmental apprehension, as they are hazardous to human being and other biota. The chelates react with the heavy metals and free that metal from the cation exchange sites and resulting the metal chelated species and move readily into the soil. For this purpose, a hydroponic stud was conducted in wire house of Saline Agricultural Research Center to monitor the potential of Maize (*Zea mays* L.) cultivars against cadmium (Cd) and lead (Pb) toxicity. A chelated source Ethylene Diamine Tetra Acetic acid (EDTA) was used to evaluate the uptake of Cd and Pb. The experiment was comprised of nine treatments. As T₁= Control, T₂= Cd 25 ppm, T₃= Pb 50 ppm, T₄= Cd 50 ppm, T₅= Pb 100 ppm, T₆= Cd 25 ppm+EDTA, T₇= Cd 50 ppm+EDTA, T₈= Pb 50 ppm+EDTA and T₉= Pb 100 ppm+EDTA. Two maize cultivars Syngenta-8711 and 33H-25 were used. It was concluded that the parameters like shoot fresh and dry weight, chlorophyll content (SPAD value) and relative water content decreased under toxic level of Cadmium (Cd) and Lead (Pb). The addition of EDTA increased the uptake of Cd and Pb which ultimately reduced the overall the plant growth. Among the maize hybrids, maximum Cd concentration was observed in Syngenta-33-H-25 while minimum in Syngenta-8711 at all cadmium stress levels. At all the lead stress levels the maximum Pb concentration was observed in Syngenta-33-H-25. The minimum Pb concentration was recorded in Syngenta-8711 at all lead stress levels.

Keywords: Cadmium; Chelated; Chlorophyll content; Lead; Maize

Introduction

Maize is one of the most important food crop in the world and together with rice and wheat provides at least 30% of the food calories to more than 4.5 billion people in 94 developing countries and worldwide its production was

1,123.65 million tons [1]. Pakistan produces an annual 3.1 million tons of maize covering an area of 1.04 million hectares giving 2,984 kg/ha grain yield on average, making it the third most valuable cereal of the country and the production has increased from 0.38 to

3.037 million tons during the last 6 decades [2].

The substantial metal incorporates naturally essential, for example, cobalt (Co), copper (Cu), chromium (Cr), manganese (Mn) and zinc (Zn) and non-essential. For example the components of mercury (Hg), lead (Pb) and cadmium (Cd). The non-essential metals are generally known as poisonous components for plants, human and creatures. Both the groups are dangerous to plants, creatures and people at higher concentration [3]. Heavy metals are actually present in soils in low focus yet there is consistent flood through atmospheric deposition, utilization of metal tainted sewage slime, fertilizers [4] and effluent irrigation water [5]. Many experts in the world have turned out to be cognizant about the immediate or backhanded impact of heavy metals on human health, spread of irresistible diseases brought on by fungi, bacteria and other worm infections [6-7].

Out of 6634 registered industries, 1228 are considered highly polluting in Pakistan [8]. Furthermore, 90% cities lack the facility of effluent treatment. Untreated city effluent is generally used for irrigating vegetables, wheat, rice, fodder and legumes. There is no comprehensive figure about the extent of wastewater used for irrigation. However, available total estimate of effluent irrigated area is 20 mha in the world. In Pakistan, effluent irrigated area is about 32,500 ha [9] Peri-urban areas in big cities like Lahore, Faisalabad, Gujranwala, Multan, Rawalpindi, Peshawar and Hyderabad are receiving raw sewage for irrigation. Easy access, ensured supply, and nutrient value of raw effluents attract its use; despite having higher levels of heavy metals, soluble salts, pathogens. Although the concentration of heavy metals in sewage effluents is low, long-term use for irrigation often results in buildup of metals in soils which is ultimately taken up by plants and enters into food chain [10].

Accessibility of metals to plants is an intricate capacity of many elements including inception of metals, aggregate fixation, metal speciation, temperature, physical and additionally concoction properties of soils and plant genetic makeup [11]. Also, metal conduct and bioavailability is alert in the earth and is impacted by soil properties, for example, pH, natural matter, soil arrangement ionic quality, Mn and Fe oxides, redox potential and nature of sorbing surfaces [12-13]. Metals have diverse affinities for various ligand particles in this way impacting particle combine and complex particle development which influence their mobility and solubility [14]. Chelation means "to grab" or "to bind." When EDTA is injected into the veins, it "grabs" heavy metals and minerals such as lead, mercury, copper, iron, arsenic, aluminum, and calcium and removes them from the body. Except as a treatment for lead poisoning, chelation therapy is controversial and unproved [15]. Our present understanding is that effluent is a huge source of irrigation water with some to considerable plant nutrition. If not properly used, it is of environmental concern. Heavy metal buildup in soils is another serious issue and needs action of industries owners, policy makers and farmers to reduce the risk of contamination. More site specific research is needed to overcome future crises of water shortage along with its monitoring and safe use. The scientists should go to the technology package investigation for reducing the metal contamination in food chain. Soil is an ultimate sink for contaminants so the social attitude should be positive for effluent farming. Phosphorus fertilizers are another source of heavy metals such as Cd, so its long term use needs the understanding in addition to its effect on uptake of other metals [16-17].

The contamination with heavy metals has become a serious threat to the environment and food security because of rapid

development in agriculture and industry, as well as disturbance of the natural ecosystem due to the enormous growth in world population [18]. Soil heavy metal pollution has become a worldwide environmental issue that has attracted considerable public attention largely from the increasing concern for the security of agricultural products [19]. Globally, there are 5 million sites of soil pollution covering 500 million ha of land, in which the soils are contaminated by different heavy metals or metalloids, with the present soil concentrations higher than the geo-baseline or regulatory levels [20]. Heavy metal pollution in soil has a combined worldwide economic impact estimated to be in excess of US\$10 billion per year [21].

Cadmium is highly mobile in soil and its concentration regarding uncontaminated soils usually varies from 0.5 to 3.0 mg kg⁻¹ [22]. However, Pb concentration in the Earth's crust is generally < 50 mg kg⁻¹. Pb has strong affinity with organic and/or colloidal materials in the soil and is generally available in low concentration for plant uptake. Literature shows that metal-organic matter complexes in soil solution are involved in significant environmental and ecological processes such as availability, solubilization, and transport of metals [23]. Thus the objectives of the study were to the effect of moderate and higher doses of Cd and Pb on maize cultivars and to study the effect of synthetic chelator (EDTA) in the translocation and uptake of Cd and Pb.

Materials and methods

Experimental conditions and plant material

To assess the capability of Maize (*Zea mays* L.) cultivars against cadmium (Cd) and lead (Pb) harmfulness a hydroponic study was conducted. At first maize seeds grown in plate (sand culture) in a green house. For water system refined water utilized amid development of maize seedlings. Following ten days old Maize seedlings of comparable

size was transplanted into plastic tubs (containing arrangement culture). The ½ quality Hoagland's solution [24] was connected to satisfy supplements request of the plant. The pH of the solution was checked and kept up in the range of 6.0 to 6.5 by utilizing 0.1N HCl and 0.1N NaOH. In the hydroponic framework, maize plants was evaluated at 0, 25 and 50 mg L⁻¹ of Cd and 0, 50 and 100 mg L⁻¹ of Pb in the nonappearance and nearness of EDTA @ 0.25 g L⁻¹. All the plastic tubs were circulated air through tenderly with an air compressor for 24 h. The treatment of Pb were made by using lead nitrate salt (Pb(NO₃)₂) having 98% purity while Cd treatments were formulated by using Cadmium Chloride salt CdCl₂ having 99.90 % purity. One and half months old maize seedlings were subjected to diagnostic and development parameters as per standard methods as given below.

Plant parameters

After the duration of 45 days, plants were harvested, and left for sun drying. Samples were oven dried to constant weight and weighed with a spring balance and yield per plant was recorded. After oven drying, samples were grinded and one gram sample was digested with a mixture of digested in a di-acid (HNO₃:HClO₄) mixture [25]. Potassium and sodium in the digested material were determined with a flame photometer (Jenway, PFP-7). The Cd and Pb concentration in the digest was estimated by atomic absorption spectrophotometer (AAS).

Statistical analysis

All values reported in this study are means for three replicates. A two-way analysis of variance (ANOVA) was performed utilizing a measurable bundle, SPSS form 16.0 (SPSS, Chicago, IL). LSD test was done to compare the contrast between the treatments [26].

Results and discussion

Shoot fresh weight (g plant⁻¹)

Plant biomass was severely affected due to toxic effect of heavy metals level

consequently; shoot fresh weight was reduced. The data regarding the means of shoot fresh weight of maize genotypes under control and different levels of Cd and Pb are presented in (Fig. 1). Four weeks of heavy metals stress strongly reduced shoot fresh weight. The impact of heavy metals toxicity, however, differed substantially between maize cultivars. Based on this data, it was observed that maize cultivar Syngenta-8711 showed less reduction (on an average, 72% and 79% of control shoot fresh weight at toxic levels of Cd and Pb respectively). Maize cultivar Syngenta-33H-25, on the contrary, showed much greater sensitivity to Cd and Pb (on an average 90% and 92% of control shoot fresh weight at toxic level of Cd and Pb respectively). In general, shoot fresh and dry weight of Syngenta-8711 was less affected as compared to Syngenta-33H-25.

The addition of EDTA significantly decreased the plant biomass due to more uptake of Cd and Pb. Syngenta-8711 (on an average, 69% and 72% of control shoot fresh weight at toxic levels of Cd and Pb respectively) while Syngenta-33H-25, on the contrary, showed much greater sensitivity to Cd and Pb on an average 87% and 92% of control shoot fresh weight at toxic levels of Cd and Pb respectively).

Shoot dry weight (g plant⁻¹)

Four weeks of heavy metals stress strongly reduced both shoot dry weight (Fig. 2). Plant biomass was severely affected due to toxic effect of heavy metals level which ultimately reduced the shoot dry weight. A significant difference was observed among the maize hybrids. It was observed that the maize cultivar Syngenta-8711 showed less reduction (on an average, 97% and 89% of control shoot dry weight at toxic levels of Cd and Pb respectively). Maize cultivar Syngenta-33H-25, on the contrary, showed much greater sensitivity to Cd and Pb (on an average 92% and 93% of control shoot dry weight at toxic level of Cd and Pb

respectively). In general, shoot fresh and dry weight of Syngenta-8711 was less affected as compared to Syngenta-33H-25.

The addition of EDTA significantly decreased the plant biomass due to more uptake of Cd and Pb. Syngenta-8711 (on an average, 86% and 93% of control shoot dry weight at toxic levels of Cd and Pb respectively) while Syngenta-33H-25, on the contrary, showed much greater sensitivity to Cd and Pb on an average 84% and 88% of control shoot dry weight at toxic levels of Cd and Pb respectively).

These results are also in agreement with the findings of some other researches. They observed that maize biomass production adversely reduced due to the toxicity of Cd and Pb which results actually due to the inhibiting effect of Cd and Pb on plant metabolic and photosynthetic process which considered as most important process of plant growth. Similarly findings was also reported by [27-28] in case of maize crop. EDTA addition to the treatment systems increased the uptake of heavy metals by plants and decreased the shoot fresh weight which was also supported by the findings of [29], EDTA much pronounced with Cd and Pb. However, the pattern of uptake by plants was similar as that of heavy metals without EDTA amendments [30].

These results are also in agreement with the findings of some other researches [31-32]. They observed that maize biomass production adversely reduced due to the toxicity of Cd and Pb which results actually due to the inhibiting effect of Cd and Pb on plant metabolic and photosynthetic process which considered as most important process of plant growth. EDTA addition to the treatment systems increased the uptake of heavy metals by plants and decreased the shoot fresh weight. EDTA much pronounced with Cd and Pb. However, the pattern of uptake by plants was similar as that of heavy metals without EDTA amendments [33].

Chlorophyll content (SPAD)

Means of chlorophyll content of maize genotypes under control and different levels of Cd and Pb are presented in (Fig. 3). Plants chlorophyll content was severely affected due to toxic effect of heavy metals level consequently; shoot and root fresh weight was reduced. Four weeks of heavy metals stress strongly reduced chlorophyll content. The impact of heavy metals toxicity, however, differed substantially between maize cultivars. Based on this data, we identified the maize cultivar Syngenta-8711 showed less reduction (on an average, 86% and 82% of control chlorophyll content at toxic levels of Cd and Pb respectively). Maize cultivar Syngenta-33H-25, on the contrary, showed much greater sensitivity to Cd and Pb (on an average 83% and 85% of control chlorophyll content at toxic level of Cd and Pb respectively). In general, chlorophyll content of Syngenta-8711 was less affected as compared to Syngenta-33H-25.

The addition of EDTA significantly decreased the chlorophyll content due to more uptake of Cd and Pb. Syngenta-8711 (on an average, 79% and 73% of control chlorophyll content at toxic levels of Cd and Pb respectively) while Syngenta-33H-25, on the contrary, showed much greater sensitivity to Cd and Pb on an average 73% and 78% of control chlorophyll content at toxic levels of Cd and Pb respectively).

Relative water content (RWC)

Means of relative water content of maize genotypes under control and different levels of Cd and Pb are presented in (Fig. 4). Plant biomass was severely affected due to toxic effect of heavy metals level consequently; relative water content was reduced. Four weeks of heavy metals stress strongly reduced both shoot and root fresh weight. The impact of heavy metals toxicity, however, differed substantially between maize cultivars. Based on this data, we

identified the maize cultivar Syngenta-8711 showed less reduction (on an average, 82% and 83% of control relative water content at toxic levels of Cd and Pb respectively). Maize cultivar Syngenta-33H-25, on the contrary, showed much greater sensitivity to Cd and Pb (on an average 91% and 90% of control relative water content at toxic level of Cd and Pb respectively). In general, relative water content of Syngenta-8711 was less affected as compared to Syngenta-33H-25.

The addition of EDTA significantly decreased the relative water content due to more uptake of Cd and Pb. Syngenta-8711 (on an average, 72% and 75% of control relative water content at toxic levels of Cd and Pb respectively) while Syngenta-33H-25, on the contrary, showed much greater sensitivity to Cd and Pb on an average 70% and 66% of control relative water content at toxic levels of Cd and Pb respectively).

The different results for chlorophyll content were presented by [34] who investigated the effect of Cd^{+2} in different concentrations for content of maize chlorophyll content. The reduction in chlorophyll content was as reported by some other researches [35] observed that Cd is more toxic to reduce the chlorophyll content compared to Pb. The addition of EDTA also caused a significant reduction due to more uptake of Cd and Pb which ultimately reduce the chlorophyll content which is as in the agreement with the findings of [36].

A decrease of water content in plants under the influence of HM was observed by many researchers [37]. The effect of HM on water exchange was evidenced by the reduction of leaf size and stomata [38], and by a decrease of turgor which resulted in a reduction of the water potential and probably was the reason for growth inhibition [39].

Cadmium concentration (mg kg^{-1}) in shoot
Maximum concentration of Cd was observed in the treatment where Cd was applied at higher concentration compared to the control

treatment. The concentration of Cd are presented in (Fig. 5). Maximum concentration of Cd in shoot were recorded up to 8.86 mg kg^{-1} (168%) in response to 50 ppm Cd + EDTA While, minimum Cd concentration was observed in 25 ppm Cd treatment. By increasing the concentration of Cd level, concentration also increased. Maximum concentration in shoot was observed by the combination of higher dose of Cd and EDTA.

An increase in Cd concentration was observed by increasing the level of Cd however the addition of EDTA increased the Cd concentration as compared to no EDTA. The chelators increased the uptake of heavy metals and the use of chelators mobilizes the metals to the root zone [40]. Some other researchers [41-42] also reported that the addition of EDTA increased the metal uptake in maize crop by making a complex with metal [43] also reported that the addition of EDTA increased the Cd concentration in broad bean.

Lead concentration (mg kg^{-1}) in shoot

The concentration of Pb in shoot were recorded up to 75.00 mg kg^{-1} (145%) in response to 100 ppm Pb + EDTA While, minimum Pb concentration was observed in 50 ppm Pb treatment. Results about Pb concentration in shoot are presented in (Fig. 6). By increasing the concentration of Pb level, concentration also increased. Maximum concentration in shoot was observed by the combination of higher dose of Pb and EDTA.

Once the metal has been expelled from the cation exchange sites, it can be taken up by plant roots. A standout amongst the most capable and regularly utilized chelating operators is ethylene diamine tetra acidic corrosive (EDTA), which shapes edifices with a considerable lot of the metal contaminants inside the natural environment. The most ordinarily utilized and compelling chelator is EDTA [44]. Although powerful at

preparing metals, and increased the Pb concentration and uptake indifferent pant parts. Same findings were also reported by [45] in rice crop they observed that the addition of EDTA increased the Pb uptake and ultimately reduced the whole plant growth.

Cadmium uptake (mg Plant^{-1}) and in shoot

Maximum uptake of Cd in shoot was recorded up to $0.93 \text{ mg plant}^{-1}$ (163%) in response to 50 ppm Cd + EDTA while minimum Cd uptake was observed in 25 ppm Cd treatment. By increasing the concentration of Cd level, uptake also increased. Maximum uptake in shoot was observed by the combination of higher dose of Cd and EDTA (Fig. 7).

Cadmium uptake increased by increasing the level of Cd and the addition of EDTA increased the uptake as compared to EDTA. [46] also observed that the application of EDTA was ineffective uptake of Cd, with the addition of EDTA Cd uptake was higher in the stems and lower in the roots.

Lead uptake (mg Plant^{-1}) and in shoot

Maximum uptake of Pb in shoot were recorded up to $7.60 \text{ mg plant}^{-1}$ (124%) in response to 100 ppm Pb + EDTA While, minimum Pb concentration was observed in 50 ppm Pb treatment. By increasing the concentration of Pb level, uptake also increased. Maximum uptake in shoot was observed by the combination of higher dose of Pb and EDTA (Fig. 8).

The chelating agents enhanced the transport of Pb from roots to shoots, such as H-EDTA and EDTA, while the concentration of Pb in the roots decreased sharply. So Pb can be easily transported in plants in the forms of Pb chelators complexes [47, 48] identified that Pb-EDTA complex was transported through plant and accumulated in the leaves. In rice, it was also reported that the root structure was damaged by excessive Na, resulting in

enhanced flow of Na into the xylem vessels and increased accumulation of Na in the shoot [49].

Figure 1. Effect of different concentrations of cadmium (Cd) and lead (Pb) on shoot fresh weight of Maize cultivars

Figure 2. Effect of different concentrations of cadmium (Cd) and lead (Pb) on shoot dry weight of Maize cultivars

Figure 3. Effect of different concentrations of cadmium (Cd) and lead (Pb) on chlorophyll content of Maize cultivars

Figure 4. Effect of different concentrations of cadmium (Cd) and lead (Pb) on relative water content of Maize cultivars

Figure 5. Effect of different concentrations of cadmium (Cd) on Maize cultivars

Figure 6. Effect of different concentrations of lead (Pb) on Maize cultivars

Figure 7. Uptake of different concentrations of cadmium (Cd) in Maize cultivars

Figure 8. Uptake of different concentrations of lead (Pb) in Maize cultivars

Conclusion

In the present study the toxic effect of heavy metals reduced the plant fresh and dry biomass, chlorophyll contents and relative

water contents. The addition of chelating agent like EDTA increased the uptake of heavy metals which were observed in the term of Cd and Pb uptake. It was concluded

that the addition of Cd and Pb decreased the overall plant biomass. The addition of EDTA increased the concentration of Cd and Pb. The maize cultivar Syngenta-33H-25 accumulated more Cd and Pb as compared to maize cultivar Syngenta-8711. The Cd concentration of all maize cultivars was increased with increase in cadmium concentration and in the presence of EDTA as compared to no EDTA. At all the cadmium stress levels the maximum Cd concentration was produced in Syngenta-33-H-25. The minimum Cd concentration was recorded in Syngenta-8711 at all cadmium stress levels. The Pb concentration of all maize cultivars was increased with increase in lead concentration and in the presence of EDTA as compared to no EDTA. At all the lead stress levels the maximum Pb concentration was produced in Syngenta-33-H-25. The minimum Pb concentration was recorded in Syngenta-8711 at all lead stress levels.

Authors' contributions

Conceived and designed the experiments: M Rasool & MA Haq, Performed the experiments: M Rasool & M Jan, Analyzed the data: M Jan & J Iqbal, Contributed reagents/ materials/ analysis tools: J Akhtar & M Ibrahim, Wrote the paper: M Rasool & M Jan.

Acknowledgement

The author wish to thanks Higher Education Commission (HEC) Islamabad, Pakistan for the provisions of funds from HEC funded indigenous scholarship.

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