The Phytotoxic Effect of Lead (Pb) and Arsenic (As) on Physiological and Biochemical Parameters of Trigonella Foenum-GraceumL Seedlings

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Abstract

The biosphere is constantly polluted by accumulation of heavy metal contamination. Synthetic materials and industrial products lead to increase the heavy metals in environment. These contaminants also harmful for Agricultural crops. Lead (Pb) is extensively used in electronic and other industries. Arsenic is used in paper and textile industries.The present study was conducted to determine the phytotoxic effect of lead and arsenic on *Trigonella foenum-graceum*L. seedling. The different concentrations of lead and Arsenic ranging from 25ppm; 50ppm; 75ppm and 100ppm were used for 10 days. Effects of varying concentrations on germination of seed is taken into consideration. Various physio-biochemical parameters i.e., fresh and dry weights, water content and length, chlorophyll content is measured. The data suggested that lead and arsenic found to be toxic for germination of seeds.

Keywords: Heavy metal, lead, arsenic, seedling germination, phytotoxicity

Introduction

Heavy metal contamination in agriculture can have serious effectson the environment and human health. Heavy metals are naturally occurring elements that can be found in soil, water, and air. However, human activities such as mining, industrial activities, and the use of agrochemicals have contributed to an increase in heavy metal contamination in agricultural soils. In many parts of the world, agricultural soils are slightly to moderately contaminated by heavy metal toxicity such as Zn, Ni, Co, Cd, Cu, Cr, Pb, and As. Because industrial waste, dust from smelters, sewage sludge application, watering practices in agricultural lands, extended use of phosphatic fertilizers (Bell et al., 2001; Schwartz et al., 2001; Passariello et al., 2002).

All forms of life, including microorganisms, plants, and animals, are affected by metal toxicity, but the degree of toxicity varies for different organisms. Chemical, biological, and physical processes combine under certain circumstances to concentrate metals rather than dilute them (Igwe et al., 2005). Lead is a highly toxic heavy metal that can have significant negative effects on human health, especially when ingested or inhaled. Concentrations of lead are rapidly increasing in agricultural soil (McGrath et al., 1995). Arsenic contamination in soil can be toxic to crops, leading to reduced yields and poor-quality produce. Crops such as rice, wheat, and vegetables can accumulate arsenic, which can then be ingested by humans and animals through the food chain. Considering these, we have examined the toxicity of lead and arsenic on *Trigonella foenum-graceum* L. seedlings.

Metal-contaminated soil affects life indirectly via edible parts of crop plants. Especially at low concentrations of heavy metal accumulation these plants may not show any symptoms of toxicity, but eventually result in transferring heavy metals into the food chain slowly and creates risks for the human health as well as the entire ecosystem (Hart et al., 2006; Zhai et al., 2015; Gall et al., 2015). Excessive heavy metals in soil can also result in decreased soil microbial activity and soil fertility (Gao et al., 2010; Yuan et al., 2015). The heavy metals do not decompose at all and do not disappear from soil easily, though their release to the environment is restricted and limited (Monteiro et al., 2012) because they have low solubility and strong binding capacity with soil colloids. Therefore, they are persistent in both water and soil and cannot be converted into harmless compounds via biological processes (Aldoobie and Beltagi, 2013). Lead's half-life is quite long compared with other elements in nature (150-5000 years) and can be retained in the environment for ever. Therefore, the effect of lead contamination in agricultural areas can be quite devastating and last for many years (El-Beltagi and Mohamed, 2010; Truta et al., 2011; Hu et al., 2012; Romero-Freire et al., 2015).

*Trigonella foenum-graceum*L commonly known as fenugreek seeds having high value but low volume crop with many medicinal properties. Fenugreek is mainly used as spice and due to high medicinal value for diabetes and heart ailments. Fenugreek is a leguminous annual plant that grows to around 60 cm tall. It is cultivated worldwide as semi-arid crop. The aim of present study is to assess the effect of different concentrations of heavy metal lead on *Trigonella foenum-graceum*L.seed. Considering to this, in the present study following objectives were designed.

- Studies the growth parameter i.e. Fresh weight, Dry weight, water content and length of control and treated seedlings.
- Estimation of chlorophyll a, chlorophyll b and total chlorophyll content

Material and Methods

Test Plant

Certified seeds of *Trigonella foenum-graceum*L. were purchased from the local market, Junagadh. Equal size seeds were screened and washed with tap water for 3-4 times and soaked in distilled water for 2 h. Seeds were surface sterilized with 0.1% mercuric chloride (HgCl2) to prevent any fungal contamination. Seeds were washed with 4-5 times double distilled water immediately before use.

Heavy metals and their different concentrations

The stock solutions of lead (Pb) and arsenic (As) were prepared at concentrations of 25ppm, 50ppm, 75ppm, and 100ppm by using standard APHA methods (1998). Distilled water was used as control. The seeds were then allowed to germinate in sterilized Petri dishes on Whatmann filter-paper moistened with 5 ml of selected heavy metal test solution and kept in dark for 36 h. Each Petri dish was contained 25 seeds. The experiment was conducted in a growth room at 20 ± 25°C for 7 days under white light, 12 h photoperiod. For each metal concentration and control groups 10 seedlings were used.

Growth analysis

Growth is measured in the terms of fresh weight, dry weight, water content and seedling and root length. The length of seedling and root were recorded by using a centimetre scale. For the measurement of fresh and dry weights, freshly harvested shoot and root were taken. Freshly separated shoot and root were weighed before and after oven drying to a constant weight at 65°C for 72 hours. Water content of each stage was determined by difference in fresh and dry weights. Data were taken in 10 replicates and the mean value was calculated with + standard deviations.

Tolerance Index (TI)

Tolerance index (TI) was determined as suggested by Iqbal and Rahmati (1992) using the following formula:

TI = RLs *100 / RLc

RLs=Average root length in stress

RLc= Average root length in control

Phytotoxicity

The phytotoxicity (%) for shoot and root of 7 days old seedlings were calculated by the formula given by Chou and Lin (1976).

$$\% \text{ Phytotoxicity of Shoot} = \frac{\text{Shoot length of control - Shoot length of treatment X 100}}{\text{Shoot length of control}}$$

$$\% \text{ Phytotoxicity of Root} = \frac{\text{Root length of control - Root length of treatment X 100}}{\text{Root length of control}}$$

Determination of Chlorophyll content

Chlorophyll content was determined according to Arnon (1949) spectrophotometrically. Data were recorded at two wavelengths i.e., 645 and 663nm. Chlorophyll content was calculated using the following equation:

mg Chlorophyll-a / leaves=12.7 [(D 663) - 2.69(D 645)] × V/1000

mg Chlorophyll-b/ leaves=22.9[(D 645) - 4.68(D 663)] × V/1000

mg Total Chlorophyll/leaves=20.2(D 645) + 8.02(D 663) × V/1000

In the above equation D represents the optical density of the chlorophyll extract at the specific indicated wave-length through 1cm cell of spectrophotometer and methanol as blank. V is the final volume of chlorophyll extract.

Results and Discussion

Results showed considerable differences in germination. Germination rate was decreased with increasing heavy metal concentration.Growth is measured by physical attributes which are fresh and dry weights and watercontent is shown in Figure 1-3. In control seedlingFresh weight was recorded 70.70 mg/seedling and then decreased with increasing the concentration of Pb (Figure1). Similarly, dry weight in control seedling was recorded 4.85 mg/seedling which decreased with increasing the concentration of Pb treatment. Maximum dry weight was observed in control and minimum in 100 ppm treated plant (Figure 2). Water content in control seedling was 20.85mg/seedling and then decreased in treated plant up to 0.89 mg in 100 ppm (Figure 3).Moreover, in As treatment control seedling fresh weight was recorded 80.30 mg/seedling and then decreased with increasing concentration of As(Figure1). Dry weight in control seedling was recorded 4.31 mg/seedling which decreased with increasing the concentration of Astreatment. Maximum dry weight was observed in control and minimum in 100 ppm treated plant (Figure 2). Water content in control seedling was 30.99 mg/seedling and then decreased in treated plant up to 1.46 mg in 100 ppm (Figure 3).

The average Pb treated seedling length is decreased from 50.0 -1.8 cm, due to increased concentration of Pb (Figure 4). In in As treatment seedling length is decreased from 5.2- 1.3 cm owing to enhanced concentration of Arsenic (Figure 4).

The phytotoxicity of seedling is presented in figure (Figure 5). Phytotoxicity of seedling was decreased at lower concentration (25 ppm) and increased at higher concentration (100 ppm). The phytotoxicity of seedlingwas increased from 12-34 %, due to increased concentration of Pb as well as 15-36% in As.

Tolerance index calculated on the basis ratio of root length ratio of experimental tothat of control shows gradual reduction of tolerance index during growth in alltreatments. The seedlings were tested for tolerance to heavy metals, using different aqueous concentrations of lead and arsenic. The figure-6 shows indices of tolerance for seedling and root at different concentration treatments of Pb an As. The Tolerance index of seedling and root was decreased from 92 to 36 in Pb and 80-23.63 in As, respectively due to treatments with increased concentrations of both metals. (Figure 6).

The changes in chlorophyll a, chlorophyll b and total chlorophyll content are presented in figures (Figure 7, 8, 9). In this work the content of chlorophyll a, chlorophyll b and total chlorophyll was significantly decreased as the both heavy metal concentrations increased. In Pb treated seedlings control leaf shows 7.8 ± 3.4 and 2.9 ± 1.2 and 10.7 ± 4.6 mg g-1chlorophyll a, b and total contents respectively in lead treated seedlings. As treated control leaf shows 7.8 ± 3.8 and 2.6 ± 1.1 and 10.4 ± 4.9 mg g-1chlorophyll a, b and total contents respectively.

The present studyexposed the detrimental effect of heavy metals lead and arsenic by showing its toxic impact on seedling growth of *Trigonella foenum-graceum*. Increase in the concentration lead and arsenic changes in most of the growth parameters of plant studied during the investigation. High concentrations of Pb and As treatment is found responsible for decreasing the percentage of tolerance indices in *Trigonella foenum-graceum* and that was clearly evident from the inhibition of seedling growth.

In *Glycine max* (Amin et al., 2014) and in *Oryza sativa* (Panda, 2007) similar effects were observed when exposed to chromium stress. By roots, a high rate of metal uptake and translocation to shoots might be the cause of a reduction in seedling growth and biomass production, and therefore the potential of root growth has been proven to be an index of metal tolerance in plants (Subin and Steffy, 2013).

The present investigation revealed the detrimental effect of heavy metals Pb and As by showing its toxic impact on seedling growth of *Trigonella foenum-graceum*. Increase in the concentration lead to changes in most of the growth parameters of plants studied during the investigation. High concentrations of Pb and As treatment is found responsible for decreasing the percentage of tolerance indices and that was clearly evident from the inhibition of seedling and root growth. Pb and As uptakes by the roots and their translocation to shoots at higher concentration might be the cause of drastic reduction in seedling growth and biomass production. Lead and arsenic also inhibit chlorophyll content thus affecting photosynthetic capacity of the plant. However, more information is needed at the subcellular and molecular levels in order to get deeper insights into the mechanism explanations of heavy metal toxicity.

Figures and Plates

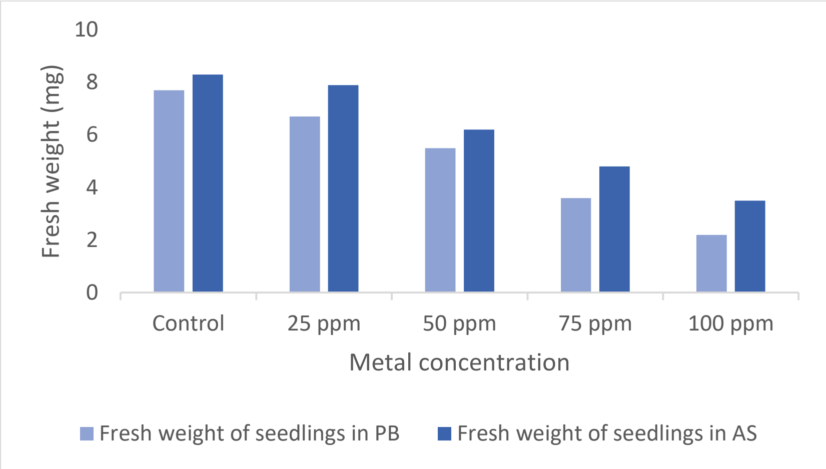


Figure 1: Changes in Fresh weight of control and treated *Trigonella foenum-graceum* seedling exposed to lead and arsenic

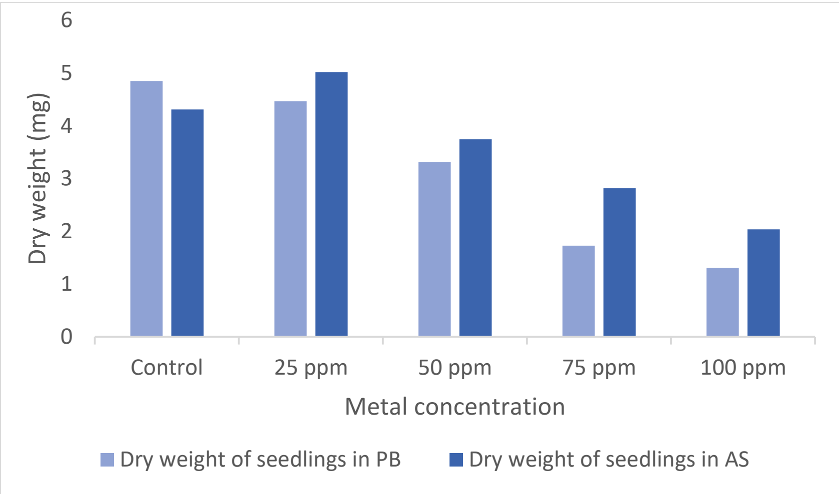


Figure 2: Changes in Dry weight of control and treated *Trigonella foenum-graceum* seedling exposed to lead and arsenic

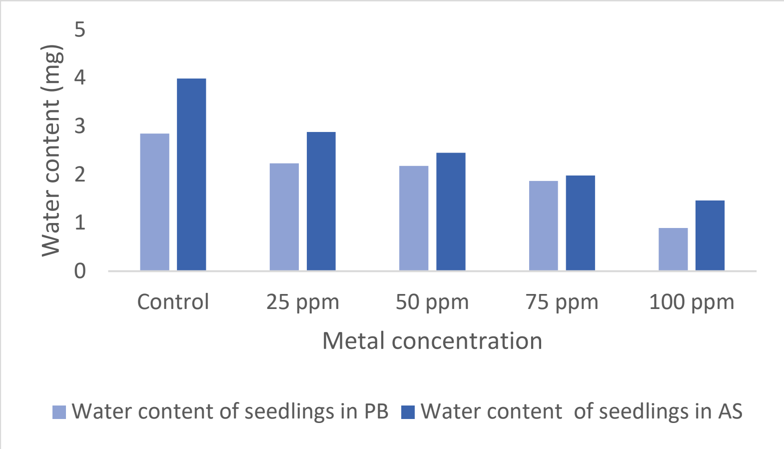


Figure 3: Changes in Water content of control and treated *Trigonella foenum-graceum* seedling exposed to lead and arsenic

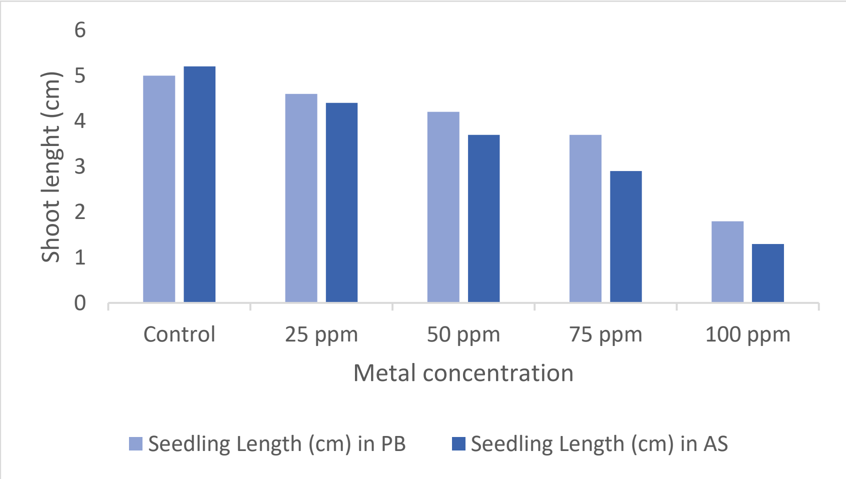


Figure 4: Changes in Seedling length of control and treated *Trigonella foenum-graceum* seedling exposed to lead and arsenic

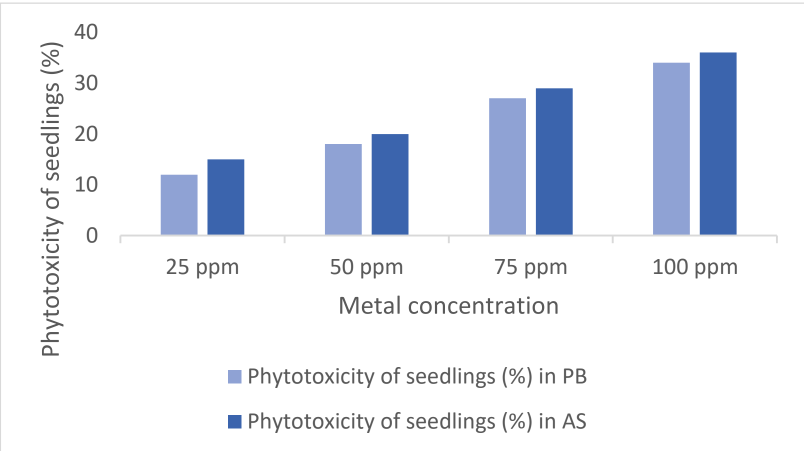


Figure 5: Changes in Phytotoxicity of control and treated *Trigonella foenum-graceum* seedling exposed to lead and arsenic

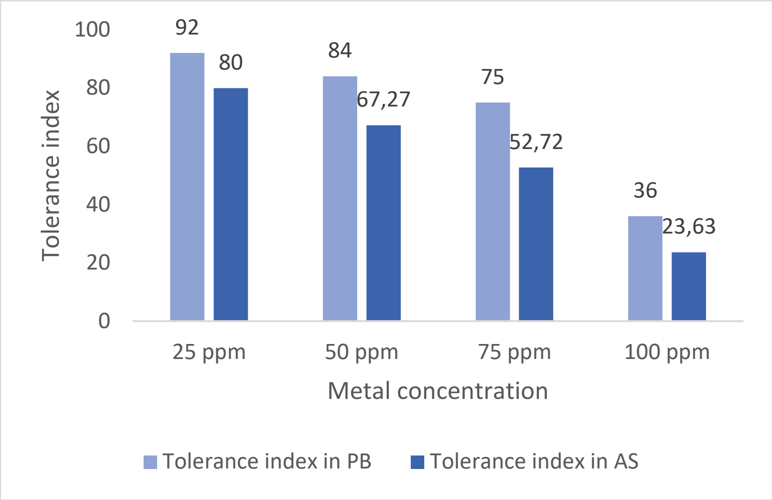


Figure 6: Changes in Tolerance index of control and treated *Trigonella foenum-graceum* seedling exposed to lead and arsenic

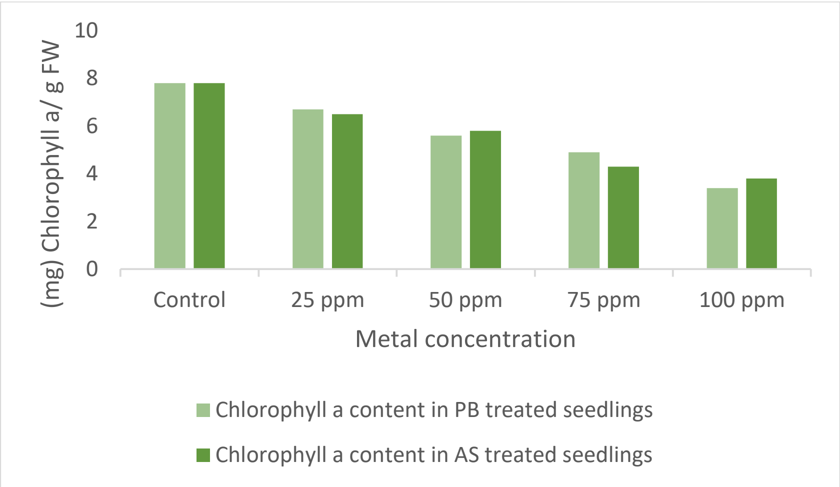


Figure 7: Changes in Chlorophyll a of control and treated *Trigonella foenum-graceum* seedling exposed to lead and arsenic

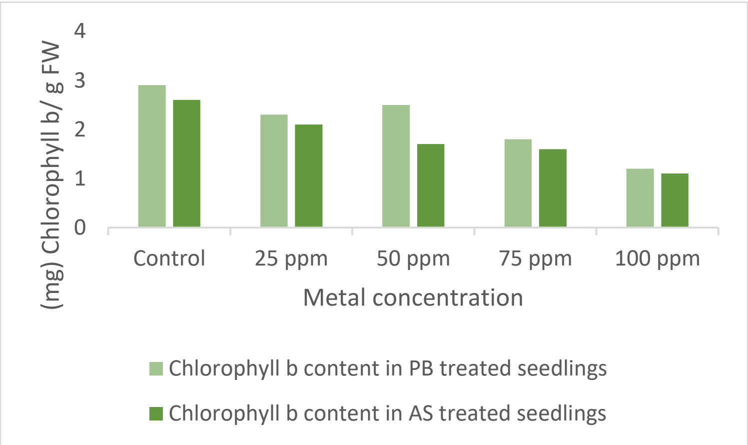


Figure 8: Changes in Chlorophyll b of control and treated *Trigonella foenum-graceum* seedling exposed to lead and arsenic

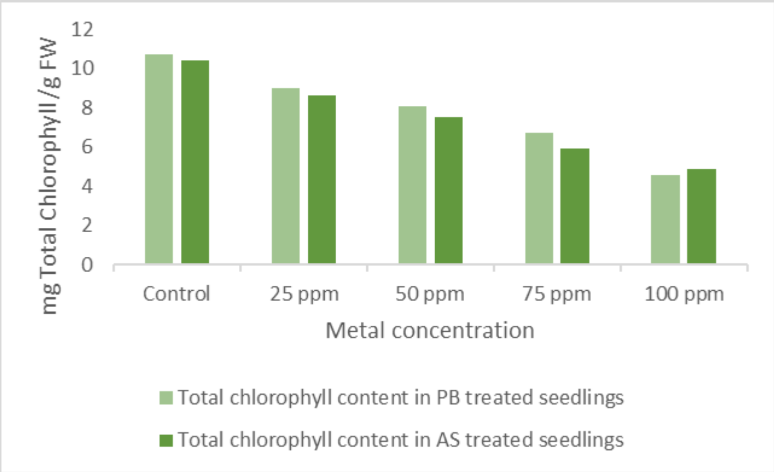


Figure 9: Changes in Total Chlorophyll of control and treated *Trigonellafoenum-graceum* seedling exposed to lead and arsenic

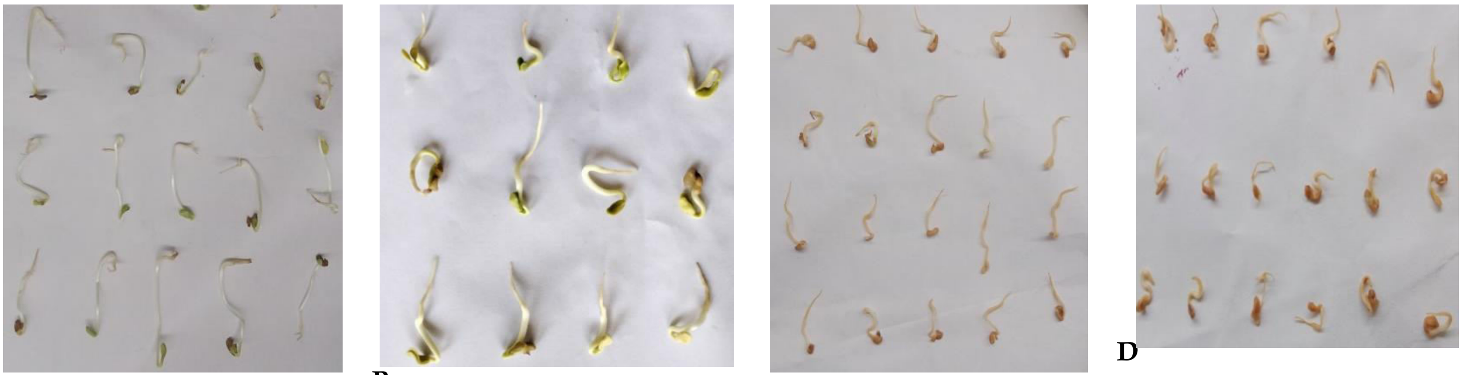


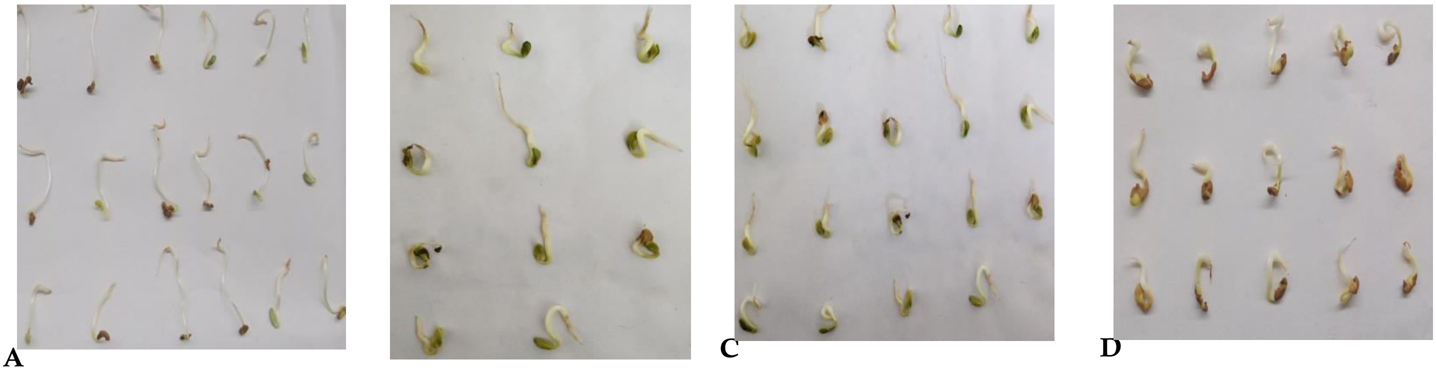
Plate 1 Changes in length of seedling treated with Pbafter germination

A. Control

B. 25 ppm

C. 75 ppm

D. 100 ppm



A B C D

Plate 2 Changes in length of seedling treated with Asafter germination

A. Control

B. 25 ppm

C. 75 ppm

D. 100 ppm

References

1. Aldoobie NF and Beltagi MS (2013). Physiological, biochemical and molecular responses of common bean (*Phaseolus vulgaris* L.) plants to heavy metals stress. African Journal of Biotechnology, 12(29): 4614-4622.
2. Amin H, Arain BA, Amin F and Surhio MA (2014). Analysis of growth response and tolerance index of *Glycine max* (L.) Merr. under hexavalent chromium stress. Advancements in Life Sciences, 1:231-41.
3. APHA (1998). Standard Methods for the Examination of Water and Wastewater. American Public Health Association, 20th edition, Washington.
4. Arnon DI (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. Plant Physiology, 24: 1.
5. Bell FG, Bullock SET, Hälbich TFJ and Lindsay P (2001). Environmental impacts associated with an abandoned mine in the Witbank Coalfield, South Africa. International Journal of Coal Geology, 45(2): 195-216.
6. Chou CH and Lin HJ (1976). Autointoxication mechanism of *Oryza sativa* L. Phytotoxic effects of decomposing rice residues in soil. Journal of Chemical Ecology, 2(3): 353-367.
7. El-Beltagi HS and Mohamed AA (2010). Changes in non-protein thiols, some antioxidant enzyme activity and ultrastructural alteration in radish plant (Raphanus sativus L.) grown under lead toxicity. NotulaeBotanicae Horti Agrobotanici Cluj-Napoca, 38(3): 76-85.
8. Gall JE, Boyd RS and Rajakaruna N (2015). Transfer of heavy metals through terrestrial food webs: a review. Environmental Monitoring and Assessment, 187(4): 201.
9. Gao S, Ou-yanga C, Tanga L, Zhub JQ, Xua Y, Wanga SH and Chen F (2010). Growth and antioxidant responses in Jatropha curcas seedling exposed to mercury toxicity. Journal of Hazardous Materials, 182: 591-597.
10. Hart AJ, Hester T, Sinclair K, Powell JJ, Goodship AE, Pele L, Fersht NL and Skinner J (2006). The association between metal ions from hip resurfacing and reduced T-cell counts. The Journal of Bone and Joint Surgery. British, 88(4): 449-454.

11. Hu R, Sun K, Su X, Pan YX, Zhang YF and Wan XP (2012). Physiological responses and tolerance mechanisms to Pb in two xerophils: Salsola passerina Bunge and Chenopodium album L. Journal of Hazardous Materials, 205-206:131-138.
12. Igwe, JC, Nnorom IC and Gbaruko BCG (2005). Kinetics of radionuclides and heavy metals behaviour in soils: Implications for plant growth. African Journal of Biotechnology, 4(B): 1541-1547.
13. Iqbal MZ and Rahmati K (1992). Tolerance of Albizia lebbeck to Cu and Fe application. Ekológia, ČSFR, 11(4): 427-430.
14. McGrath SP, Chaudri AM and Giller KE (1995) Long-term effects of metals in sewage sludge on soils, microorganisms and plants. Indian Journal of Microbiology, 14:94–104
15. Monteiro C, Santos C, Pinho S, Oliveira H, Pedrosa T and Dias MC (2012). Cadmium induced cyto- and genotoxicity are organ-dependent in lettuce. Chemical Research in Toxicology, 25(7): 1423-1434
16. Panda SK (2007). Chromium-mediated oxidative stress and ultrastructural changes in root cells of developing rice seedlings. Journal of Plant Physiology, 164:1419- 28.
17. Passariello B, Giuliano V, Quaresima S, Barbaro M, Caroli S, Forte G, Carelli G and Iavicoli I (2002). Evaluation of the environmental contamination at an abandoned mining site. Microchemical Journal, 73(1): 245-250
18. Romero-Freire A, Martin Peinado FJ and Van Gestel CA (2015). Effect of soil properties on the toxicity of Pb: assessment of the appropriateness of guideline values. Journal of Hazardous Materials, 289: 46-53.
19. Schwartz C, Gérard E, Perronnet K and Morel JL (2001). Measurement of in situ phytoextraction of zinc by spontaneous metallophytes growing on a former smelter site. Science of The Total Environment, 279(1): 215-221.
20. Subin MP and Steffy F (2013). Phytotoxic effects of cadmium on seed germination, early seeding growth and antioxidant enzyme activities in Cucurbita maxima duchesne. International Research Journal of Biological Sciences, 2(9): 40-47.
21. Truta E, Rosu CM and Bara IC (2011). Lead-induced genotoxicity in wheat. Analelestiintifice ale Universitatii "AlexandruIoanCuza". SectiuneaGeneticasiBiologieMoleculara TOM XII. 51–58.
22. Yuan L, Zhi W, Liu YS, Karyala S, Vikesland PJ, Chen X and Zhang HS (2015). Lead toxicity to the performance, viability and community composition of activated sludge microorganisms. Environmental Science and Technology, 49(2): 824- 830.
23. Zhai QX, Narbad A and Chen W (2015). Dietary strategies for the treatment of cadmium and lead toxicity. Nutrients, 7(1): 552-571.