Life Science Informatics Publications
Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences
Journal Home page http://www.rjlbpcs.com/
RJLBPCS
ISSN 2454-6348

Original Research Article **DOI - 10.26479/2018.0402.29**

EFFECT OF HEAVY METALS CADMIUM, NICKEL AND LEAD ON THE SEED GERMINATION AND EARLY SEEDLING GROWTH OF *PISUM SATIVUM*

Meena Deswal, J.S. Laura*
Department of Environmental Sciences, MDU, Rohtak, Haryana, India.

ABSTRACT: The heavy metals, Cd^{2+}, Ni^{2+}, and Pb^{2+} quantitative accumulation occurred in the order radical>plumule>leaves. Seed germination percentage was affected only at relatively higher concentrations. At the lower concentration of the heavy metals 0.1 mM for Cd^{2+}, 1 mM for Ni^{2+} and Pb^{2+} there was only delay in the germination. Early seedling growth was however, quite sensitivity even at low concentrations. As compared to plumule, the growth of the radical was affected to a relatively greater extent, by all the three heavy metals. There was a pronounced effect on elongation of radical. The fresh and dry weights of different plant organs were considerably reduced in heavy metal treated plants and this decrease, increase with concentration of heavy metal applied. The dry mass of embryonic axis increases with the seedling age and is associated with decline in cotyledonary dry mass. The embryonic axis dry weight as % of total dry weight decrease in case of heavy metals. The heavy metals resulted in a decrease in the nitrogen (%) of the various plant parts as compared to control. The percentage of nitrogen in radical and plumule decreased with the increase in concentration of the heavy metals. However in case of cotyledons the percentage of nitrogen increased with increase in concentration of the heavy metals. The protein content of plumule and radical increased with the heavy metal concentration, the reverse was observed in case of cotyledons. In the present investigation the amount of the total chlorophyll and carotenoids was reduced by the heavy metals and the reduction in the total chlorophyll and carotenoids was concentration dependent.

KEYWORDS: Heavy metal, seed germination, Chlorophyll, seedling growth, protein, nitrogen.

***Corresponding Author: Dr. J.S. Laura** Ph.D.
Department of Environmental Sciences, MDU, Rohtak, Haryana, India
*Email Address: jslmdu@gmail.com

1.INTRODUCTION

The release of toxic waste in the environment is increasing day by day [1-3]. Potentially hazardous chemicals are being discharge into the atmosphere which causes disease or abnormal conditions in humans, animals and vegetation [4-5]. The uncontrolled disposal of the industrial effluent and the solid wastes has become a matter of great concern as these are known to contain heavy metals such as Cd^{2+}, Pb^{2+}, Ni^{2+} etc [6]. Amongst the heavy metals cadmium and lead are major pollutants followed by nickel. Plants is a member of food chain and creates a risk for man and animals through contamination of food supplies. The food contaminated with heavy metals causes various diseases in human being and animals [7-9]. Besides endangering human health the heavy metals are likely to jeoparadize the welfare of manking by their impact on productivity The need of food for the growing population is not met since cultivable land is being degraded due to severe heavy metal contamination[10-12]. Although the heavy metals are not essential for plant growth they are readily taken up directly from air or indirectly through the contaminated soil and water by the plant species and get accumulated in various parts. It is reported that heavy metals are phytotoxic [3]. The amount of heavy metals absorbed by the plants tend to increase with increase in concentration of the heavy metals in the soil [13-14]. Seed germination is a crucial phenomenon in the life cycle of angiosperms and heavy metals are known to exert a deleterious effect on it [15]. The present work is designed to see the effect of cadmium, nickel, and lead on the percentage germination, biomass production, chlorophyll content, total nitrogen content, and to determine the accumulation and distribution in the various plant parts of *Pisum sativum.*

2. MATERIALS AND METHODS

Pea seed (*Pisum sativum*, L.cv.Azd p-1) were purchased from a local seed dealer. Seed were surface sterilized with 2% Sodium hypochloritefor five minutes, thoroughly washed and rinsed several times with sterile water. Hoagland solution (Half strength) was used for growing seedlings. Placed two layers of moistened whatman no.1 filter paper disc in petri dishes. Added 15 ml. of Hoagland's solution (half strength) for control. Added 15 ml each of cadmium chloride, lead acetate and nickel sulphate solution (all prepared in Hoagland solution) of 0.1 mM, 1mM, 3mM, and 5mM concentration in petri dishes. Placed ten seeds of uniform size in each of the petridish. Streptomycine sulphate (25µg/ml) was included in all solutions to suppress microbial growth. Seeds were germinated at 25 ^{0}C in an incubator, in the dark for 48 hrs, followed by germination in continuous light.

Measurement of seed germination and plant growth parameters

Percentage of seed germination- The number of seeds which sprouted by 3^{rd}, 5^{th}, 7^{th}, and 9^{th} day were calculated.

Growth of Embryonic axis- The effect of Cd^{2+}, Pb^{2+}, and Ni^{2+} on growth of embryonic axis of

germination pea seeds was examined by determining the length, fresh weight and dry weight of plumule and radical. All the parameters were determined by harvesting the seedlingson the 7th day of germination.The amount of chlorophyll and carotenoids was determined by the method of Arnon (1949) [16]. 50 mg of fresh leaf material was hand homogenized. The final volume was made 10 ml. The absorbance was taken at 480, 510, 645, 652, and 663 nm wavelengths using spectrophotometer (Hitachi model U-2000)
The chlorophyll content was calculated using following formulae-

$$\text{Chlorophyll a (mg/g)} = 12.7\ (A_{663}) - 2.69\ (A_{645}) \times \frac{V}{1000 \times W}$$

$$\text{Chlorophyll b (mg/g)} = 22.9\ (A_{645}) - 4.68\ (A_{663}) \times \frac{V}{1000 \times W}$$

$$\text{Total chlorophyll (mg/g)} = \frac{A652 \times 1000}{34.5} \times \frac{V}{1000 \times W}$$

$$\text{Carotenoids (mg/g)} = 7.6\ (A_{480} - 1.49 \times A_{510}) \times \frac{V}{1000 \times W}$$

A = Absorbance
V = Final volume of 80% acetone
W = Fresh weight of sample taken.

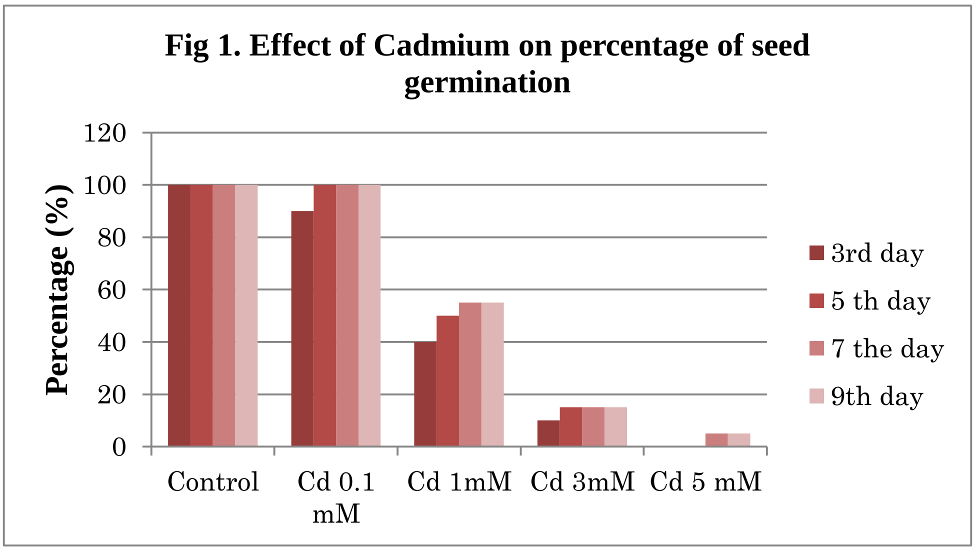
Protein estimation-Protein was estimated by following method of Lowry et al. 1951 [17]. The amount of protein was calculated from a standard curve prepared by using bovine serum albumin.

Estimation of Nitrogen-Total nitrogen was determined by the Micro-Kjeldahlmethod [18-19]. Digested a fixed amount of dried plant material in conc. H_2SO_4 and $HClO_4$(4:1). 0.5 ml of digested plant sample was taken for estimation.

Estimation of heavy metals-To determine the heavy metals in different plant parts A known volume of the digested plant solution from the previous step was made with distilled water and the heavy metals were determined by using Atomic Absorption spectrophotometer (Electronic Corporation of India Ltd (ECIL), model AAS4141).

3. RESULTS AND DISCUSSION

Results of seed germination are presented in Figure-1, 2, and 3. The seed germination was adversely affected by the heavy metals. The intensity of this adverse effect was concentration dependent, increasing with increase in concentration of each heavy metal [20]. The order of toxicity for metal ions on germination was $Cd^{2+} > Ni^{2+} > Pb^{2+}$. Except Ni^{2+}at the lower concentration all the metals showed delay in germination. In case of Pb^{2+}at 1mM concentration there was a delay in germination and at 3mM and 5mM on 6th day the germination was 90% and 80% respectively. With Cd^{2+} the delay in germination was up to a concentration of 0.1mM Cd^{2+} at the higher concentration there was a drastic decrease in seed germination, on 6th day for 1mM $Cd^{2+,}$ 3mM Cd^{2+} and 5mM Cd^{2+}recorded 55%, 15%, 5% germination respectively. In the case of Ni^{2+} there was no deleterious effect on germination at the lowest concentration of 0.1mM Ni^{2+}, at 1 mM Ni^{2+} there was a slight delay in germination and at 3mM Ni^{2+} and 5mM Ni^{2+} the value of % germination on 6th day were 80 and 25 respectively. Result of the present investigation on seed germination are in conformity of earlier workers, percentage seed germination of various plant species was depressed only at relatively higher concentration of the heavy metals like Cadmium, nickel, mercury, chromium and arsenic. At lower concentration of heavy metals up to 0.1 mM for Cd, 1mM for Ni^{2+} and Pb^{2+} there was only delay in the germination. Similar results were obtained by [8,4,20- 23]. Inhibition of early seedling growth by heavy metals has been noted earlier by several workers [6,5, 24-27].



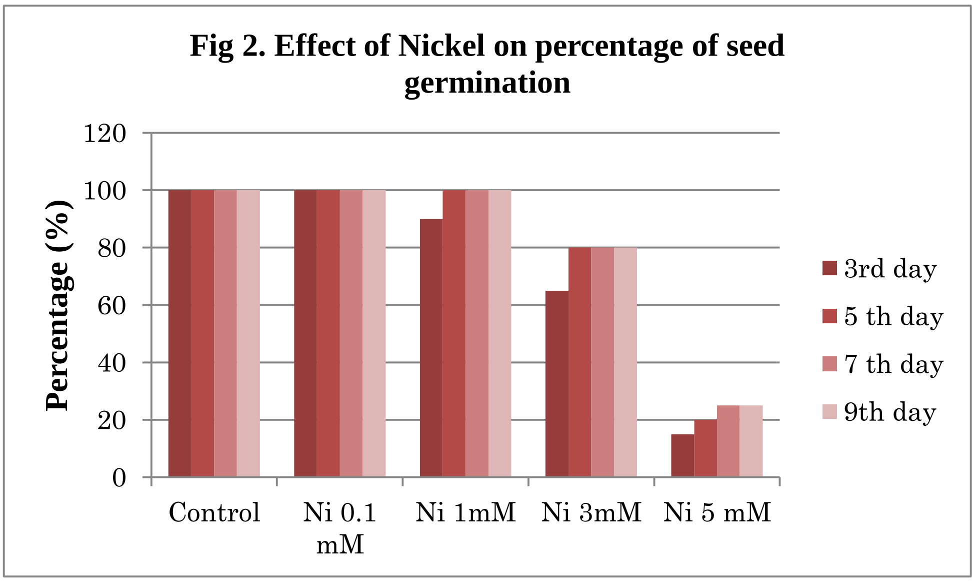
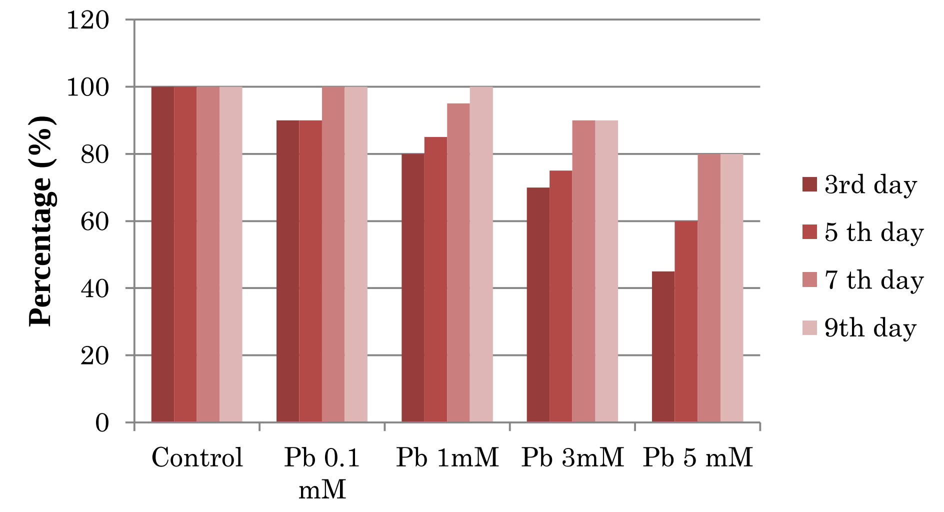


Fig. 3 Effect of lead on percentage of seed germination



The effect of the heavy metals on seedling growth is given Table-1. The growth of the embryonic axis was adversely affected by all the metals. This reduction in embryonic axis growth was concentration dependent as seen visually in Figure-4. The order of toxicity was Cd^{2+}> Ni^{2+}> Pb^{2}. The reduction in embryonic length at the lowest concentration of the metals (0.1mM) was 27.2% for Cd^{2+}, 5.4 % for Pb^{2+} and 1.9% for Ni^{2+}. However, at the highest concentration (5mM) of the metal these values were 89.1% for Cd^{2+}, 66.4% for Pb^{2+} and 87.2% for Ni^{2+}. For the metals cadmium and lead the reduction in embryonic length was more than the reduction in fresh weight. However, in case of Ni^{2+} the reduction in embryonic length and fresh weight was to the same extent. In general, the growth of radical was inhibited to a greater extent than that of plume by these heavy metals. Inhibition of early seedling growth by heavy metals has been noted earlier by several workers [27-30]. As compared to plumule, the growth of the radical was affected to a relatively greater extent, by all the three concerned heavy metals. A similar differential impact of heavy metals on these two parts of growing embryonic axis in germinating seeds has been observed by various workers [31-33]. The more pronounced effect on elongation of radical was probably due to its being in direct contact with the heavy metals containing solution resulting in a greater accumulation of heavy metals in the organ as is evident from the present investigation (Table 10) as well as by those reported by other workers [34-36]. The reduced growth of the embryonic axis may be due to the curtailed mobilization of the food reserves by the heavy metals from the cotyledons [37]. The more pronounced reduction of the radical may be due to the heavy metals affecting differently the mobilization of cotyledonary reserves to this part of the embryonic axis [38]. The result suggested that seedling emerging in heavy metal contaminated soils are likely to have poorly developed roots systems [39]. This would restrict the capacity of the seedlings to absorb nutrient and moisture from soil which may hamper their further growth [40].

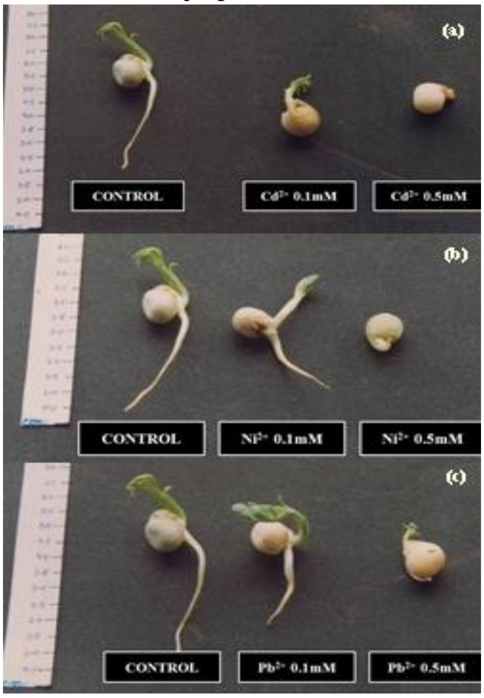


Figure-4. Effect of heavy metals on seedling, (a) Cadmium, (b) Nickel, (c) Lead.

The effect of the heavy metal on dry weight of various plant parts is given in Table-2. The general trend for all the heavy metal is a decrease in dry weight of both radical and plumule. The dry weight of both plumule and radical is reduced by all the heavy metals and the severity of this reduction increases with the concentration of each heavy metal. However the decrease in plumule dry weight is more drastic as compared to radical. The dry weight of cotyledons however increases with the increase in the concentration of the heavy metals. When the dry weight of each organ is calculated as % of total for each concentration, it is observed that there is greater retention of dry matter in the cotyledons with increase in concentration of the metal [41-42]. The proportion of the dry matter in the embryonic axis decreases with increased concentration of the metals. Such deleterious effects of heavy metals have been recognized [42-43]. The dry mass of embryonic axis increases with seedling age and is associated with decline in cotyledonary dry mass [44].The effect of the heavy metals on pigments is given in Table-3. Treatment of the seedlings with heavy metals resulted in reduced total chlorophyll content at all concentrations tested for each heavy metal. The order of toxicity to total chlorophyll was $Cd2^{+}$> Ni^{2+}> Pb^{2+}. The values as % of control for 1mM concentration of the heavy metals Cd^{2+}, Ni^{2+}, Pb^{2+} were 26%, 43% and 68% respectively. The content of chlorophyll decreased rapidly with increase in concentration of each heavy metal. The decrease in chlorophyll content may be due to reduced synthesis [2, 42, 45-46]. The trends for carotenoids were similar to total chlorophyll. The extent of decrease in content was 14% of control for Cd^{2+}, 32% control for Ni^{2+} and 65% of control for Pb^{2+}. On the other hand although there was a decrease in concentration of Chlorophyll b with increase in concentration of each heavy metal, this decrease in chlorophyll b was more drastic than chlorophyll a. This may be ascribed to the higher sensitivity of chlorophyll b to various types of stress including heavy metals. The corresponding value being 44%, 68% and 73% of control for Cd^{2+}, Ni^{2+},and Pb^{2+} respectively. However, at the lowest Ni^{2+} concentration i.e. 0.1mM there was a slight increase in chlorophyll b content. The order of toxicity is also same Cd^{2+}> Ni^{2+}> Pb^{2+}. The trend of carotenoids were similar. Similar results on the effects of heavy metals on the pigments of plant have been reported in the literature [47-53].Total organic nitrogen**-**The nitrogen percentage of all the plant parts is less than that of control at all the concentrations of heavy metals (Table-4). The percentage of nitrogen in plumule and radical decreases with the increase in the concentration of the heavy metal, however the decrease in percentage nitrogen is more severe in case of plumule as compared to radical. On the other hand the percentage of nitrogen in the cotyledons increases with the increase in the concentration of the heavy metal. It can be seen that the nitrogen content per seedling decreases with the increases with the increase in the concentration of the heavy metals was 99.8%, 55% and 94.2% of control for Cd^{2+}, Ni^{2+}, Pb^{2+} (Table-5). The nitrogen content of cotyledons increased with increase in concentration of the heavy metals. Whereas the nitrogen content of radical and plumule decreased with increase in heavy metal concentration. This decrease was more pronounced for plumule than for radical. Decrease in nitrogen content of various plant parts has been reported earlier by various workers [54-58].Protein-The trends are more or less as similar to those observed for nitrogen per plant part. The protein per seedling decreased with the increase in concentration of the heavy metal (Table-6). The value as % of control for the heavy metal $Cd^{2+}$and Ni^{2+} at 1mM concentration are 69.4% and 73.1 % respectively. However in case of Pb^{2+} there was a slight increase, the value being 102.5% of control. The results of the effect of heavy metals on the protein content is given in Table-7. As can be seen from the table that there is an increase in the protein content of both plumule and radical with the increase in the concentration of the heavy metal, an increase in protein of these organs could be taken as an indication of the synthesis specific metal binding peptides called phytochelatins [59-60]. This may results in increase in protein content of these organs, where as the protein content of cotyledons decreases with the increase in heavy metal concentration [61-65].The accumulation of the heavy metals is more in the radical as compared to the other parts (Table-8). The order of accumulation is Cd^{2+}, Ni^{2+}, Pb^{2+}. In case of the plumule the accumulation is more in case of Ni^{2+} followed by Cd^{2+} and Pb^{2+}. The accumulation in the cotyledons follows a similar trend i.e. Ni^{2+} >Cd^{2+}>Pb^{2+}.

4. CONCLUSION

The result of the present investigations shows that the heavy metals studied i.e. Cd^{2+}, Ni^{2+}, Pb^{2+} are readily taken up and accumulated in the plant parts and are phytotoxic. The toxicity is manifested in reduced germination and reduced growth of the seedlings. The heavy metals reduced growth of the embryonic axis. There was a more pronounced effect on the radical, probably due to its being in direct contact with the heavy metals containing solution, resulting in greater accumulation from the heavy metals containing solution. Larger quantity of the heavy metals in this organ leads to grater toxicity to this organ. This would restrict the capacity of the seedling to absorb moisture from the solution which hampers further growth of the seedling. This reduction in growth may be due to the reduced mobilization of material (as is evident from the discussion pertaining to dry weight, nitrogen and protein contents of various plant parts) from the cotyledons due to heavy metal toxicity.

REFERENCES

1. Kumar NM, Tomar M, Bhatnagar AK. Influence of cadmium on growth and development of Vicia faba Linn. In Indian Journal of Experimental Biology, 2000, 38(8):819–823.
2. Yousefi Z, Lolahi M, Majd A, Jonoubi P. Effect of cadmium on morphometric traits, antioxidant enzyme activity and phytochelatins synthase gene expression (SoPCS) of Saccharum officinarum var. cp 48-103 in vitro. Ecotoxicology and Environmental Safety, 2018, 157:472-481.
3. Shamshed S, Shahis M, Ratia M, Khalid S, Dumat C, Sabir M, Farooq ABU, Shah NS. Effect of organic amendment on cadmium stress to pea: A multivariate comparison of germination vs young seedlings and younger vs older leaves. Ecotoxicol Environ. Saf., 2018, 151:91-97.
4. Ahmad MSA, Ashraf M, Tabassam Q, Hussain M, Firdous H. Lead (Pb) induced regulation in growth, photosynthesis, and mineral nutrition in maize (*Zea mays* L.) plants at early growth stages. *Biol. Trace Elem. Res.*, 2011b,144: 1229-1239.
5. Nie J, Pan Y, Shi J, Guo Y, Yan Z, Duan X, Xu M. A comparative study on the uptake and toxicity of nickel added in the form of different salts of Maize seedlings.Int. J. Environ. Res. Public Health, 2015, 30(12):15075-87.
6. Ahmad I, Zahir Z, Jamil,A. Effect of cadmium on seed germination and seedling growth of four wheat (Triticum aestivum L.) cultivars. Pakistan Journal of Botany, 2012, 44(5):1569-1574.
7. Singh KJ, Thakur AK. Graviperceptional changes in the roots of cadmium treated soybean seedlings. Curr. Sci., 2014,107(8):1294−1298.
8. Jun-yu H, Yan-fang R, Cheng Z, De-an J. Effects of cadmium stress on seed germination, seedling growth and seed amylase activities in rice (*Oryza sativa*). *Rice Sci.,* 2008, 15: 319-325.
9. Raziuddin, Farhatullah, Hassan G, Akmal M, Shah SS, Mohammad F, Shafi M, Bakht J, Zhou W. Effects of cadmium and salinity on growth and photosynthesis parameters of brassica species. *Pak. J. Bot.*, 2011, 43(1): 333-340.
10. Liu J, Reid RJ, Smith FA. The mechanism of cobalt toxicity in mung beans. In. Physiologia Plantarum, 2000, 110(1):104–110.
11. Lux A, Šottníková A, Opatrná J, Greger M. Differences in structure of adventitious roots in Salix clones with contrasting characteristics of cadmium accumulation and sensitivity. In. Physiologia Plantarum, 2004, 120(4):537–545.

12. Tiwari S, Bajpai S, Srivastava N. Some Morphological and Biochemical Changes in Gram Seedlings Under Cadmium Stress. J. Bioremediat Biodegrad, 2017, 8:403.
13. Begonia GB, Davis CD, Begonia MFT, Gray CN. Growth responses of Indian mustard (Brassica juncea L.c zern.) and its phytoextraction of lead from a contaminated soil. Bull. Environ. Contam. Toxicol., 1998, 61:39-43.
14. Procházková D, Haisel D, Pavlíková D, Száková J, Wilhelmová N. The impact of increased soil risk elements on carotenoid contents. In Central European Journal of Biology, 2014, 9(7):678–685.
15. Mayer AM, Mayber AP. In the germination of seeds. Perma Press, New York, 1989.
16. Arnon DI. Copper enzymes in isolated chloroplast. Polyphenol oxidase in Beta Vulgaris. Plant Physiol., 1957, 24:51-57.
17. Lowery, oH, Rosebrrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J.Biol. Chem., 193:265-275.
18. Barbano, D.M., Clark, J.L., Chapman, E.D., Fleming, J.R., 1990. J. Assoc. Off. Anal. Chem, 73:849-859.
19. Purificación, S.P., María, J.N., Sławomir, W., Tadeusz, M., Agustín, G.A., 2013. An Overview of the Kjeldahl Method of Nitrogen Determination. Part II. Sample Preparation, Working Scale, Instrumental Finish, and Quality Control, Critical Reviews in Analytical Chemistry, 43:4, 224-272, DOI: 10.1080/10408347.2012.751787.
20. An YJ. Soil ecotoxicity assessment using cadmium sensitive plants. *Environ. Pollut.,* 2004,127: 21-26.
21. Zhang S, Hu J, Chen ZH, Chen JF, Zheng YY, Song WJ. Effects of Pb pollution on seed vigour of three rice varieties. *Rice Sci*., 2005b, 12: 197-202.
22. Correa AXR, Rorig LR, Verdinelli MA, Cotelle S, Ferard JF, Radetski CM. Cadmium phytotoxicity: Quantitative sensitivity relationships between classical endpoints and antioxidative enzyme biomarkers. *Sci. TotalEnviron.*, 2006, 357: 120-127.
23. Ahmad MSA, Hussain M, Ijaz S, Alvi AK. Photosynthetic performance of two mung bean (*Vignaradiata* (L.) Wilczek) cultivars under lead and copper application. *Int. J. Agric. Biol.*, 2008, 10(2): 167-176.
24. Titov AF, Talanova VV, Boeva NP. Growth responses of barley and wheat seedlings to lead and cadmium. *Biol. Plan.*, 1996, 38(3): 431-436.
25. Aydinalp C, Marinova S. The effects of heavy metals on seed germination and plant growth on alfalfa plant (*Medicago sativa*). *Bulgarian J. Agri. Sci.,* 2009,15: 347-350.
26. Raziuddin, Farhatullah, Hassan G, Akmal M, Shah SS, Mohammad F, Shafi M, Bakht J, Zhou W. Effects of cadmium and salinity on growth and photosynthesis parameters of brassica species.

Pak. J. Bot., 2011, 43(1): 333-340.

27. Datta JK, Bandhyopadhyay A, Banerjee A, Mondal NK. Phytotoxic effect of chromium on the germination, seedling growth of some wheat (Triticum aestivum L.) cultivars under laboratory condition. International Journal of Agricultural Technology, 2011, 7(2), 395−402.
28. Gang A, Vyas A, Vyas H. Toxic effect of heavy metals on germination and seedling growth of wheat. J. Environ. Res. Develop., 2013, 8(2):206−213.
29. Acharya S, Sharma DK. Study on the effect of heavy metals on seed germination and plant growth on Jatropha curcas. International Journal of Agricultural Science Research, 2014, 3(3):031−034. http://academeresearchjournals.org/journal/ijasr.
30. Rasafi TE, Nouri M, Bouda S, Haddioui A. The effect of Cd, Zn and Fe on seed germination and early seedlinf growth of wheat and bean. The journal of Institute of Landscape Ecology of Slovak Academy of Sciences, 2016, 35(3):213-223.
31. Moosavi SA, Gharineh MH, Afshari RT, Ebrahimi A. Effects of some heavy metals on seed germination characteristics of canola (Barassica napus), wheat (Triticum aestivum) and safflower (Carthamus tinctorious) to evaluate phytoremediation potential of these crops. J. Agric. Sci., 2012, 4(9):11−19.
32. Shaikh IR, Shaikh PR, Shaikh RA, Shaikh AA. Phytotoxic effects of heavy metals (Cr, Cd, Mn and Zn) on wheat (Triticum aestivum L.) seed germination and seedlings growth in black cotton soil of Nanded, India. Research Journal of Chemical Sciences, 2013, 3(6):14−23.
33. Singh R, Biharti N, Kumar G. Differential toxicity of heavy metals to growth and nitrate reductase activity of *Sesamum indicum* seedlings. *Phytochemistry,* 1994, 35: 1153–1156.
34. He JY, Ren YF, Cheng ZH, Jiang DA. Effects of cadmium stress on seed germination, seedling growth and seed amylase activities in rice (*Oryza sativa*). Rice Sci., 2008, 15: 319-325.
35. Bahmani R, Bihamta MR, Habibi D, Forozesh P, Ahmadvand S. Effect of Cadmium Chloride on Growth Parameters of Different Bean Genotypes (Phaseolus vulgaris L.). ARPN Journal of Agricultural and Biological Science, 2012, 7: 35-40.
36. Tiwari S, Bajpai S, Srivastava N. Some Morphological and Biochemical Changes in Gram Seedlings Under Cadmium Stress. J. Bioremediat Biodegrad, 2017, 8:403.
37. Hirve M, Bafna A. Effect of Cadmium exposures on growth and biochemical parameters of Vigna radiata seedlings. International Journal of Environmental Sciences, 2013, 4: 312-322.
38. Seevers PM, Daley JM, Catedral FF. The sole of peroxidise irozyme in resistance to wheat stem rust diseases. Plant Physiol. 1971, 48:358-360.
39. Saha S, Bhattacharjee C. Effect of lead acetate on Bengal gram seeds. International Journal of Clinical and Diagnostic Research, 2014, 2: 1-10.
40. Ashraf MY, Sadiq R, Hussain M, Ashraf M, Ahmad MSA. Toxic effect of nickel (Ni) on growth

and metabolism in germinating seeds of sunflower (*Helianthus annuus L.*). Biol Trace Elem Res, 2011, 143: 1695-1703.

41. Herath D, Bandara DC, Weerasinghe PA, Wijayawardhana D, Iqbal MCM. Effect of Cadmium on growth parameters and plant accumulation in different Rice (Oryzasativa L.) varieties in Sri Lanka. Tropical Agriculture Research, 2014, 25(4):432-442.
42. Paunov M, Koleva L, Vassilev A, Vangronsveld J, Goltsev V. Effects of different metals on photosynthesis: Cadmium and Zinc affect chlorophyll fluorescence in Durum Wheat. International Journal of Molecular Sciences, 2018, 19-787.
43. Honda R, Swaddiwudhipong W, Nishjom, Mahasakpan P, Teeyakasem W, Ruangyuttikarn W, Satarug S, Padungtod C, Nakagawa H. Cadmium Induced Renal Dysfunction among Residents of Rice Farming Area Downstream from Zinc– Mineralized belt in Thailand, Toxicology letters., 2010, 198, 26 - 32.
44. Guo-sheng S, Ming-xue C, Xiu-fu Z.Cadmium Accumulation and Its Toxicity in Brittle culm1 (bc1), A Fragile Rice Mutant.Rice Science. 2007,14(3):217 - 222.
45. Lichtenthaler HK, Wellburn AR. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. In. Biochem. Society Transactions, 1983, 11(5):591– 592.
46. Muradoglu F, Gundogdu M, Sezai E, Tarik E, Balta F, Jaafar HZE, Ziaul-Haq M. Cadmium toxicity affects chlorophyll a and b content, antioxidant enzyme activities and mineral nutrient accumulation in strawberry. In Biological Research, 2015, 48(11).
47. Myśliwa-Kurdziel B, Strzałka K. Influence of metals on biosynthesis of photosynthetic pigments. In PRASAD, M.N.V. – STRZABKA, K. Physiology and biochemistry of metal toxicity and to lerance in plants. Dordrecht : Kluwer Academic Publishers, 2002,pp. 201–227.
48. Hattab S, Dridi B, Chouba L, Kheder MB, Bousetta H. Photosynthesis and growth responses of pea Pisum sativum L. under heavy metals stress. In Journal of Environmental Science, 2009,21(11):1552–1556.
49. Pietrini F, Zacchini M, Iori V, Pietrosanti L, Ferretti M, Massacci A. Spatial distribution of cadmium in leaves and on photosynthesis: examples of different strategies in willow and poplar clones. In. Plant Biology, 2010, 12(2):355–363.
50. De Maria S, Puschenreiter M, Rivelli AR. Cadmium accumulation and physiological response of sunflower plants to Cd during the vegetative growing cycle. In Plant Soil Environment, 2013, 59(6):254–261.
51. Parmar P, Kumari N, Sharma v. Structural and functional alterations in photosynthetic apparatus of plants under cadmium stress. In Botanical Studies, 2013, 5: 45.
52. Dobroviczká T, Piršelová B, Mészáros P, Blehová A, Libantová J, Moravčíková J, Matušíková I. Effects of cadmium and arsenic ions on content of photosynthetic pigments in the leaves of

Glycine max (L.) Merrill. In Pakistan Journal of Botany, 2013,45(1):105–110.

53. Pirselova B, Kuna R, Lukas P, Havrlentova M. Effect of cadmium on growth, photosynthesis, pigments, iron and cadmium accumulation of FABA Bean, (VICIA FABULA CV. ASTAR). Agriculture (Polnohospodarstvo), 2016, 62(2):72-79.
54. Petrovic N, Kastori R, Rajcan I. The effect of cadmium on nitrate reductase activity in sugar beet (*Beta vulgaris*). *In* Plant Nutrition: Physiology and Applications. Edited by Van Beusichem, M.L., 1990, pp. 107–109. Kluwer Academic, Dordecht.
55. Chugh LK, Gupta VK, Sawahney SK. Effect of cadmium on enzymes of nitrogen metabolism in pea seedlings. *Phytochemistry,* 1992, 31: 395–400.
56. Kolloffel L. Respiration rate and mitochondriaial activity in the cotyledons of Pisum sativum L. during germination. Acta Bot. Neere, 1967, 16:111-112.
57. Kochlar S, Ahmad G, Kochlar VK. Amelioration of Cd^{++} toxicity by Ca^{++} on germination, growth and changes in anti-oxidant and nitrogen assimilation enzymes in mungbean (Vigna mungo) seedlings. In. Journal of Plant Biotechnology, 2004, 6(4):259–64.
58. Chaffei C, Pague K, Suzuki A, Gouia H, Gharbel MH, Masclaux-Daubresse C. Cadmium toxicity induced changes in Nitrogen Management in Lycopersicon esculantum leading to a metabolic safeguard through an amino acid storage. Plant and Cell Physiology., 2004, 45(11):1681-1693.
59. Grill E, Winnacker EL, Zenk MN. Phytochelatin a class of heavy metal binding peptides from plants are functionally analogous to metallothioneins. Proc. Natl. Acad. Sci. U.S.A., 1987, 84:439-444.
60. Steffens JC. The heavy metals binding peptides of plants. Ann. Rev. Plant Physiol. Mol. Biol., 1990, 41:553-575.
61. Romero-Puertas MC, Palma JM, Gómez M, del Rio LA, Sandalio LM.Cadmium causes the oxidative modification of proteins in pea plants. *Plant Cell Environ.,* 2002,25: 677–686.
62. Bavi K, Kholdebarin B. Effect of cadmium on growth, protein content and peroxidase activity in pea plants. Pakistan Journal of Botany, 2011, 43(3):1467-1470.
63. Jie Z, Enrico M, Youngsook L. Vacuolar Transporters for Cadmium and Arsenic in Plants and their Applications in Phytoremediation and Crop Development, *Plant and Cell Physiology*, pcy006, 2018, https://doi.org/10.1093/pcp/pcy006
64. Kummerová M, Zezulka Š, Kráľová K, Masarovičová E. Effect of zinc and cadmium on physiological and production characteristics in Matricaria recutita. In Biologia Plantarum, 2010, 54(2):308–314.
65. Pichtel J, Bradway DJ. Conventional crops and organic amendments for Pb, Cd and Zn treatment at a severely contaminated site. In Bioresourse Technology, 2008, 99(5):1242–1251.

Table-1. Effect of Cd^{2+}, Ni^{2+} and Pb^{2+} on radical and plumule length and fresh weight of pea seedling.

Treatment	Radical		Plumule		Embryonic Axis	
	Length (cm)	**Fresh wt.**(mg)	**Length** (cm)	**Fresh wt.** (mg)	**Length** (cm)	**Fresh wt.** (mg)
Control	4.0±0.3 (100)	44.3±9.1(100)	2.5±0.2(100)	72.7±24.4(100)	6.5±0.1(100)	116.9±31.1(100)
Cd 0.1mM	2.6±0.7(64.6)	38.6±5.4(87.2)	2.1±0.4(83.7)	56.8±9.6(78.1)	4.7±0.6(72.5)	95.4±10.8(81.6)
Cd 1mM	0.8±0.2(20.2)	10.5±7.8(23.7)	0.9±0.6(39.3)	15.3±11.1(21.1)	1.8±0.3(27.6)	25.8±19.7(22.1)
Cd 3mM	0.6±12.5(13.6)	9.0±1.9(20.3)	0.3±0.1(12.2)	4.0±1.6(5.5)	0.8±0.2(13.2)	13.0±3.5(11.1)
Cd 5mM	0.4±0.0(9.9)	6±0.0(13.6)	0.3±0.0(12.2)	2.0±0.0(2.7)	0.7±0.0(10.9)	8.0±0.0(6.8)
Ni 0.1mM	3.9±0.7(98.5)	43.9±6.9(99.2)	2.3±0.2(93.5)	65.1±18.2(59.8)	6.3±0.8(98.1)	109±20.7(93.2)
Ni 1mM	2.7±1.3(66.1)	29.3±7.1(66.2)	1.7±0.2(67.9)	43.5±16.8(59.8)	4.3±0.9(67.4)	72.8±20.4(62.2)
Ni 3mM	1.6±0.5(39.98)	16.0±6.5(36.2)	1.2±0.6(49.8)	26.5±14.1(36.5)	2.8±0.8(43.5)	42.5±21.6(36.3)
Ni 5mM	0.9±0.3(23.1)	13.7±2.9(30.9)	0.5±0.1(21.7)	15±3.1(20.6)	1.5±0.3(22.8)	28.7±3.6(24.5)
Pb 0.1mM	3.7±0.6(91.8)	42.6±7.5(96.3)	2.4±0.4(96.7)	62.0±9.5(85.3)	6.1±0.9(94.6)	104.6±9.5(89.4)
Pb 1mM	3.5±0.3(86.4)	39.4±7.5(89)	2.1±0.5(86.6)	55.4±21.5(76.2)	5.6±0.1(87.3)	94.8±22.5(81.1)
Pb 3mM	2.2±0.7(54.5)	34.3±8.5(77.6)	1.9±0.6(77.2)	42.0±9.6(57.8)	4.1±0.4(63.7)	76.3±10.6(65.3)
Pb 5mM	1.4±0.7(33.7)	29.5±11.3(66.7)	1.5±0.3(58.9)	38.4±12.2(52.8)	2.8±0.2(43.6)	67.9±24.5(58.1)

Mean of ten replicates ± Standard deviation (S.D); Values in parenthesis denote length and fresh weight of plant part as % of total.

Table-2. Effect of Cd^{2+}, $Ni^{2+}$and Pb^{2+} on dry weight of pea seedlings.

Treatment	**Plumule Dry wt.**(mg)	**Radical Dry wt.** (mg)	**Coty. Dry wt.** (mg)	**Total Dry wt.** (mg)
Control	6.1±1.6 (4.4)	6.8±1.8 (4.8)	126.5±8.2 (90.7)	139±6.9 (100)
Cd 0.1mM	4.4±1.3 (3.1)	4.2±0.5 (2.9)	132.9±11.6 (93.9)	141.5±10.3 (100)
Cd 1mM	1.0±0.2 (0.7)	2.1±0.6 (1.4)	140.8±5.9 (97.9)	143.8±8.4 (100)
Cd 3mM	N.O.	N.O.	N.O.	N.O.
Cd 5mM	N.O.	N.O.	N.O.	N.O.
Ni 0.1mM	6.1±2.3 (4.1)	6.5±0.6 (4.37)	126.6±3.6 (85.83)	147.5±5.6 (100)
Ni 1mM	1.5±0.6 (1)	3.2±0.6 (2.2)	141.6±11.6 (96.8)	146.3±15.6 (100)
Ni 3mM	1.3±0.3 (0.9)	2.6±0.6 (1.7)	148.8±12.3 (97.4)	152.8±11.3 (100)
Ni 5mM	N.O.	N.O.	N.O.	N.O.
Pb 0.1mM	5.4±0.8 (4.1)	5.9±1.6 (4.5)	120.8±6.9 (91.4)	132.1±8.6 (100)
Pb 1mM	5.0±1.6 (3.6)	5.6±2.6 (3.9)	129.8±11.6 (92.4)	140.4±15.6 (100)
Pb 3mM	4.8±0.7 (3.4)	4.4±1.3 (3.2)	130.5±4.6 (93.4)	139.7±5.9 (100)
Pb 5mM	1.1±0.4 (0.8)	1.3±0.5 (0.9)	137.9±9.6 (98.36)	140.2±7.9 (100)

Mean of ten replicates ± S.D; N.O. - No Observation; Values in parenthesis denotes dry weight of plant part as % of total.

Table-3. Effect of Cd^{2+}, $Ni^{2+}$and Pb^{2+} on chlorophyll and carotenoid content in pea plant.

Treatment	Chlorophyll a (µg/ fresh wt.)	Chlorophyll b (µg/ fresh wt.)	Total Chlorophyll (µg/ fresh wt.)	Carotenoids (µg/ fresh wt.)
Control	626±21.8	478±22.6	1041±79.8	279±30.3
Cd 0.1mM	342±18.7	312±21.5	701±77.9	113±12.8
Cd 1mM	89±5.6	167±11.6	269±11.6	92±4.6
Cd 3mM	N.O.	N.O.	N.O.	N.O.
Cd 5mM	N.O.	N.O.	N.O.	N.O.
Ni 0.1mM	326±16.9	393±11.6	809±50.4	186±27.9
Ni 1mM	198±13.6	256±16.9	446±39.8	157±15.4
Ni 3mM	33±5.6	132±12.6	166±37.9	68±69.6
Ni 5mM	N.O.	N.O.	N.O.	N.O.
Pb 0.1mM	457±19.9	370±41.3	933±7.5	124±12.3
Pb 1mM	404±21.3	275±25.3	704±22.8	159±23.6
Pb 3mM	216±14.6	127±18.3	394±32.1	98±21.6
Pb 5mM	154±20.3	134±9.7	339±39.6	71±12.3

Mean of four replicates ± S.D; N.O.- No observation.

Table-4. Total organic nitrogen content (%) of various plant parts of pea seedling treated with heavy metals (Cd^{2+}, Ni^{2+}, Pb^{2+}).

Treatment	Organic nitrogen percentage		
	Plumule	Radical	Total
Control	0.064	0.057	0.51
Cd 0.1mM	0.061	0.052	0.399
Cd 1mM	0.011	0.023	0.468
Cd 3mM	N.O.	N.O.	N.O.
Cd 5mM	N.O.	N.O.	N.O.
Ni 0.1mM	0.053	0.035	0.224
Ni 1mM	0.021	0.043	0.246
Ni 3mM	0.01	0.018	0.301
Ni 5mM	N.O.	N.O.	N.O.
Pb 0.1mM	0.067	0.057	0.42
Pb 1mM	0.065	0.055	0.474
Pb 3mM	0.062	0.058	0.501
Pb 5mM	0.046	0.037	0.492

Mean of six replicates; N.O.- No observation.

Table-5. Effect of cadmium, nickel, lead on total organic nitrogen(mg/plant part dry wt.) in pea seedling.

Treatment	Plumule	Radical	Cotyledons	Total
Control	0.026±0.010	0.026±0.007	3.263±0.300	3.316±0.294
Cd 0.1mM	0.015±0.002	0.011±0.002	2.636±0.487	2.662±0.470
Cd 1mM	0.002±0.001	0.002±0.0	3.3±0.289	3.311±0.241
Cd 3mM	N.O.	N.O.	N.O.	N.O.
Cd 5mM	N.O.	N.O.	N.O.	N.O.
Ni 0.1mM	0.017±0.004	0.012±0.001	1.424±0.045	1.453±0.054
Ni 1mM	0.001±0	0.003±0	1.827±0.148	1.831±0.189
Ni 3mM	0.001±0.001	0.006±0	2.186±0.214	2.193±0.214
Ni 5mM	N.O.	N.O.	N.O.	N.O.
Pb 0.1mM	0.018±0.005	0.017±0	2.581±0.254	2.616±0.268
Pb 1mM	0.017±0.003	0.014±0.005	3.084±0.554	3.115±0.552
Pb 3mM	0.019±0.010	0.008±0.004	3.246±0.134	3.495±0.345
Pb 5mM	0.001±0	0.001±0	3.255±0.217	3.256±0.217

Mean of six replicates ± S.D.; N.O. - No observation.

Table-6. Effect of cadmium, nickel and lead on protein(mg/plant part fresh wt.) in pea seedling.

Treatment	Plumule	Radical	Cotyledons	Total
Control	0.46±0.06	0.26±0.05	3.40±0.75	4.12±0.77
Cd 0.1mM	0.18±0.09	0.14±0.06	2.14±0.59	2.47±0.59
Cd 1mM	0.16±0.04	0.27±0.10	2.64±0.57	2.85±0.39
Cd 3mM	N.O.	N.O.	N.O.	N.O.
Cd 5mM	N.O.	N.O.	N.O.	N.O.
Ni 0.1mM	0.17±0.03	0.14±0.03	2.06±0.35	2.37±0.36
Ni 1mM	0.29±0.08	0.25±0.09	2.47±1.65	3.01±1.59
Ni 3mM	0.22±0.06	0.16±0.07	2.97±1.74	3.24±1.68
Ni 5mM	N.O.	N.O.	N.O.	N.O.
Pb 0.1mM	0.51±0.13	0.32±0.09	3.29±0.83	4.16±0.73
Pb 1mM	0.76±0.17	0.59±0.19	3.81±1.96	5.16±2.19
Pb 3mM	0.63±0.16	0.43±0.06	3.82±1.22	4.88±1.35
Pb 5mM	0.42±0.07	0.21±0.04	3.95±1.18	4.45±1.22

Mean of ten replicates ± S.D.; N.O.- No observation.

Table-7. Effect of cadmium, nickel and lead on protein (mg/g fresh wt.) in pea seedling.

Treatment	**Plumule**	**Radical**	**Cotyledons**
Control	11.08±3.30	8.72±2.44	7.34±1.77
Cd 0.1mM	6.74±4.51	6.59±2.98	4.47±1.31
Cd 1mM	20.94±6.04	27.97±8.97	6.19±1.04
Cd 3mM	N.O.	N.O.	N.O.
Cd 5mM	N.O.	N.O.	N.O.
Ni 0.1mM	4.31±1.22	3.97±1.21	4.69±1.04
Ni 1mM	11.2±9.45	7.28±2.09	5.28±4.17
Ni 3mM	6.95±5.11	16.22±12.11	17.77±6.67
Ni 5mM	N.O.	N.O.	N.O.
Pb 0.1mM	14.35±4.44	12.5±5.14	7.14±1.68
Pb 1mM	16.14±5.71	16.65±3.24	7.25±3.77
Pb 3mM	16.13±2.55	17.08±12.47	7.67±3.56
Pb 5mM	18.07±11.12	18.53±7.62	8.29±2.54

Mean of ten replicates ± S.D.; N.O.- No observation.

Table-8. Heavy metal accumulation in plant part of pea seedling after 7 days of germination

	Heavy Metal content (μg/g dry wt.)		
Treatment	**Radical**	**Plumule**	**Cotyledons**
Cd 1mM	194±22	88±15	38±8
Ni 1mM	168±30	96±12	45±9
Pb 1mM	103±19	71±13	33±8

Mean of four replicates ± S.D