ISSN (Print): 0974-6846
Indian Journal of Science and Technology, Vol 11(35), DOI: 10.17485/ijst/2018/v11i35/131555, September 2018 ISSN (Online): 0974-5645

Response of the Germination of Bean (*Vigna unguiculata* L. (Walp) to the Toxic Effects of Heavy Metals

Katiuska Acosta-Marcano[1], Cateryna Aiello-Mazzarri[2], Katheryn Atencio-Acosta[3], Geomar Molina-Bolivar[4] and Iris Jimenez-Pitre[4]

[1]Facultad de Agronomia, Laboratorio de Microbiologia Agricola, Universidad del Zulia; kacosta@fa.luz.edu.ve
[2]Facultad de Ingenieria, Universidad del Zulia-Venezuela; caiello@fing.luz.edu.co
[3]Facultad de Ingenieria, Universidad "Rafael Urdaneta"; katheryatecio@hotmail.com
[4]Group of Rechearch BIEMARC, Universidad de La Guajira; gmolina@uniguajira.edu.co, iajimenez@uniguajira.edu.co

Abstract

Objective: To evaluate the toxic effects of metals on the crop Beans (*Vigna unguiculata* L. (Walp). **Materials and Methods:** assays of seed germination, elongation of the root, growth of hypocotyl and radicle in these plants as biomonitoring parameters varying concentrations of metals. The metals selected for this study were Cd and Pb. **Findings**: The seeds of V. unguiculata were sensitive to the presence of metals in comparison to the behavior of plants witnesses, showing all the biomarkers evaluated sensitivity to Cd and Pb however did not complete blockade of the germination and initial growth of seedlings, being the effect more dramatic by the presence of cadmium than with lead, observing a maximum values in the germination rate of 63 and 80%, respectively, in comparison with the control treatment, which were correlated with strongly to changes in morphology and anatomy of the seed. **Application/Improvements**: The Bean was able to survive and tolerate these polluting metals, so it could be used for the ecotoxicological monitoring of contaminated soils for future studies of phytoremediation.

Keywords: Heavy Metals, Seed Germination, Toxic Effects, *Vigna unguiculata*

1. Introduction

The contamination of agricultural soils by heavy metals has grown abundantly in recent decades, adversely affecting the soil-plant system, as these tend to accumulate in the soil from waste, irrigation of wastewater sewage treatment, extensive use of chemicals, fertilizers and improvements in agricultural practices, representing a greater risk to human health when the plants develop tolerance mechanisms against metals and when these plants are incorporated into the food chain, representing a risk to consumers at primary, secondary, and human[1]. Seed germination and subsequent growth of the embryo are important stages of plant life and highly sensitive to fluctuations of the surrounding environment, in these stages there are numerous physiological processes, exchange of materials between the development cycle of the plant and the environment, so the presence of toxic substances can interfere, altering the survival and normal development of the plant[2].

The covers present in seeds constitute barriers to metals and allows you to prevent contamination of the

*Author for correspondence

embryo until the root embryonic. The toxicity of heavy metals affects the germination process by inhibiting the absorption of water[3, 4], competition for the absorption of nutrients[5, 6], alteration of metabolism, the photosynthetic system, breathing[7]; permeability of membranes and the activity of enzymes linked to the membrane[8], seed viability, alteration of the process of cell division of the root apices, delaying the process of mitosis and altering the process of root elongation, arriving to cause the death of the embryo[9], in addition to fault in the morphology of the same.

However, the effects of the metals in the germination of seeds depend on the differences between species in the structure and anatomy of the seed[3], type, concentration and distribution of metal[9], stage of development and maturity of the organs of the plant and may induce only a delay in the process of germination, without lead directly to the loss of seed viability[10, 11]. Different metals have been associated with alterations in plant germination, previous studies have shown that mercury (Hg), zinc (Zn), cadmium (Cd), cobalt (Co), plumbum (Pb) and nor reduce germination in lentils, radishes, mustard and rice seeds. It has been observed that the Pb has a genotoxic effect, which leads to reduce the potential for germination through inhibition of the mitotic index and cytogenetic errors in the seedlings, leading to chromosomal abnormalities in the mitotic stage in the seeds of *Vigna mungo* (4). Cadmium (Cd) and plumbum (Pb) induced reductions. Arsenic toxicity induced by significantly reducing the percentage of germination in a 70% of rice seed and a 9% in the root length[12].

Although there is research on the effect of metal toxicity in plants, are there is very little information on how heavy metals affect the physiology of the seed, so have reported different indexes to biomonitorear during the life cycle of cultivation, the effects of phytotoxicity of the tensions caused by the stress of heavy metals, which have a great influence in the early stages on seeds and its adverse effects on the process of germination, elongation of the radicle and hypocotyl, index of tolerance, root length of seedlings during germination and in the development of the seedlings during the first days of growth[1], since the response of plants to these adverse conditions during germination and early growth stages are considered to be fundamental to the successful establishment of agricultural crops under conditions of stress[13].

2. Materials and Methods

The experiment was conducted in the Phytosanitary Technical Unit, Faculty of Agronomy, University of Zulia, Municipality of Maracaibo, Zulia State. The climate is semi-arid. The village is classified as an area of life for very dry tropical forest (BMS-T), with a regime of soil moisture aridico with average annual rainfall of 600mm, average annual temperature of 28.1°C, average annual evapotranspiration of 1800mm and average relative humidity of 88%. Seeds of the cultivar black eye of *V. unguiculata* (L) provided by the seed bank of the association of producers of the state of Portuguesa (PAI) and certified with more than 90% of germination. The damaged seeds were discarded and used the same size. For the disinfection of the seeds will be conducted a pre-treatment with a solution of ethanol 70%, subsequently were rinsed with distilled water and dried in an oven at 30°C for 20 min. It was determined the concentration of metal in which was observed the alteration or changes in the physiological process of the plant, discarding those lethal concentrations that entailed upon the death of the crop. To that end, solutions were prepared mothers of the two metals to be used lead chloride ($PbCl_2$) and Cadmium Chloride ($CdCl_2$) to 100 mg of the metal L-1 under the following concentrations (Table 1).

The bean seeds are disinfect previously with 10 mL of sodium hypochlorite at 10% for 5 minutes, rinsed five times with sterile distilled water and were placed in Petri dishes with a disc of the filter paper to which was added 3 ml of dilution of heavy metal, keeping plates without the addition of the heavy metal how to control. The bioassays were carried out with five replicates (10 seeds/plates) for each experiment incubating the plates at 37°C for 5 days. It was determined the concentration of metal in which was observed the alteration or changes in the physiological process of the plant, discarding those lethal concentrations that led to the death of the seed. Once selected, we proceeded to the stage of greenhouse gas

Table 1. Concentrations of $CdCl_2$ and $PbCl_2$ used in the tests of tolerance of the bean seeds (*V. unguiculata* L (walp)

formulate	Concentration of metals					
$PbCl_2$	mg L^{-1}	40	80	160	200	250
$CdCl_2$		40	80	160	200	250

emissions, where pots of 1 kg capacity were filled with topsoil, previously sieved and sterilized at one atmosphere of pressure at 121° C for one hour in autoclave conditions. To determine the percentage of germination, we recorded the number of seeds that germinated, considering as a criterion for germination the appearance visible in the radicle. (≥1 mm) The germination percentage was determined by means of the following equation:

$$\%\ G:\ \ PG/\ PT$$

Where:

% G: percentage of germination
PG: number of germinated plants
PT: total number of plants evaluated

Elongation of the radicle and hypocotyl was determined using a graph paper, medium with vernier the length of the radicle (from the knot (thickened region of transition between the radicle and hypocotyl) up to the radicular apex) and of the hypocotyl (from the hub until the insertion site of the two cotyledons) of each of the seedlings for each concentration of metal and control. For the determination of the tolerance from the measurement taken of the radical lengths and the hypocotyl, both of the plants treatments such as the plants controls, was made the determination of tolerance through the following expression[11]:

$$IT = Lrm\ /Lrc$$

Where:

LRm: radical length of the seedlings that grew in the presence of the metal
LRc: radical length of the seedlings grown in the absence of the metal.

3. Results and Discussion

The early outbreaks in the seeds is appreciated to the 24 hours, the germination frequency was independent of dose. However after 48 hours the viability of the same deteriorated in the highest concentrations of metals, being observed a direct relationship between the frequency of mortality and the dose of treatment, appreciating degraded, items and seeds with softening of the tissues. In the case of lead the seeds become inhibited, increasing the size to then form mucilage of strong odor and abort the germination, showing greater sensitivity to cadmium where it was observed that the seeds departed, dismembered, burned and with necrotic radicular apex.

The data obtained showed significant reductions in the percentage of germination, elongation of the radicle and hypocotyl, length of roots and index of tolerance in comparison with the control treatment. This effect of toxicity was strongly correlated to changes in morphology, anatomy of the seed, which may infer an effect on the decrease of the digestion and mobilization of food reserves as proteins and carbohydrates in seeds for the realization of the germination process. The analysis of variance showed significant differences ($p<0.05$) for the percentage of germination of bean seeds in relation to the different concentrations of lead and cadmium evaluated. Appreciating in Figure 1 for the case of lead that the highest values were achieved by the seedlings with the lowest concentration with 80.8% of germination, no differences between the average values obtained at concentrations of 80 and 160 mg L-1 with values of 66 and 64%, respectively. Similarly the germinative capacity of the bean seeds was most drastically diminished by the presence of cadmium, observing a maximum value in the germination rate of 57% with the concentration of 80 mg L-1, corresponding to a decrease of 59% of germination when compared with the witnesses. The lowest values were reached with the levels of this metal to 40 and 160 mg L-1 with 55 and 45%, respectively (Figure 2).

There is a decrease in germination with both metals at higher concentrations, although the germination

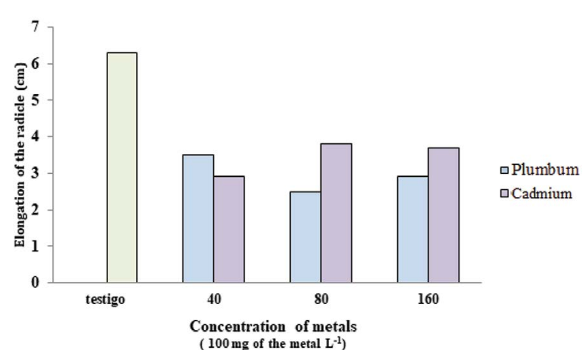

Figure 1. The radicle elongation in seedling of beans (*V. unguiculata* L (Walp) exposed to different doses of plumbum and cadmium.

was never completely inhibited. There is a decrease in germination with both metals at higher concentrations, although the germination was never completely inhibited[14] and the alterations of the selective properties of permeability of the membrane, as has been pointed out, that for a seed germinate, the water potential of the embryo needs to pass a critical threshold which is affected under these stress conditions[15]. In relationship has indicated that cadmium is a strong inhibitor of the germination process[16, 17], which inhibits the imbibition of seeds, Percentage, Index and speed of germination[18], by limiting the amount of water of the seeds exposed to this metal, reducing the water content of the future seedlings[19], appreciating a direct correlation as it increases the concentration of metal, coming to inhibit the germination of 77%[20]. Similarly, there has been decline in the growth of the seedlings and total reduction in germination[16, 21], given that this element to be absorbed and distributed quickly through the vascular system causing in general form a depression of growth and even death of plants[22].

It should be noted that there is a slight stimulation of germination to the concentration of 80 mgL-1 of cadmium, in relation to this has been pointed out that cadmium is a non-essential element that may not be toxic at low concentrations[23], appreciating stimulation of germination and the thermodormancia[9, 24], which has been attributed to an overproduction of reactive oxygen species (ROS) and reactive nitrogen (RNS) such as nitric oxide (NO) in plants stressed by metals[9, 16]; resulting in a slightly higher level of oxidative stress that stimulates germination[19]. The bean seeds tolerate concentration of cadmium of 200mg/l in the field, and to concentrations of 400mg/l as much soil as the seeds may show decreased biological diversity as it does not tolerate such heavy concentrations of this metal contaminants, as has been observed this effect in other leguminous plants such as chickpea (*Cicerarietinum*) and mung beans (*Vignaradiata*)[25-28]. The bean is highly sensitive to lead which alters the physiological processes of soaking, germination and subsequent growth of the plant, however at low doses this element does not affect the viability of seeds which could infer the presence of the same in edible tissues of the crop[29].

Significant differences ($p<0.05$) for the growth of the radicle of bean seedlings in relation to the different concentrations of lead and cadmium. Appreciating in Figure 3 that the highest values were achieved by the seedlings with the lowest concentration of lead with 3.5 cm, not differences between the average values achieved by the seedlings treated with values of 80 and 160 mg L-1 with values of 2.5 and 2.9 cm respectively. An inverse behavior was observed in the behavior of this variable in relation to cadmium, where the highest values were achieved by the seedlings treated with higher levels of this metal with values of radicle growth of 3.8 and 3.7 cm, while the seedlings subjected to concentrations of 40 mgL-1 showed an elongation of 2, 9 cm. In both cases, the development of the radicle was below the value displayed by plants witnesses whose value was 6.3 cm, with a significant difference 55% for the lead and 60% for cadmium (Figure 1).

According to the cover of the seeds of the majorities of the species show permeability to Pb, making the seeds to become more permeable to Pb during the latter stages of the imbibition, delaying germination[30]. The tissues that cover the embryo in the seed play a role in the selective permeation of different metals, this was suggested by the fact that the seeds still germinate in the presence of high concentration of metal[31].

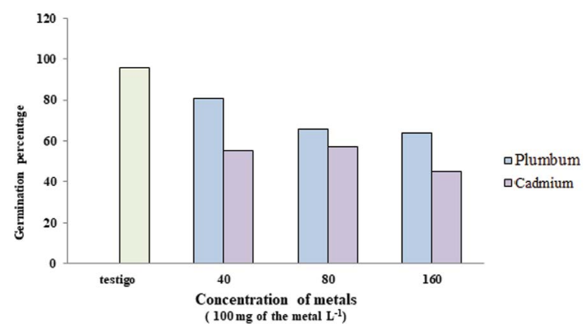

Figure 2. Percentage of germination of bean (*V. unguiculata* L (Walp) exposed to different doses of plumbum and cadmium.

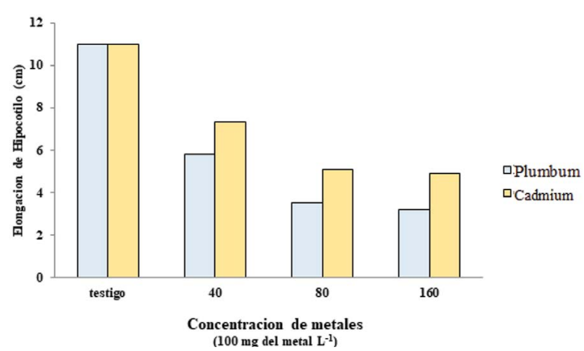

Figure 3. Elongation of the hypocotyl in seedling of beans (*V. unguiculata* L (Walp) exposed to different doses of plumbum and cadmium.

In Figure 3, describes the results for the variable elongation of the hypocotyl of bean seedlings, the analysis of variance showed significant differences ($p<0.05$) for the different concentrations of lead and cadmium evaluated. The highest values were achieved by the seedlings treated with the lowest concentration of lead with values of 5.8 cm, observing the same trend in behavior for the previous variable, since there were no significant differences between the average values achieved by the seedlings treated with values of 80 and 160 mg L^{-1} $PbCl_2$ with values of 3.5 and 3.2 cm respectively, indicating a difference of 52% when compared with the seedlings witnesses which showed a value of 11 cm for the growth of the hypocotyl. In the case of the treatments undergoing $CdCl_2$, showed a direct correlation, appreciating the growth of the hypocotyl decrease with the increase of the concentration of cadmium, the values ranged from 7.3 to 4.9 cm, which were in a 66% below the value achieved by the control plants whose value was 11 cm.

In Figures 4-5 shows some indicators of phytotoxicity or abnormal growth in bean seedlings exposed to $PbCl_2$ and $CdCl_2$, seeds with germination process, where the abnormal symptoms several from necrotic tissue in the seeds, cotyledon emergence or hypocotyl only, but without the emergence of the radicle, which could be inferred in the low capacity of development of the future plant. It is noted that in spite of the cytotoxic effect, the concentrations of metals evaluated were able to slow down or completely inhibit the processes of the radicle elongation of the hypocotyl or but they were not enough to inhibit the germination and the death of the seedling.

In Figure 6 shows, the results for the length of roots developed by bean seedlings exposed to concentrations of lead and cadmium. The analysis of variance showed no significant differences for this variable in the seedlings of the treatments with metals, whose values ranged from 6.1 to 4.1 cm; however, when compared with the control plants, which showed growth of 9.7 cm is a decline of 53% for the case of $PbCl_2$ and 62% for $CdCl_2$. The behavior remained the same trend seen in the other variables evaluated, with a detriment as it increases the concentration of the metal, but a slight stimulus when the seedlings are exposed to levels of 80 and then rests.

For the plant, the length of the root is an important factor for the transport of water and nutrients, because the reduction of this variable is associated to inhibition directly or indirectly from the physiological processes reduce the uptake of water and nutrients, reduction of water transpiration and alteration in the water balance, inhibition of the enzyme activities by inhibiting the activity of the cell and its enlarged, resulting in poor growth and low production of radical biomass[26, 32, 33].

(4) (5)

Figure 4 and 5. Necrotic Areas or dead tissue in the bean seeds (*V. unguiculata* L (Walp) exposed to different doses of lead and cadmium, evidenced as localized spots brown to blackish with sinking and perforation of the tissue.

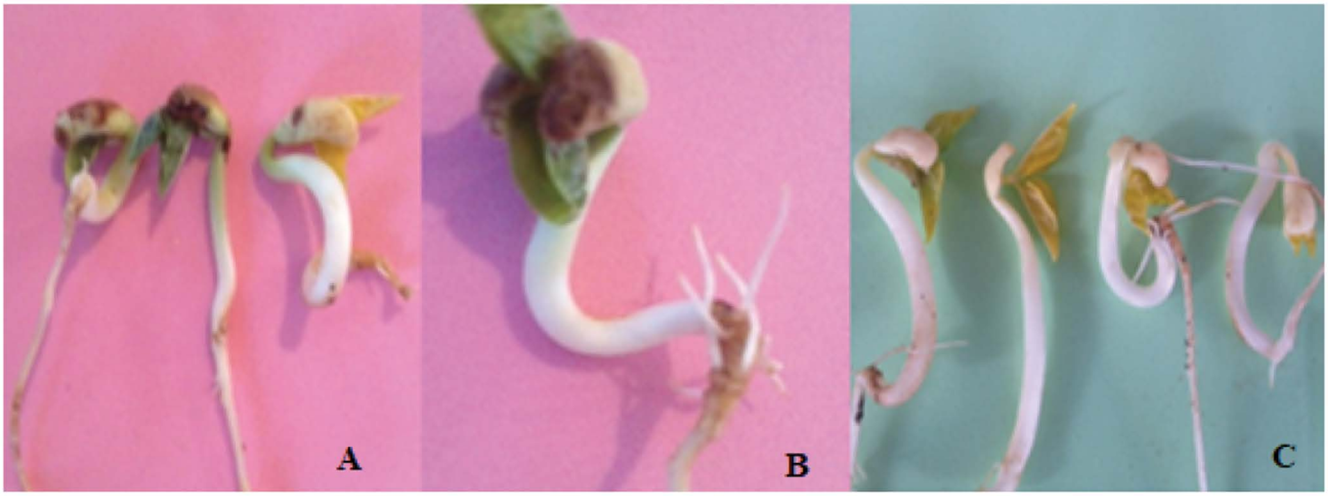

Figure 6. The growth of the root and hypocotyl were severely affected. To: necrotic tissue burned and in the cotyledons of beans (*V. unguiculata* L (walp), B: apices root canals with necrosis, C: absence or little development of hairs, Ridicules with curly-encurvadas growth, yellowing chlorotic cotyledons and wrinkling of the leaves primaries.

In the same sense it has been pointed out that the inhibitory effect of stress metal in the elongation of the root may be due to the high accumulation of the metal in the outbreak of the roots, motivated to competition for absorption of nutrients cations in the cells of the root, direct interaction with functional proteins that lead to a disturbance in its structure and function, inhibition of the activity of the enzyme amylase that lead to delay in transportation of sugars, interference in cell division that leads to chromosomal aberrations and abnormal mitosis[9, 20, 34-38].

The inhibition of the elongation of the root has been shown to be one of the first and different symptoms of toxicity of Cd, which could be attributed to the microtubule depolymerization of the cytoskeleton and cell formation of chromosome aberrations that result in mitotic activities rates of meristematic cells[39, 40]. In the same way it has been suggested that the primary site of toxicity of Pb is the root, causing serious reductions in root growth, loss of apical dominance (shown by an increase in the branching), the formation of hyper located behind the tips of the roots (due at the beginning of the lateral roots) and the bending of some tips of the roots, to accumulate mainly within the cell walls and intercellular spaces[41]. Significant differences were found for the index values of tolerance obtained for the seedlings of beans split both metals at various concentrations of Cd and Pb, ranging between 52 to 62% for the lead and 42 to 53% for cadmium. Appreciating a direct relationship between the dose of metal and the decline in tolerance (Figure 7).

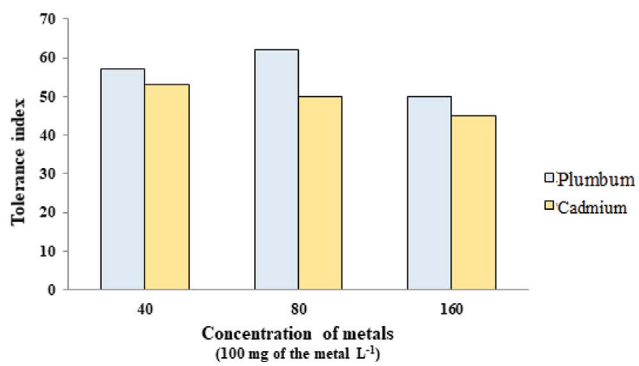

Figure 7. Index of tolerance of bean seedlings (*V. unguiculata* L (Walp) exposed to different doses of plumbum and cadmium.

4. Conclusion

The seeds of *V. unguiculata* showed sensitivity to the presence of metals Cd and Pb, in comparison to the behavior of plants witnesses, showing decreased in all the parameters evaluated, directly correlated with increasing concentrations of Cd and Pb in the ground, without which they will lead to the total inhibition of the germination process. However the bean is able to survive and tolerate these pollutants for future studies of phytoremediation, so it could be used for the ecotoxicological monitoring of contaminated soils, we were unable to identify that the bean presents a tolerance to cadmium concentrations lower than with lead, enduring concentrations up to 160 mg/l but with notable declines, showing selectivity for the ability to tolerate and absorb this metal. These biological responses are indicators of toxicity to plants exposed to metals and a useful tool for environmental monitoring. The bean is a plant of consumption and vegetable protein source, future research is needed to assess the response considering the full life-cycle of plants, to verify the bioconcentration of metals in their tissues and in grains, which could be an intermediate stage to the incorporation of the same to the food chain which would represent a risk to be consumed.

5. References

1. Semida WM, Hemida KA, Rady MM. Sequenced ascorbate-proline-glutathione seed treatment elevates cadmium tolerance in cucumber transplants. Ecotoxicology and Environmental Safety. 2018; 154:171-9. https://doi.org/10.1016/j.ecoenv.2018.02.036 PMid:29471279
2. Mozafariyan M, Pessarakli M, Saghafi K. Effects of selenium on some morphological and physiological traits of tomato plants grown under hydroponic condition. Journal of Plant Nutrition. 2017; 40(2):139-44. https://doi.org/10.1080/01904167.2016.1201500
3. Munzuroglu O, Geckil H. Effects of Metals on Seed Germination, Root Elongation, and Coleoptile and Hypocotyl Growth in Triticum aestivum and Cucumis sativus. Archives of Environmental Contamination and Toxicology. 2002; 43(2):203-13. https://doi.org/10.1007/s00244-002-1116-4 PMid:12115046
4. Siddiqui MM, Abbasi BH, Ahmad N, Ali M, Mahmood T. Toxic effects of heavy metals (Cd, Cr and Pb) on seed germination and growth and DPPH-scavenging activity in Brassica rapa var. turnip. Toxicology and Industrial Health. 2014; 30(3):238-49. https://doi.org/10.1177/0748233712452605 PMid:22872632
5. DalCorso G, Manara A, Furini A. An overview of heavy metal challenge in plants: from roots to shoots. Metallomics. 2013; 5(9):1117-32. https://doi.org/10.1039/c3mt00038a PMid:23739766
6. Kaur G. pb-induced toxicity in plants: effect on growth, development, and biochemical attributes. Journal of Global Biosciences. 2014; 3(6):1-9.
7. Lu Q, Zhang T, Zhang W, Su C, Yang Y, Hu D. Alleviation of cadmium toxicity in Lemna minor by exogenous salicylic acid. Ecotoxicology and Environmental Safety. 2018; 147:500-8. https://doi.org/10.1016/j.ecoenv.2017.09.015 PMid:28915397
8. Sandbichler A, Hockner M. Cadmium Protection Strategies - A Hidden Trade-Off? International Journal of Molecular Sciences. 2016; 17(1):139. https://doi.org/10.3390/ijms17010139 PMid:26805823 PMCid:PMC4730378
9. Kranner I, Colville L. Metals and seeds: Biochemical and molecular implications and their significance for seed germination. Environmental and Experimental Botany. 2011; 72(1):93-105. https://doi.org/10.1016/j.envexpbot.2010.05.005
10. Shamshad S, Shahid M, Rafiq M, Khalid S, Dumat C, Sabir M, et al. Effect of organic amendments on cadmium stress to pea: A multivariate comparison of germinating vs young seedlings and younger vs older leaves. Ecotoxicology and Environmental Safety. 2018; 151:91-7. https://doi.org/10.1016/j.ecoenv.2018.01.002 PMid:29329098
11. Escalante-Campos S, Rodriguez-Dorantes A, Vasquez-Murrieta MS, Rodriguez-Tovar AV, Guerrero-Zu-iga LA, Perez NO. Evaluacion del efecto de cadmio sobre la germinacion y elongacion radical de semillas bacterizadas de Axonopus affinis y Festuca rubra. Polibotanica. 2012; 34:17.
12. Debojyoti M, Dibakar G, Subhas Chandra S. Evaluation of effectiveness of seed priming with selenium in rice during germination under arsenic stress. Plant Physiology and Biochemistry. 2016; 109:571-8. https://doi.org/10.1016/j.plaphy.2016.11.004 PMid:27838598
13. He S, Yang X, He Z, Baligar VC. Morphological and Physiological Responses of Plants to Cadmium Toxicity: A Review. Pedosphere. 2017; 27(3):421-38. https://doi.org/10.1016/S1002-0160(17)60339-4
14. Houshmandfar A, Moraghebi F. Effect of mixed cadmium, copper, nickel and zinc on seed germination and seedling growth of safflower. African Journal of Agricultural Research. 2011; 6(5):1463-8.
15. Moosavi SA, Gharineh MH, Afshari RT, Ebrahimi A. Effects of some heavy metals on seed germination characteristics of canola (Brassica napus), wheat (Triticum aestivum) and safflower (Carthamus tinctorious) to evaluate phytoremediation potential of these crops. The Journal of Agricultural Science. 2012; 4(9):11.
16. Smiri M, Chaoui A, El Ferjani E. Respiratory metabolism in the embryonic axis of germinating pea seed exposed to cadmium. Journal of Plant Physiology. 2009; 166(3):259-69. https://doi.org/10.1016/j.jplph.2008.05.006 PMid:18760497
17. Rafiq M, Shahid M, Abbas G, Shamshad S, Khalid S, Niazi N, et al. Comparative effect of calcium and EDTA on arsenic uptake and physiological attributes of Pisum sativum. International Journal of Phytoremediation. 2017; 19(7):662-9. https://doi.org/10.1080/15226514.2016.1278426 PMid:28084804
18. Hassan S, Abdulhakeem O. Effect Of Copper And Cadmium On Germination And Anatomical Structure Of Leaf And

Root Seedling In Maize (Zea mays L). Australian Journal of Basic and Applied Sciences. 2013; 7(1):548-55.
19. Lefèvre I, Marchal G, Correal E, Zanuzzi A, Lutts S. Variation in response to heavy metals during vegetative growth in Dorycnium pentaphyllum Scop. Plant Growth Regulation. 2009; 59(1):1-11. https://doi.org/10.1007/s10725-009-9382-z
20. Alaraidh IA, Alsahli AA, Abdel Razik ES. Alteration of antioxidant gene expression in response to heavy metal stress in Trigonella foenum-graecum L. South African Journal of Botany. 2018; 115:90-3. https://doi.org/10.1016/j.sajb.2018.01.012
21. Escalante-Campos S, Rodriguez-Dorantes A, Vasquez-Murrieta M, Rodriguez-Tovar A, Guerrero-Zu-iga L, Perez N. Evaluación del efecto de cadmio sobre la germinacion y elongacion radical de semillas bacterizadas de Axonopus affinis y Festuca rubra. Polibotanica. 2012; (34):205-21.
22. Shahid M. Cadmium Bioavailability, Uptake, Toxicity and Detoxification in Soil-Plant System. Reviews of Environmental Contamination and Toxicology. 2016; 241:73-137. https://doi.org/10.1007/398_2016_8 PMid:27300014
23. Manquian-Cerda K, Escudey M, Zu-iga G, Arancibia-Miranda N, Molina M, Cruces E. Effect of cadmium on phenolic compounds, antioxidant enzyme activity and oxidative stress in blueberry (Vaccinium corymbosum L.) plantlets grown in vitro. Ecotoxicology and Environmental Safety. 2016; 133:316-26. https://doi.org/10.1016/j.ecoenv.2016.07.029 PMid:27485373
24. Deng B, Yang K, Zhang Y, Li Z. Can heavy metal pollution defend seed germination against heat stress? Effect of heavy metals (Cu2+, Cd2+ and Hg2+) on maize seed germination under high temperature. Environmental Pollution. 2016; 216:46-52. https://doi.org/10.1016/j.envpol.2016.05.050 PMid:27239687
25. Rahoui S, Adbelilah C, Ezzeddine E. Differential sensitivity to cadmium in germinating seeds of three cultivars of faba bean (Vicia faba L). Acta Physiologiae Plantarum. 2008; 30(4):451-6. https://doi.org/10.1007/s11738-008-0142-x
26. Liu S, Yang C, Xie W, Xia C, Fan P. The effects of cadmium on germination and seedling growth of Suaeda salsa. Procedia Environmental Sciences. 2012; 16:293-8. https://doi.org/10.1016/j.proenv.2012.10.041
27. Vijayaragavan M, Prabhahar C, Sureshkumar J, Natarajan A, Vijayarengan P, Sharavanan S. Toxic effect of cadmium on seed germination, growth and biochemical contents of cowpea (Vigna unguiculata l.) plants. International Multidisciplinary Research Journal. 2011; 1(5):1-6.
28. Wani P, Khan M, Zaidi A. Synergistic effects of the inoculation with nitrogen-fixing and phosphate-solubilizing rhizobacteria on the performance of field-grown chickpea. Journal of Plant Nutrition and Soil Science. 2007; 170(2):283-7. https://doi.org/10.1002/jpln.200620602
29. Isaza Guzman G. Efecto del plomo sobre la imbibicion, germinacion y crecimiento de Phaseolus vulgaris L. y Zea mays L. Biotecnologia Vegetal. 2013; 13(3):161-7.
30. Kuriakose S, Prasad M. Cadmium stress affects seed germination and seedling growth in Sorghum bicolor (L.) Moench by changing the activities of hydrolyzing enzymes. Plant Growth Regulation. 2008; 54(2):143-56. https://doi.org/10.1007/s10725-007-9237-4
31. Li W, Li Y, Gao F. Abelson, enabled, and p120catenin exert distinct effects on dendritic morphogenesis in Drosophila. Developmental Dynamics. 2005; 234(3):512-22. https://doi.org/10.1002/dvdy.20496 PMid:16003769
32. Kumari M, Kumar M, Srivastava A, Singh V. Cytogenetic Effects of Individual and Combined Treatment of Cd2+, Cu2+ and Zn2+ in Vigna radiata (L.) Wilczeck. Journal of Phytology. 2011; 3(8):38-42.
33. Souguir D, Ferjani E, Ledoigt G, Goupil P. Sequential effects of cadmium on genotoxicity and lipoperoxidation in Vicia faba roots. Ecotoxicology (London, England). 2011; 20(2):329-36. https://doi.org/10.1007/s10646-010-0582-0 PMid:21153701
34. Schutzendubel A, Polle A. Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. Journal of Experimental Botany. 2002; 53(372):1351-65. https://doi.org/10.1093/jexbot/53.372.1351 https://doi.org/10.1093/jxb/53.372.1351 PMid:11997381
35. Farooq M, Ali S, Hameed A, Bharwana S, Rizwan M, Ishaque. Cadmium stress in cotton seedlings: physiological, photosynthesis and oxidative damages alleviated by glycinebetaine. South African Journal of Botany. 2016; 104:61-8. https://doi.org/10.1016/j.sajb.2015.11.006
36. Mohamed H. Molecular and biochemical studies on the effect of gamma rays on lead toxicity in cowpea (Vigna sinensis) plants. Biological Trace Element Research. 2011; 144(1-3):1205-18. https://doi.org/10.1007/s12011-011-9058-1 PMid:21509597
37. Pavlova D. Nickel effect on root-meristem cell division in Plantago lanceolata (Plantaginaceae) seedlings. Australian Journal of Botany. 2017; 65(5):446-52. https://doi.org/10.1071/BT17054
38. Rizwan M, Ali S, Qayyum M, Ok Y, Adrees M, Ibrahim M. Effect of metal and metal oxide nanoparticles on growth and physiology of globally important food crops: A critical review.

Journal of Hazardous Materials. 2017; 322:2-16. https://doi.org/10.1016/j.jhazmat.2016.05.061 PMid:27267650

39. Lux A, Martinka M, Vaculik M, White P. Root responses to cadmium in the rhizosphere: a review. Journal of Experimental Botany. 2010; 62(1):21-37. https://doi.org/10.1093/jxb/erq281 PMid:20855455
40. Shanying H, Xiaoe Y, Zhenli H, Virupax C. Morphological and Physiological Responses of Plants to Cadmium Toxicity: A Review. Pedosphere an International Journal. 2017; 27(3):421-38. https://doi.org/10.1016/S1002-0160(17)60339-4
41. Kopittke P, Asher C, Kopittke R, Menzies N. Toxic effects of Pb2+ on growth of cowpea (Vigna unguiculata). Environmental Pollution. 2007; 150(2):280-7. https://doi.org/10.1016/j.envpol.2007.01.011 PMid:17379363