

## Zinc Biofortification as Treatment of Lead-Contaminated Soils

BASIM H. FARAJ, SABA R. KHUDHAIER<sup>1</sup>, SEDIK A. K. AL-HIYALY, SALIH A. AL-SALIH, DHUHA J. MAHDI, M. T. A. MOHAMMED\* AND MOHANAD R. DHEYAB

*Environment Research Center, University of Technology, Iraq, Baghdad, Iraq*  
*\*(e-mail: Mohammed.M.TA@uotechnology.edu.iq; Mobile: +964 77072 53359)*

(Received: May 15, 2022; Accepted: June 16, 2022)

---

### ABSTRACT

Metal biofortification may effectively improve plant productivity via overcoming the various plant growing constraints. This work was designed to examine the impact of zinc biofortification on soils synthetically contaminated by various lead concentrations by growing mungbeans. Silty clay loam soil samples in plastic pots were artificially contaminated with five lead levels and each Pb level received three zinc concentrations. Ten mungbean seeds were planted in each pot during the growing summer season of 2019. There were significant differences between all examined crop characters, and this reflected the capability of zinc biofortification in reducing lead toxicity. This indicated that certain metal biofortification might be the alternative technique to overcome soil toxicity due to heavy metal contamination.

**Key words :** Zn biofortification, Pb toxicity, mungbean, soil contamination, silty clay loam soils

### INTRODUCTION

Plant food crop productivity is significantly affected by several growth factors such as micronutrient deficiency, salinity, heavy metal contamination, lack of water and drought (Rui and Ricardo, 2017). Therefore, huge attention was concentrated on controlling this crop growth affecting factors by applying various techniques such as chemical synthetic and organic fertilizers, amelioration of soil salinity, heavy metal toxicity and modern watering techniques (Sahin *et al.*, 2014; Shrivastava and Kumar, 2015), selecting most evolved and adapted crops and metal biofortification (Malagoli *et al.*, 2015). The soil heavy metal toxicity was remediated by several methods such as biosurfactant techniques (Sarubbo *et al.*, 2015; Da Rocha Junior *et al.*, 2019), microbial polymers (Ayangbenro and Babalola, 2018), immobilization technology (Nejad *et al.*, 2018), sewage sludge (Placek *et al.*, 2015) and water containing hydrogen nan bubbles (Kim and Han, 2020). On the other hand, biofortification was applied to subsidize the plant crop with certain micronutrient elements by improving malnutrition and enhancing growth and productivity (Jha and Warkentin, 2020; Haider *et al.*, 2021). Both

biofortification and phytoremediation seem to have an almost similar target to assist the plant in overcoming the growth constraints (Wu *et al.*, 2015; Prahara *et al.*, 2021). Several studies have examined the ability of micronutrient elements such as selenium and zinc to enhance plant crops grown on soil contaminated with cadmium and lead (Hu *et al.*, 2014; Qaswar *et al.*, 2017). Also, they reported that such micronutrient biofortification had enhanced plant growth and prevented the accumulation of Cd and Pb in rice and wheat crops. So, the current work was designed to examine the effect of zinc biofortification on mungbeans grown on synthetically lead-contaminated soils.

### MATERIALS AND METHODS

The study was carried out using plastic pots of 30 cm diameter (almost 3 kg) containing loam soil. The soil was synthetically contaminated by lead as PbNO<sub>3</sub> at five concentrations (0.0, 100, 200, 300 and 400 µg/kg). Ten mungbean seeds were planted in each pot during the growing season of summer 2019. Zinc sulphate was sprayed as foliage application at three levels (0.0, 3.0 and 5.0 mg ZnSO<sub>4</sub>/l). The experiment was laid out as a completely

---

<sup>1</sup>Biology Department, College of Science, Mustansiriya University, Baghdad, Iraq.

randomized block design covering 15 treatments with three replications of each treatment. After seed germination, only four healthy plants were left in each pot, and the remaining seedling was removed. Zinc application was sprayed weekly after 20 days of the seedling stage. The experiment was left under natural conditions but with regular watering. By the end of the growing season, several plant characters such as plant height (cm), pod length (cm) per plant, seed number per pod, seed index (100-grain weight g), seed yield per pot (g) and straw dry weight per plot (g) of plants grown on each pot were measured. The collected results were statistically subjected to analysis of variance (F test), and the least significant difference was calculated for each examined variable.

## RESULTS AND DISCUSSION

Zinc biofortification had significantly enhanced mungbean growth cultivated in lead-contaminated soils in all examined plant characters. Furthermore, the higher zinc concentration obviously increased the mean values of these examined plant variables, but there were detectable impacts of soil lead concentrations on these characters. In general, this work found that plant length, seed number/pod and straw dry weight/plot of plants grown with 400 ppm of lead and biofortification with five ppm zinc had mean values higher than those of plants grown on lead-free soils with no added zinc. For plant height, the highest mean value of  $87.6 \pm 4.6$  cm was recorded for mungbean plants grown on free Pb soil and biofortification with 5.0 mg Zn/l, while the lowest mean value of  $33.1 \pm 2.0$  cm was measured for those plants grown on 400  $\mu$ Pb/kg but was not zinc biofortification (Fig. 1). The analysis of the variance test showed significant differences between grown mungbean plants in terms of lead soil contamination and zinc biofortification, and LSD values for both Pb and Zn were 10.7 and 15.6, respectively.

Regarding pod number per plant, the plants grown on uncontaminated soil with lead receiving 5.0 mg Zn/l gave the highest mean value ( $16.6 \pm 1.0$ ), while similar plants grown on soil contaminated with 400  $\mu$ g/kg without zinc biofortification gave the lowest mean value of  $3.7 \pm 0.6$  (Fig. 2).

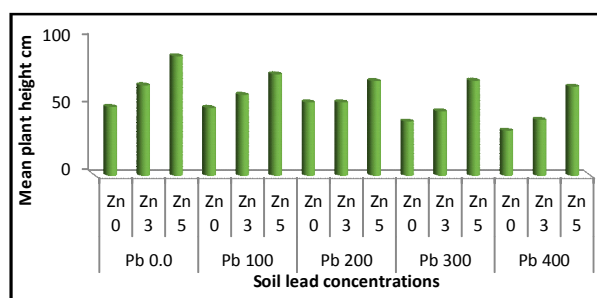


Fig. 1. Mean plant height (cm) of mungbean grown on soils contaminated with different lead concentrations and biofortification with three zinc levels.

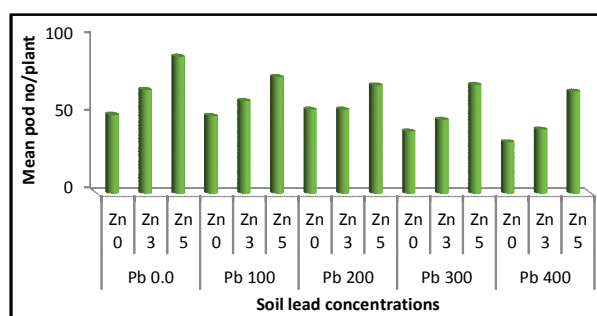


Fig. 2. Mean pod number per plant of mungbean grown on soils contaminated with different lead concentrations and biofortification with three zinc levels.

Statistical test of these data revealed clear significant effects of both soil lead concentrations and zinc spray applications. The least significant difference (LSD) values obviously confirmed these effects, which were 3.54 and 5.49 for soil lead and zinc spraying, respectively. Also, seed number per pod was significantly affected by zinc spray application where the plants grown on free lead soil and sprayed with 5.0 mg Zn/l gave the highest mean value of  $8.2 \pm 0.6$ . In contrast, those raised on lead-contaminated soil of 400  $\mu$ g/kg but not zinc biofortification had the lowest mean value of  $2.6 \pm 0.6$  (Fig. 3).

Analysis of variance of these data displayed clear effects of both increased soil lead content and the levels of zinc biofortification, where such differences were backed by calculated LSD of both Pb and Zn, which were 3.07 and 4.67, respectively (Table 2). In addition, seed indices (100-seed weight g) of the examined plants were found to vary significantly in terms of soil lead and zinc spraying, where those grown on uncontaminated soil receiving 5.0 mg Zn/l had the highest mean value ( $7.2 \pm 0.4$ ), while similar plants grown on soil contaminated with 400  $\mu$ g/kg and not zinc

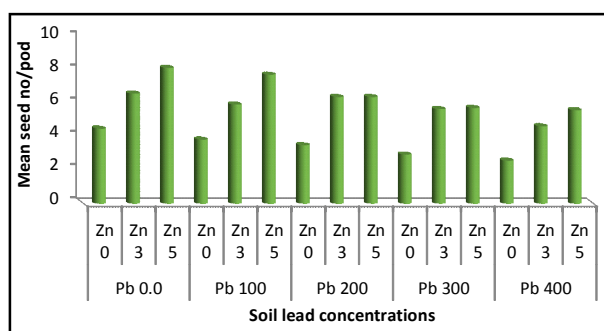


Fig. 3. Mean seed number per pod of mungbean grown on soils contaminated with different lead concentrations and biofortification with three zinc levels.

biofortification gave the lowest mean value of  $3.2 \pm 0.1$  (Fig. 4). However, the analysis of variance showed significant differences between mean values of grown mungbean plants for both lead soil contamination and zinc biofortification. These differences were clearly confirmed by LSD values of both Pb and Zn, which were 3.2 and 4.96, respectively.

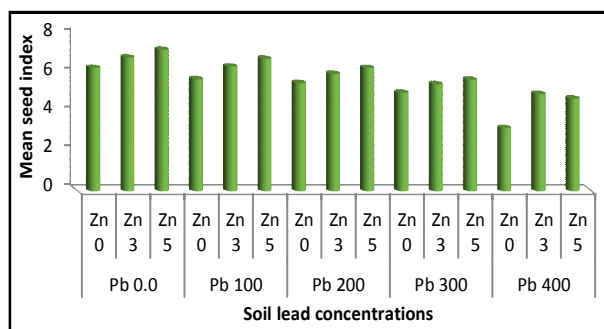


Fig. 4. Mean 100-seed weight (g) of mungbean grown on soils contaminated with different lead concentrations and biofortification with three zinc levels.

Regarding plant seed yield weight (g) per pod, there were obvious effects of zinc biofortification on all examined plants. Still, those grown particularly on contaminated soils by the lead of 300 and 400  $\mu\text{g}/\text{kg}$  were severely affected despite the fact that they were sprayed by 3.0 and 5.0 mg Zn/l. However, the mean values of plant seed yield weight per pod (g) were found to range from the minimum value of  $2.2 \pm 0.6$  g to the maximum value of  $34.8 \pm 4.3$  g of seeds planted on 400  $\mu\text{g}$  Pb/kg contaminated soil, but unsprayed by zinc and those planted on control soil but biofortification by 5 mg Zn/l, respectively (Fig. 5).

Statistical test of these data revealed clear significant effects of both soil lead concentrations and zinc spray applications. The

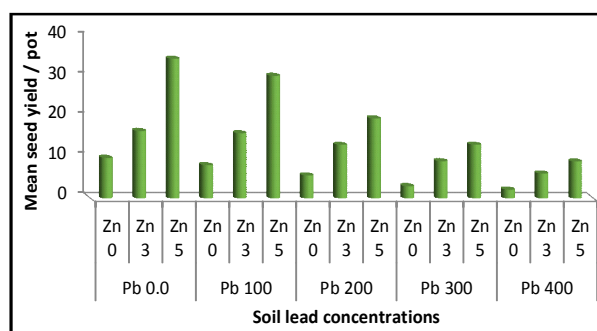


Fig. 5. Mean seed yield weight (g) per pod of mungbean grown on soils contaminated with different lead concentrations and biofortification with three zinc levels.

least significant difference (LSD) values obviously confirm these effects, which were 4.829 and 7.485 for soil lead and zinc application, respectively. In the case of straw dry weight per pod (g), this work found that the highest mean value of  $45.9 \pm 4.4$  g was recorded for seeds grown on control soil but biofortification by 5.0 mg Zn/l. In comparison, the lowest mean value of  $12.1 \pm 2.9$  g was detected in plants grown on 400  $\mu\text{g}$  Pb/kg of contaminated soil and unsprayed by zinc (Fig. 6).

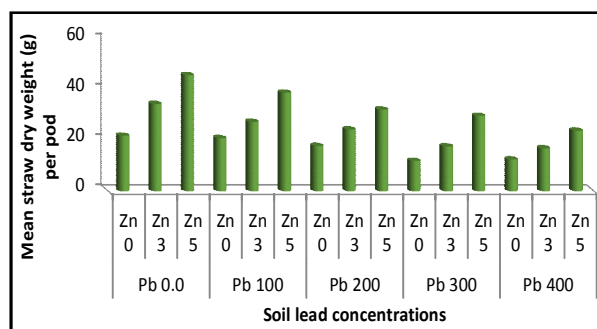


Fig. 6. Mean straw dry weight (g) per pod of mungbean grown on soils contaminated with different lead concentrations and biofortification with three zinc levels.

Analysis of variance of these data displayed clear effects of both increased soil lead content and the levels of zinc biofortification, where such differences were backed by calculated LSD of both soil Pb and Zn biofortification which were 6.70 and 10.39, respectively. In fact, there was a lack of such similar studies, but both phytoremediations of heavy metals contaminated soils and application of various micronutrient elements biofortification had shown similar findings (Laghlimi *et al.*, 2015; Shrestha *et al.*, 2019; Jha and Warkentin, 2020). From these results, it seems that there

was a certain limitation of zinc biofortification in the heavy metal contaminated environment despite the improvement of plant growth and productivity. However, still, the plant suffers from increased lead concentrations. However, such limitation was associated with both phytoremediation nutrient biofortification processes, as suggested by other studies (Mahar *et al.*, 2016; Singh *et al.*, 2016; Dias *et al.*, 2018).

## REFERENCES

- Ayangbenro, A. and Babalola, O. O. (2018). Metal(loid) bioremediation: Strategies employed by microbial polymers. *Sustainability* **10**: 3028. <https://doi.org/10.3390/su10093028>.
- Da Rocha Junior, R. B., Meira, H. M., Almeida, D. G., Rufino, R. D., Luna, J. M., Santos, V. A. and Sarubbo, L. A. (2019). Application of a low-cost biosurfactant in heavy metal remediation processes. *Biodegradation* **30**: 215-233.
- Dias, D., Costa, N. M. B., Nutti, M., Tako, E. and Martino, H. (2018). Advantages and limitations of *in vitro* and *in vivo* methods of iron and zinc bioavailability evaluation in the assessment of biofortification program effectiveness. *Crit. Rev. Food Sci. Nut.* **58**: 2136-2146.
- Haider, M. U., Hussain, M., Farooq, M., Ul-Allah, S., Ansari, M. J., Alwahibi, M. S. and Farooq, S. (2021). Zinc biofortification potential of diverse mungbean [*Vigna radiata* (L.) Wilczek] genotypes under field conditions. *PLoS One* **16**: 0253085. <https://doi.org/10.1371/journal.pone.0253085>.
- Hu, Y., Norton, G. and Duan, G. (2014). Effect of selenium fertilization on the accumulation of cadmium and lead in rice plants. *Plant and Soil* **384**: 131-140.
- Jha, A. and Warkentin, T. (2020). Biofortification of pulse crops: Status and future perspectives. *Plants* **9**: 73. <https://doi.org/10.3390/plants9010073>.
- Kim, D. and Han, J. (2020). Remediation of copper contaminated soils using water containing hydrogen nanobubbles. *Appl. Sci.* **10**: 2185. <https://doi.org/10.3390/app10062185>.
- Laghlimi, M., Baghdad, B., El-Hadi, H. and Bouabdli A. (2015). Phytoremediation mechanisms of heavy metal contaminated soils: A review. *Open J. Ecol.* **5**: 375-388.
- Mahar, A., Wang, P., Ali, A., Awasthi, M. K., Lahori, A. H., Wang, Q., Li, R. and Zhang, Z. (2016). Challenges and opportunities in the phytoremediation of heavy metals contaminated soils: A review. *Ecotoxicology and Environ. Safety* **126**: 111-121.
- Malagoli, M., Schiavon, M., Acqua, S. and Pilon-Smits, E. A. H. (2015). Effects of selenium biofortification on crop nutritional quality. *Front. Plant Sci.* **6**: 280. <https://doi.org/10.3389/fpls.2015.00280>.
- Nejad, Z. D., Jung, M. C. and Kim, K. (2018). Remediation of soils contaminated with heavy metals with an emphasis on immobilization technology. *Environ. Geochemistry and Health* **40**: 927-953.
- Placek, A., Grobelak, A. and Kacprzak, M. (2015). Improving the phytoremediation of heavy metals contaminated soil by use of sewage sludge. *J. Phytoremediation* **18**: 605-618.
- Prahara, S., Skaltra, M., Maltra, S., Bhadra, P., Shankar, T., Brestic, M., Hejank, V., Vachova, P. and Hossain, A. (2021). Zinc biofortification in food crops could alleviate the zinc malnutrition in human health. *Molecules* **26**: 3509. <https://doi.org/10.3390/molecules26123509>.
- Qaswar, M., Hussain, S. and Rengel, Z. (2017). Zinc fertilization increases grain zinc and reduces grain lead and cadmium concentrations more in zinc-biofortified than standard wheat cultivar. *Sci. Total Environ.* **605**: 454-460.
- Rui, M. A. M. and Ricardo, P. S. (2017). Soil salinity: Effect on vegetable crop growth, management practices to prevent and mitigate soil salinization. *Horticulturae* **3**: 30. <https://doi.org/10.3390/horticulturae3020030>.
- Sahin, U., Ors, S., Kiziloglu, F. M. and Kuslu, Y. (2014). Evaluation of water use and yield responses of drip-irrigated sugar beet with different irrigation techniques. *Chilean J. Agric. Res.* **74**: 302-310.
- Sarubbo, L. A., Rocha, Jr. R. B., Luna, J. M., Rufino, R. D., Santos, V. A. and Banat, I. M. (2015). Some aspects of heavy metals contamination remediation and role of biosurfactants. *Chem. Ecol.* **31**: 707-723.
- Shrestha, P., Belliturk, K. and Görres, J. H. (2019). Phytoremediation of heavy metal-contaminated soil by switchgrass: A comparative study utilizing different composts and coir fiber on pollution remediation, plant productivity and nutrient leaching. *Int. J. Environ. Res. Public Health* **16**: 1261. <https://doi.org/10.3390/ijerph16071261>.
- Shrivastava, P. and Kumar, R. (2015). Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. *Saudi J. Biol. Sci.* **22**: 123-131.
- Singh, U., Prahara, C. S., Chaturvedi, S. K. and Bohra, A. (2016). *Biofortification: Introduction, Approaches, Limitations and Challenges. Biofortification of Food Crops*. pp. 3-18. Springer, New Delhi.
- Wu, Z., Bañuelos, G. S., Lin, Z., Liu, Y., Yuan, L., Yin, X. and Li, M. (2015). Biofortification and phytoremediation of selenium in China. *Front. Plant Sci.* **6**: 136. <https://doi.org/10.3389/fpls.2015.00136>.