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Assessment of Zinc Bioaccumulation in Soybean

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ABSTRACT
In the present work, Soybean (*Glycine max* (L.) Merr.) plants were exposed to different concentrations of zinc up to 1250 mg/kg of soil to understand how they bioaccumulate into different plant parts. The zinc concentration in the plant parts was estimated at the pre-flowering, peak-flowering, and post-flowering stages. Our results directly correlate soil zinc concentration and bioaccumulation by soybean plants. There is a distinct accumulation hierarchy: roots accumulate the highest levels of zinc, followed by leaves, stems, and seeds. Important for human health, our study revealed that zinc content in soybean seeds was close to the permitted level of 500 mg/kg and thus safe for human consumption. We also analyzed translocation factors (TFs) and bioconcentration factors (BCFs) for zinc to understand the dynamics of zinc translocation within the plant. As both the TF and BCF values obtained were < 1, it is recommended that the Soybean crop should not be used for zinc phytoremediation.

Key words: Bioaccumulation factor, Atomic Absorption Spectrometry, Heavy metal, Translocation factor

INTRODUCTION

The escalating contamination of agricultural soils with heavy metals represents a significant challenge to food safety and ecosystem health. Among these metals, zinc, a crucial trace element required for numerous biochemical and physiological functions in plants, can become problematic in excessive amounts. Although zinc is essential for plant growth and development, its overaccumulation in soils can lead to toxic levels in crops, potentially compromising their safety for human consumption. The sources of excessive zinc in soil are multifaceted, including using sewage sludge or urban composts, fertilizers, emissions from waste incineration, mining residues, and metal smelting (Ali et al. 2023). Once zinc is present in the soil, its availability to plants is influenced by various factors, including soil physicochemical properties, root architecture, soil pH, organic matter content, and microbial interactions (Saleem et al. 2022). This dynamic interaction can lead to zinc mobilization from the soil into plant tissues, where it may accumulate to levels that disrupt essential physiological processes such as photosynthesis, nutrient uptake, and enzyme function (Chakraborty and Mishra 2020). In the context of major crops like soybean, known globally as a critical source of vegetable oil and protein, excessive zinc uptake can adversely affect yield and nutritional quality. Soybean plants, though resilient, are sensitive to imbalances in zinc levels, which can manifest in reduced growth, impaired reproductive development, and diminished seed quality (Gupta and Meena 2024). As the global population expands, ensuring the health and productivity of staple crops like soybean is paramount. This study aims to delve into zinc distribution and accumulation patterns within different parts of soybean plants. By investigating the correlation between zinc levels in soil and its concentration in grains, the research seeks to elucidate the impact of zinc contamination on crop quality and safety.

MATERIALS AND METHODS

Experimental set-up

The experiments were conducted in April at the Department of Botany, University of Rajasthan, Jaipur greenhouse, under natural outdoor conditions with a 12-hr photoperiod and an average temperature of 30°C. Pots filled with 4 kg of garden soil at 30 cm height and 25 cm diameter were used, with five zinc concentrations (250, 500, 750, 1000, 1250 mg/kg of soil) applied as zinc sulfate. No other supplement nutrients were applied. Pots without added zinc were taken as controls. The soybean seeds underwent surface sterilization using 0.1% mercuric chloride ($HgCl_2$) for two minutes, followed by thorough washing with distilled water (DW). Ten sterilized soybean seeds were sown equidistant at 2cm deep in each pot. Three replicates were used for each concentration. Watering was done on alternate days. The plant samples were collected at pre-, peak, and post-flowering stages to analyze zinc contents.

Estimation of zinc

The wet digestion method was adopted to extract heavy metal (zinc) from various plant parts (Anonymous 1984). Samples were dried in a hot air oven set at 80°C for 7 hrs to determine the amount of metal absorbed. One gram of each sample was weighed into a conical flask, then 10 mL of HNO_3 was added, followed by 3 mL of $HClO_4$(2:1), and left for 2-3 hrs in a water bath. 10 ml of HCl was then added to dissolve inorganic salts and oxides. The digested samples were filtered through 0.45 ìm pore size Millipore filter paper and made up to 50 mL with distilled water before the metal contents were determined through Atomic Absorption Spectrometry (AAS) Perkin Elmer (model 1100B).

Bioaccumulation and translocation factor

The bioaccumulation factor estimates the relative availability of HMs in soil and the plant's ability to uptake a particular metal. This factor is often calculated based on the ratio between concentrations in the seed and the available HM fraction in the soil (Salazar et al. 2012). After entering the plant's root system, metals can either stay in the roots or go to the shoot system, which can be estimated using the translocation factor (TF) (Marchiol et al. 2004).

$$TF = C_{shoot}/C_{root}$$

Where, C_{root} is Zn concentration in the root and C_{shoot} in the soybean shoot.

Statistical analysis

Statistical analysis was conducted using SPSS version 25.0 and Microsoft Office Excel 2016. The data were calculated by variance analysis (ANOVA). The significance of differences between control and treatment was determined at the 0.05, 0.01 and 0.1 level of probability. Data presented in this study were expressed as mean ± standard error (S.E.).

RESULT AND DISCUSSION

The soil physic-chemical proterties of the soil used in the present research are presented in Table 1. The pH of the soil used for the study is slightly alkaline and almost neutral; thus, it was found to be within a range that is good for the cultivation of crops (Sirisuntornlak et al. 2021). The soil's electrical conductivity (EC) was within the acceptable range (0 – 8 dS/m), indicating that the soil is not saline and fertile (1.1 - 5.7 dS/m), i.e., the optimal range in the soil. More so, the macro-nutrient element results were adequate for producing soybeans.

Soybean seeds from plants grown in soil amended with zinc @1250 mg/kg of soil had the highest zinc concentration, followed by those from soil with @1000 mg/kg of soil (Table 2) and the lowest in soil amended with @250 mg/kg of soil. Zinc concentration in soybean seeds from plants grown in the treatments was close to the permitted level at 500 mg/kg, below the potential health hazard of direct consumption. This pattern was evidenced by lower accumulation at early growth stages and increased accumulation at maturity, reflecting the plant's distribution among organs coinciding with nutrient incorporation and remobilization. These

Table 1. Physiochemical properties of garden soil

Soil properties	**Value**
pH	7.2±0.4
Electrical conductivity (dS/m)	1.61±0.01
Organic carbon (%)	0.16±.02
N (%)	0.5
P (%)	0.92
K (%)	1.52
Ca (cmol/kg)	1.8
Mg (cmol/kg)	11.98
Iron (Fe) (ppm)	7.42±0.44
Copper (Cu) (ppm)	0.64±0.04
Maganese (Mn) (ppm)	6.46±0.70
Zinc (Zn) (ppm)	0.005±0.002

Values were expressed as mean± SEM

Table 2. Zinc content in the seed (mg/g) of *Glycine max*

Zn application to soil (mg/kg)					
Control (0)	**250**	**500**	**750**	**1000**	**1250**
0.02±0.0	0.0588±0.006 ^{b}	0.0706±0.002 ^{b}	0.378±0.030 ^{c}	0.835±0.063 ^{c}	1.128±0.17 ^{c}

Values were expressed as mean± SEM, Significance level: a = $p \le 0.1$, b = $p \le 0.05$, c = $p \le 0.01$

Table 3. Accumulation of Zinc in Leaf (mg/g) in *Glycine max* at different stages of plant growth

Treatment	Pre-flowering stage	Peak-flowering stage	Post-flowering stage
Control	0.00067±0.0	0.0004±0.0	0.002±0.0
250 mg/kg	0.7737±0.054 ^{a}	1.2538±0.22 ^{c}	1.7893±0.18 ^{c}
500 mg/kg	1.0167±0.22 ^{b}	1.3732±0.19 ^{c}	1.6372±0.23 ^{c}
750 mg/kg	1.2545±0.19 ^{c}	2.2786±0.20 ^{c}	2.6193±0.27 ^{c}
1000 mg/kg	2.2089±0.23 ^{c}	2.8673±0.25 ^{c}	3.3997±0.25 ^{c}
1250 mg/kg	2.8560±0.19 ^{c}	3.1493±0.16 ^{c}	3.7624±0.16 ^{c}

Values were expressed as mean± SEM, Significance level: a = $p \le 0.1$, b = $p \le 0.05$, c = $p \le 0.01$

observations align with findings from previous studies which also reported elevated concentrations of heavy metals in soybeans exceeding permissible limits, both in our study area and other locations (Salazar et al. 2012, Rodriguez et al. 2011).

Zinc content in the leaves exceeded the maximum allowable limits established by the Joint FAO/WHO standards (Anonymous 2007) (Table 3). Thus, consuming these crop leaves may pose health risks due to high levels of zinc, which are associated with various illnesses. Furthermore, we noted a positive correlation between the zinc concentration in leaves and higher zinc levels in the soil. This finding aligns with the conclusions of Khudsar et al. (2008), who demonstrated that the Accumulation of Zn^{2+} in different plant parts was considerably high at each developmental stage of the treated plants and showed a positive correlation with Zn in the soil.

The zinc concentration in the soybean shoots was higher than in the seeds (Table 4). Furthermore, it was observed that the zinc levels in the shoots increased with higher zinc concentrations in the soil. This trend underscores the influence of soil zinc availability on the uptake and accumulation of zinc in plant shoots. Several studies have highlighted that the uptake of metals from soil is influenced by various factors such as the solubility of metals in soil, soil pH, plant growth stages, plant species, types of fertilizers used, and soil composition (Ismail et al. 2005, Sharma et al. 2006). These factors collectively affect the bioavailability of zinc and its subsequent accumulation in plant tissues.

The results revealed that the roots of soybean

Table 4. Accumulation of zinc in shoot (mg/g) in *Glycine max* at different stages of plant growth

Treatment	Pre-flowering stage	Peak-flowering stage	Post-flowering stage
Control	0.00018±0.0	0.0003±0.0	0.0003±0.0
250 mg/kg	0.5546±0.05 ^{a}	0.9156±0.020 ^{b}	1.2834±0.19 ^{b}
500 mg/kg	0.9389±0.034 ^{b}	1.1892±0.13 ^{b}	1.3795±0.22 ^{c}
750 mg/kg	1.1257±0.19 ^{c}	1.4748±0.28 ^{c}	1.9284±0.26 ^{c}
1000 mg/kg	2.1148±0.23 ^{c}	2.2104±0.26 ^{c}	2.4018±0.24 ^{c}
1250 mg/kg	2.2045±0.19 ^{c}	2.4386±0.21 ^{c}	2.7294±0.32 ^{c}

Values were expressed as mean± SEM, Significance level: a = $p \le 0.1$, b = $p \le 0.05$, c = $p \le 0.01$

Table 5. Accumulation of zinc in root (mg/g) in *Glycine max* at different stages of plant growth

Treatment	**Pre-flowering stage**	**Peak-flowering stage**	**Post-flowering stage**
Control	0.0005±0.0	0.0008±0.0	0.004±0.0
250 mg/kg	1.2368±0.19 ^{b}	1.4958±0.29 ^{c}	1.9971±0.19 ^{c}
500 mg/kg	1.5452±0.28 ^{c}	1.9286±0.19 ^{c}	2.2392±0.20 ^{c}
750 mg/kg	3.2865±0.32 ^{c}	3.6339±0.25 ^{c}	4.0428±0.26 ^{c}
1000 mg/kg	5.0452±0.19 ^{c}	6.1954±0.23 ^{c}	6.5635±0.29 ^{c}
1250 mg/kg	5.9213±0.25 ^{c}	6.4841±0.27 ^{c}	7.0726±0.31 ^{c}

Values were expressed as mean± SEM, Significance level: a = $p \leq 0.1$, b = $p \leq 0.05$, c = $p \leq 0.01$

Table 6. Translocation Factor of zinc in *Glycine max* at different stages of plant growth

Treatment	**Pre-flowering stage**	**Peak-flowering stage**	**Post-flowering stage**
250 mg/kg	0.4484±0.024	0.6120±0.064	0.6426±0.049
500 mg/kg	0.6075±0.012	0.6160±0.065	0.6160±0.075
750 mg/kg	0.3425±0.032	0.4058±0.074	0.4770±0.081
1000 mg/kg	0.4191±0.033	0.3567±0.032	0.3659±0.089
1250 mg/kg	0.3723±0.04	0.3760±0.029	0.3859±0.090

Values were expressed as mean± SEM

plants accumulated higher concentrations of zinc than other plant parts (Table 5). However, the concentration in the roots increased with an increase in the concentration in the soil. Baker et al. (1982) reported that the populations of *Silene maritima* accumulated zinc to a high degree in the roots relative to the shoots. This trend supports findings from Kaewsringam et al. (2014), who noted a direct relationship between soil metal content and plant uptake, emphasizing that higher soil concentrations lead to increased plant metal uptake.

The translocation factor (TF), a measure of heavy metal mobility within plants (Ondo et al. 2012), obtained in this study was consistently below one (Table 6). This suggests that zinc is not easily transferred from the roots to the shoots in soybean plants. Consequently, the roots accumulate higher metal levels than other plant parts. Probst et al. (2009) similarly observed higher metal concentrations in the roots of *Vicia faba* compared to leaves and stems. Similar results were reported in wheat by Li et al. (2012) and in *Carthamus tinctorius* by Namdjoyan et al. (2017). The roots serve as a primary storage area due to their continuous soil contact and extensive root hairs, which increase surface area for metal adsorption and absorption (Street et al. 2009, Yap et al. 2010). Overall, metal concentrations were highest in the roots, followed by the leaves. The lower heavy metal content in aerial parts may be attributed to factors such as immobilization of negatively charged pectin in cell walls, sequestration in the plasma membrane, intracellular precipitation of inorganic metal salts, or vacuolar sequestration in cortical or rhizodermal cells (Mahmood et al. 2007, Kopittke

Table 7. Bio-concentration Factor of zinc in *Glycine max* at post-flowering stage

Zn application to soil (mg/kg)				
250 mg/kg	**500 mg/kg**	**750 mg/kg**	**1000 mg/kg**	**1250 mg/kg**
0.2352±0.023	0.1412±0.026	0.504±0.030	0.835±0.029	0.9024±0.023

Values were expressed as mean± SEM

et al. 2007, Jiang et al. 2010, Arias et al. 2010).

The BCF values were 0.2352, 0.1412, 0.504, 0.835, and 0.9024 for soil amended with 250, 500, 750, 1000, and 1250 mg/kg of zinc, respectively (Table 7). The BCF indicates the plant's ability to accumulate heavy metals from the soil, with higher values indicating more significant accumulation potential.

CONCLUSION

In the present work, zinc accumulation is in the decreasing order in tissues of roots > leaves > shoots > grains. However, it showed an increasing trend in all parts with age pre- > peak > post-flowering stage. Our study reported that soybean plants accumulated zinc content in seeds close to the permitted level at 500 mg/kg soil amendment treatment which is below the permissible level (0.07 mg/g) set by the World Health Organization (WHO). In addition, zinc concentration in soybean was influenced by the soil's zinc concentration and the soybean's age. Our findings suggest that soybean crops should not be used for zinc phytoremediation because the BCF and TF values were lower than 1. Overall, our research provides valuable insights into the bioavailability of zinc in soybean plants, highlighting the need for effective strategies to mitigate potential risks to human health.

Authors' contributions: Both the authors contributed equally

Conflict of interest: Authors declare no conflict of interest

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