Study of the degree of metal accumulation and toxicity of corn plants grown on heavy metals contaminated (artificially) soil

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Abstract. The Aim of this research is to examine the ability of maize (*Zea mays*) to accumulate heavy metals and assess the bio-concentration factor (BCF) by collecting, and analyzing data on heavy metal concentrations in *Zea mays*. This study assessed the accumulation of three selected heavy metals; Copper (Cu), lead (Pb), and Cadmium (Cd) in soil and the corresponding Bio concentration factor (BCF) of *Zea mays* grown on those soils using a systematic approach.

1 Introduction

Environmental problem of heavy metal pollution has reached a global scale. There is growing concern about the safety of food due to soil contamination from heavy metals released from natural and anthropogenic activities [2, 12]. The incidence of high levels of heavy metals in agricultural products due to heavy metal pollution (HMP) in the soil have drawn more and more attention nowadays [5]. In addition to posing a threat to the safety of agricultural products, heavy metal contaminants also pose a risk to human health after they have acquired them through the food chain in the ecosystem [3, 4]. The main route of health threat is the capacity of plants to accumulate metals and transfer them to the next trophic level in the food chain.

It is well known that phytoremediation is an environmentally friendly method for successfully reducing and economically re-vegetating soil that has been contaminated by heavy metals. In phytoremediation many higher plant species, referred to as hyper-accumulators, can accumulate extremely high metal concentrations in their tissues without displaying toxicity [1]. Recently, the remediation of heavy metal (HM) contaminated soils using plants has become a predominant research. Some plants that accumulate higher levels of these metals include Thlaspi sp., maize, sunflower, rice, wheat etc. The amount of metal that accumulates in plants depends on both the type of metal contaminant, the type of plant species [10] and other chemical and climatic factors.

Maize (Zea mays L.), is a versatile cereal crop that is grown extensively world-wide in a variety of agro-ecological environments. There are around 50 species that come in various sorts, textures, and grain sizes and shapes. The most prevalent colors are red, yellow, and white [13].The purpose of this research is to study the capability of maize(*zea, mays*) to

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uptake heavy metals, identify the relationship between the heavy metals BCF and their concentrations in maize and provide data to support future assessment of human health risks associated with HMP in soil in relation with the consumption of such cereals.

2 Materials and methods

This experiment was carried out in astrakhan state university, Russian federation in an Agricultural and Research laboratory center from April to June 2022 and the Materials used were Cereals, soil, auger, 2 mm sieve, plastic container, plastic bags, hand gloves, wipes marker, distilled and deionized water, oven, aquaria, conical flask, filter papers, thermometer, pH meter and digital weighing balance and atomic absorption spectrophotometer.

Maize seeds were obtained from local markets. In order to select the most viable seeds, a technique that suggests that seeds with a higher probability of germination are denser than water was used as a reference, so the seeds were immersed in a container of distilled water, and the seeds that precipitated to the bottom were selected, while those that remained on the surface were discarded (Figure 1).

The selected seeds were washed with a 1% sodium hypochlorite solution for two minutes to remove pathogens, and then cleaned with distilled water to remove chlorine residues. Following that, the seeds were wrapped in a moist paper towel and placed in a clothed petri dish before being left to germinate in a room with a constant temperature of 37^{o}C [5] (Figure 2).

Fig. 1. Selection of the most viable seeds.

Fig. 2. Seed germination.

2.1 Preparation of soil samples

According to soil sampling procedures, natural soil samples were taken from the compound field and sieved thoroughly to remove gravel and other debris. Then, fertilizer was added in the ratio 75% soil and 25% fertilizer. The final sample mixture was then transferred into

two plastic pots, and two additional plastic pots were used to store unadulterated soil samples. A mixture of heavy metals (Cu, Pb, and Cd in the forms of, $CuSO_4$,$6H_2O$, $Pb(CH_3COO)_2.3H_2O$, and CdO, respectively), were prepared which were then dissolved in water and left to homogenize for 48 hours. Finally the solution was spiked into the experimental soil to make sure the soil contains the target elements [7].

2.2 sawing the seedlings

A few well-germinated seedlings were transplanted into a plastic pot (pot#1P experimental), while a control pot (pot#1NP) was kept that didn't have any plants in it. As a control experiment, the remaining seedlings were also sown in pot #2 (natural soil) that had received standard water treatment. The plants were grown in an environment with natural light, with temperatures typically between 18 and 24 ^oC and a range of humidity between 60 and 70 % (Figure 3). The mature plants were divided into two phases of harvesting, and the roots, stems, and leaves were separated (Figure 4).They were then thoroughly washed with tab water, finishing with a rinse in deionized water, and stored at a freezer temperature of -18 ^oC until analysis After the plants were harvested, soil samples from the experiment and control pots were also taken.

Fig. 3. Plant cultivation.

Fig. 4. Plant part harvesting.

Fig. 5. Sample preparations for analysis.

2.3 Heavy metals Soil determination

The soil sample was oven dried at 70°C for 48 hours to determine the total heavy metal content. The sample was then transferred to a digestion flask and 10 ml of concentrated nitric acid was added. The sample was then heated for 15 minutes at 95°C without boiling. After 5 minutes of cooling at room temperature, 5ml of concentrated HNO_3 was added, and the sample was heated again at 95°C for 30 minutes [8]. Concentrated HNO_3 were added until no more brown fumes were released. To a level of less than 5 ml, the solution was allowed to evaporate. After cooling, 3ml of H_2O_2 and 2ml of deionized water (DI) were added, and the mixture was heated below boiling point until the effervescence subsided and the solution cooled. Until effervescence stopped, 10 ml more H_2O_2 was added. At a temperature below the boiling point, this stage was continued for 2hours. The solution was then allowed to evaporate until less than 5ml remained. 10ml of concentrated HCl was added after cooling, and the solution was then heated at 95°C for 15 min. After cooling the sample was filtered through What Man filter paper into a 50ml volumetric flask, and was made up to the mark with DI (Figure 5), and was analyzed using AAS.

2.4 Plant heavy metal determination

The tissue from individual plant parts was divided into tiny pieces, dried for two days at 80°C, and then burned to ashes. 10 ml of concentrated HNO_3 and 1g of plant material were added to a digestion tube that had been sterilized. The sample was then heated for one hour on the heating block to 95°C. After cooling, 5ml of concentrated H_2SO_4 was added, and the sample was heated to 140^0C until charring first appeared [11]. 5ml of concentrated HNO_3 was added, cooled, and then heated to 180 °C. HNO_3 was added to the sample in smaller amounts at a time until a clear or light straw color was visible. 1ml of H_2O_2 was added and heated to 200 °C after cooling. Until there were no longer any brown fumes, this process was repeated. 10 ml of DI water and 0.5ml of concentrated HNO_3 were added after cooling, and the mixture was heated to 200 °C until white fume was released. The digest was then cooled, filtered through what man filter paper into a 50 ml volumetric flask made to the proper consistency with DI water, and subjected to an AAS analysis “Quantum .Z”.

Statistical analysis was performed using R studio Software version 12.0 ANOVA. The correlation significance level ($p < 0.01$ and $p < 0.05$) between different parameters and the concentration of metals is reported based on Pearson’s correlation coefficients. Results are presented as the mean (± standard deviation) (n = 3).

3 Results and Discussion

The results indicated that the amount of HM concentration in the two types of soils differed significantly ($p<0.05$), as it can be seen from table 1. The soil where the plant was grown contained 89.1±2.250mg.kg^{-1}Cu, 2.31±0.434mg.kg^{-1}Cd, and 57.18±5.972mg.kg^{-1}Pb, whereas the soil where no plant was grown contained 127±6.45mg.kg^{-1}Cu, 4.32±0.441mg.kg^{-1}Cd, and 117±12.110mg.kg-^{1}Pb. Additionally, the plant has absorbed a substantial amount of HM in its organs in the first fifteen days($Rt_{15,}St_{15,}Lf_{15}$): Its root, stem, and leaf contained ((75.91±2.392, 46.84±1.324, 40.04±1.527) mg.kg^{-1} of copper), ((1.62±0.199, 1.59±0.025, 1.10±0.004) mg.kg^{-1} Cd), ((55.11±2.308, 43.30±1.198, 40.24±1.527) mg.kg^{-1} of Pb) respectively (Table 1). The outcome of the experiment also reveals that there are differences in the proportions of Cu, Cd, and Pb in the various parts of the maize plant, which are as follows: root > stem > leaf, a comparable pattern as demonstrated by Alina Kabata-Pendias (2007) [4] . Furthermore there result showed there is generally a decrease in the composition of target metals in the plant organs when

measured after thirty days; $Rt_{30,}$ $St_{30,}$ and Lf_{30}.This can be explained the fact that Cd and Pb are not required by the plant for any metabolic activities thus the plant prevented their excessive accumulation to avoid toxicity.

Although plants don't need Cd for metabolic purposes, their relatively easy access to it indicates a serious health risk. Its presence in food, particularly in food and feed plants, is extremely concerning. Cd content in food plants ranges from 0.005-0.4 mg kg^{-1} and is slightly higher in roots and leafy vegetables than in other plant parts. The WHO recommends a permissible limit of 0.1 mg kg^{-1} to 0.2 mg kg^{-1} of Cd in plants, but in the current study, Cd levels were higher (1.1±0.004mg kg^{-1} to 3.59±0.135mg kg^{-1}), similar to those reported by Adekiya et al. (2018) [1], which had a mean of 3.03mg kg^{-1}. These results suggest that even cereals, which are thought to be the plant organ with the most stable chemical composition, may be exposed to excessive amounts of Cd from soil that is highly contaminated with the metal. Due to the risk of food chain contamination, this discovery of Cd in food grain is also significant for the health of the general public Plants naturally contain lead, but research has not yet indicated that lead is necessary for any of the processes that make up their metabolism. Because Pb is passively absorbed by roots, the rate at which it is taken up from soils is relatively slow [9].The average Pb concentrations in maize ranged from 27.63±5.868mg kg^{-1} to 55.11±2.308mg kg^{-1} (Table 1), which is significantly higher than the WHO/FAO-permitted limit of 0.30mg kg-^{1} for the majority of food plants. Therefore, if the land on which the maize plant grows is contaminated with lead metal, eating maize could be hazardous to one's health.

Some research papers have shown that Pb cannot be absorbed from the soil by the leaves because Pb is a heavy metal with little plant motion; Pb in the crop's leaves is primarily airborne; and plant roots only absorb very small amounts of Pb [6]. However, in this experiment, the concentration of Pb found in the experimental plant could not have been solely airborne, because the experiment was conducted in a dust-free laboratory; thus the limitation of uptake of Pb by plants is likely due to other factors such as soil type, climate, and organic composition.

Copper, a crucial element in plants, is a component of several "key" enzymes and is crucial to many physiological processes. With the exception of the root, which is slightly higher at 74.91±2.392mgkg^{-1} but statistically insignificant, the overall concentration of Cu in the experimental plant (maize) in this experiment is well below the range recommended value by WHO/FAO 73 mg kg^{-1} (Table 1). This finding likely confirms that there is a low possibility of copper contamination when eating maize plants grown in copper-contaminated soil.

Table 1. Heavy metal uptake by maize plants grown in heavy metal contaminated soil (in mg.kg^{-1}).

	Cu			**Cd**			**Pb**		
	mean	**Sd**	**%**	**mean**	**Sd**	**%**	**mean**	**Sd**	**%**
Sswp	127	6.450	5.08	4.32	0.441	10.21	117.00	12.110	10.35
Rss	89.1	2.250	2.53	2.31	0.434	18.79	57.18	5.972	10.44
$Rt._{15}$	74.91	2.392	3.15	1.62	0.199	12.30	55.11	2.308	4.19
$St._{15}$	46.84	1.324	2.83	1.59	0.025	1.58	43.30	1.198	2.77
$Lf._{15}$	40.04	1.527	3.81	1.10	0.004	0.35	40.24	1.527	3.79
$Rt._{30}$	57.18	5.972	10.44	1.21	0.017	1.44	43.30	1.198	2.77
$St._{30}$	40.04	1.527	3.81	2.14	0.159	7.43	34.18	1.409	4.12
$Lf._{30}$	27.93	2.712	9.71	3.59	0.135	3.76	27.63	5.868	21.24

Sswp= Soil sample without grown plant, Rss= root soil sample, Rt =root, St=stem, Lf= leaf,the subscript 15&30 represent number of days

3.1 Bio concentration factor (BCF) Translocation factor (TF)

BCF is defined as a plant's capacity to accumulate elements from its substrate. It can be calculated for each plant part, including the roots, stems, and leaves, using equation (1). An essential tool for determining a plant's potential for phytoremediation is the translocation factor (TF). Equation (2) is used to calculate it from the ratio of the element's presence in the plant's shoots to that in the plant's roots: BCF values greater than 1 show a plant species' potential for accomplishment in phytoremediation. Metal accumulators have TF values greater than 1, while metal excluders have TF values lower than 1 [10, 11].

$$\text{BCF} = \boldsymbol{C}_{\text{plant part}}/\boldsymbol{C}_{\text{soil}} \quad (1)$$

$$\text{TF} = \boldsymbol{C}_{\text{shoot}}/\boldsymbol{C}_{\text{root}} \quad (2)$$

Where; C represents Metal concentration; E.g. $C_{plant\ part}$= Metal concentration in plant parts, C_{soil}= Metal concentration in the soil

The result in Tables 2 and 3 showed that BCF and TF are all below 1 thus maize is not good hyper accumulator but as compared to other crop plants it is relatively good in phytoreme-diation.

Table 2. Bioaccumulation factor of maize plant for target heavy metals for first 15 days.

	Cu	**Cd**	**Pb**
root	0.841	0.701	0.964
Stem	0.526	0.688	0.757
Leaf	0.449	0.476	0.704

Table 3. Bio transfer factor of maize plant for target heavy metals first 15 days.

	Cu	**Cd**	**Pb**
Root _Stem	0.625	0.981	0.786
Root_leave	0.535	0.679	0.730

4 Conclusion

Although Zea mays is not a particularly good heavy metal extractor from heavy metal-contaminated soil, according to BCF and TF calculations, its concentrations of Cu, Cd, and Pb are higher than those of other food plants. Zea mays, then, might be a reasonably good option for phytoremediation. The issue is that one of the main staple crop plants, maize, typically absorbs metals from the soil, which reduces crop productivity and interferes with physiological metabolism, resulting in unsafe food. Additionally, the buildup of heavy metals in such crop plants endangers the health of both people and the environment. The entry of toxic pollutants into the human body is facilitated by food chain contamination. Regular environmental monitoring and restoration are therefore required to ensure that soils used for maize farming are not contaminated with heavy metals, and governments should promote coordinated data collection, research, legislation, and regulations, as well as the use of indicators.

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