Effect of some heavy metals on germination and early seedling growth of *Atriplex halimus subsp. Schweinfurthii*

MESNOUA Mohammed* [1,2] and LOTMANI Brahim[2]

[1.] Scientific and Technical Research Centre for Arid Areas (CRSTRA), Biskra, Algeria.
[2.] Laboratory of Plant Protection, University of Abdelhamid Ben Badis of Mostaganem, Algeria.
*Corresponding author: email: mohamesnoua@gmail.com

Abstract: Seed is a developmental stage that is highly protective against external stresses in the plant life cycle. The aim of this study is to evaluate the effect of Cadmium (Cd), Copper (Cu) and Zinc (Zn) on germination and early seedling growth of *Atriplex halimus subsp. Schweinfurthii*. Different concentrations (50-4000 µM) of each heavy metal were analyzed. Results show that seed germination was not influenced by Zn or Cu. Whereas Cd reduced significantly seed germination at 1500 µM. Seedling growth was more sensitive to heavy metals in comparison to seed germination and was reduced significantly with increasing metal concentration. Among heavy metals, Cd showed more toxicity to seed germination and seedling growth followed by Cu than Zn. Root was the most sensitive parameter and may be used as indicators of heavy metal pollution.

[MESNOUA Mohammed and LOTMANI Brahim. **Effect of some heavy metals on germination and early seedling growth of *Atriplex halimus subsp. Schweinfurthii*.** *Life Sci J* 2017;14(7):62-67]. ISSN: 1097-8135 (Print) / ISSN: 2372-613X (Online). http://www.lifesciencesite.com. 11. doi:10.7537/marslsj140717.11.

Keywords: Cadmium, Copper, Zinc, *Atriplex halimus subsp. Schweinfurthii*, Germination.

Introduction

Heavy metal contamination is one of the most serious environmental problems that limits plant productivity and threatens human health (1). The major sources of heavy metal pollution in the world are industrial activities such as mining, the combustion of fossil fuels, fertilizers and pesticides (2). Some heavy metals, such as Fe, Mn, Zn, Cu, Mg, Mo, and Ni, are essential for micronutrients for plants growth, but are toxic to organisms at high concentrations (3,4).

The sensitivity of plants to heavy metals depends on an interrelated network of physiological and molecular mechanisms (3,5,6). The evaluation of the toxicity and stress caused by heavy metals on plants is very important part of the phytoremediation research. Several physiological parameters can be used to assess the heavy metal induced stress such as germination, plant growth and biomass production, photosynthetic pigments, antioxidant enzymes or antioxidants (7). Seed germination is the first step of the life of plant and it is one of the most sensitive physiological process in plants, affected by hormonal interactions and environmental factors, both biotic and abiotic (8). Therefore, to study the inhibition of plants exposed to contaminants in this stage is a best way to understand the toxic mechanisms of environmental contaminants to plants (9,10). The current literature suggests that seed germination is affected by metals in two ways. Firstly, by their general toxicity, and secondly, by their inhibition of water uptake (11).

Atriplex halimus subsp. schweinfurthii (Chenopodiaceae) is a xerohalophyte which is perennial and native in arid and semi arid Mediterranean regions. These specie tolerant to extremely harsh abiotic conditions such as; salinity (12), light stress (13), drought (14) and cold (15,16), moreover, a recent study reported that this species is present on heavy metal contaminated mining sites and that it also exhibits a high level of resistance to Cd and Zn (17,18).

The aim of this study was to analyze and compare the effects of selected metals on seed germination in *A. halimus subsp. Schweinfurthii.* The final germination, root elongation, and the growth of hypocotyls and cotyledons were investigated at different concentrations of Cd, Cu and Zn. According to its sensibility, agar was used as support material for seed germination (19).

Materials and methods

Seeds were collected in November 2013 from wild population grow at the experimental station of the Scientific and Technical Research Centre for Arid Areas (CRSTRA) located in Biskra (Algeria) (34°55'42"N 5°38'58"E, and 198 m elevation). Seeds were surface-sterilised by immersion in 4.3 % (v/v) sodium hypochlorite for 3 min, followed by a treatment with 70% ethanol for 30 s and rinsed three times in sterile water.

The heavy metals used in this study (Zn, Cu and Zn) were in the form of sulfate. Ten concentrations (between 0 and 4000 µM) of each metal were used in this study. For each treatment the pH was adjusted to 5.5.

Germination tests were carried out in petri dishes (120 x 15 mm). In each dish, 40 to 50 seeds were placed on the agar 1% (w/v) with metals at different concentrations. Petri dishes were then wrapped with adhesive tape to allow aeration and placed on an inclined plane (15° from the horizontal).

The seeds were maintained firstly for 24 h under dark conditions at 25°C, and then transferred under controlled-environment conditions with 10/14 h of day/night at 25°C. The light was provided by fluorescent lamps that produce a photosynthetic photon flux density of 100 µmol m^{-2} s^{-1}. Germination was monitored for 6 days with the germination in each plate recorded every day.

Germination tests were carried out using triplicate samples. Seeds were scored as germinated when the breakage of seed coat was visible. Seedling development was regarded as being inhibited 6 d after imbibition if the seed coat was visibly broken (germination), but the embryo did not grow further (3).

The germination dynamic was analyzed by taking the final germination percentage after 6 days, the time for the first seed germinated (T0) and the number of days necessary to reach 50% of the final germination percentage (T50) for each plate (20).

For each treatment, six seedlings were scanned using HP Scanjet G2710, and then the length of cotyledons, hypocotyls and roots were measured by software (Opmias Ver1.0). Theses seedlings fresh weight was measured to the nearest 0.01 mg using a Laboratory Scale - Scaltec -SBC 22.

Statistical method

Statistical analysis was performed using SPSS software. The germination rate pattern, fresh weight and the size of the seedlings were analyzed by the one-way ANOVA technique, using the student-Newman-Keuls test at the $p < 0.01$ confidence level (due to the normality and homogeneity of the data), while the T0 and T50 were analyzed by non-parametric tests (Kruskal Wallis and Mann Whitney U tests) at the $p < 0.01$ confidence level due to the no normality of the data.

Results And Discussion

Phytotoxic effects of Zn, Cu and Cd on seeds germination and germination rate are shown in (Table 1). These parameters were not affected by any concentrations of Cu or Zn. The final germination percentages were equal or very close to 100% together with non-significant differences compared to the control plants ($p > 0.01$). The germination rate values (T0 and T50) were equal to 1 day for all treatments of Cu or Zn. Cd reduced slightly the germination percentage above 1500µM. The minimum germination (79.7%) was recorded at 3000µM ($P < 0.01$). The initial germination (T0) was not affected in any of the treatments applied, but delays were observed in (T50) at 2000µM of Cd.

The seedling fresh weight, cotyledons, hypocotyls and roots length were significantly decreased with increasing of metal dose in different ways ($P < 0.01$). Zn induced a slight decrease on cotyledons length. However, its effect was more significant in seedling fresh weight, hypocotyls and roots length compared to the control (Table 2 and fig. 1A). In addition, Cu was also reduced significantly seedling growth, but its effect was more toxic, and the growth was inhibited over 2000µM (Table 2 and fig. 1B). Cd reduced cotyledons length as from 50 µM, the seedling fresh weight and the length of root were affected for 100µM and the length of the hypocotyls seemed to be affected from 250 µM. At this dose Cd suppressed growth almost completely (Table 2 and fig. 1C).

The most widespread visual evidence of metal toxicity is a reduction in plant growth as metal toxicity increases. However, as different metals have different sites of action within the plant, the overall visual toxic response differs between metals (21). Zn and Cu are essential elements to higher plants and are involved in several metabolic processes, whereas Cd is not known to have any function in plants. High concentration of Zn causes a reduction in chlorophyll synthesis and chloroplast degradation. It may also interfere with P, Mg and Mn uptake (22). Zn has been reported to reduce the germination in *Vigna mungo* at concentrations higher than 0.50 mM (23) and in alfalfa at concentrations higher than 0.3 mM (24). However, in this study no effect was observed in germination stage. In addition, negative effects on seedling weight and shoots have been observed in *Eucomis autumnalis* at levels higher than 20µM and in roots in concentration above 4µM (25). In the present study Zn affected negatively the seedling weight and the roots at levels of 50 and the shoots at 100 µM.

Cu is direct and indirect constituent of many important enzyme systems such as cyctochrome oxidase, polyphenol oxidase and superoxide dismutase (26). At high concentrations it can affect key enzymes, such as glutamine synthetase and glutamate synthetase, and change nitrogen metabolism in higher plants (27). The toxicity of this metal has been observed in germination at concentrations over 4 µM in *Eucomis autumnalis* (25). 100µM in *Vigna mungo* (23) and alfalfa at concentrations over than 300 µM (24). In addition, negative effect has been detected in the seedling weight and shoots length of *Bowiea volubilis* at concentration up to 80µM. while the root length reduced from 4µM (25). In our study no effect was observed in germination percentage. But seedling growth was reduced significantly from 50µM and inhibited completely up to 2000µM.

Cd is non-essential elements in plants and its accumulation alters mineral nutrients uptake, inhibits stomatal opening by interacting with the water balance of plant (28). Cd was the most toxic to seed germination and seedling growth of *A. halimus subsp. Schweinfurthii* among the three heavy metals tested. Seed germination was reduced at 1500µM, and Seedling growth was completely suppressed above 250µM. Cd has been also found to decrease *Suaeda salsa* seed germination at 2.75 µM (9). In addition, roots of radish and tomato were reduced at 128 and 64 µM respectively (19).

Our results clearly show that the negative impact of heavy metals was more pronounced at the post-germination stage in comparison to seed germination. Similar results have been reported for *Bowiea volubilis*, *Merwilla natalensis* (25) and *Arabidopsis thaliana* (3). Cheng and Zhou (2002)(10) reported that least sensitivity of germination might be due to greater tolerance of seeds having stored foods in themselves. However, Li et al. (2005)(3) referred this character to tissues covering the embryo witch play a role in selective penetration of different heavy metals into seeds.

Roots are the primary targets of metal anions and their growth is usually more severely affected than that of the aerial parts (20), therefore, root length is commonly used for evaluating the toxicity levels of heavy metals (29). This is confirmed by the results presented here, as the roots were affected before the hypocotyls or cotyledons in all the metals tested

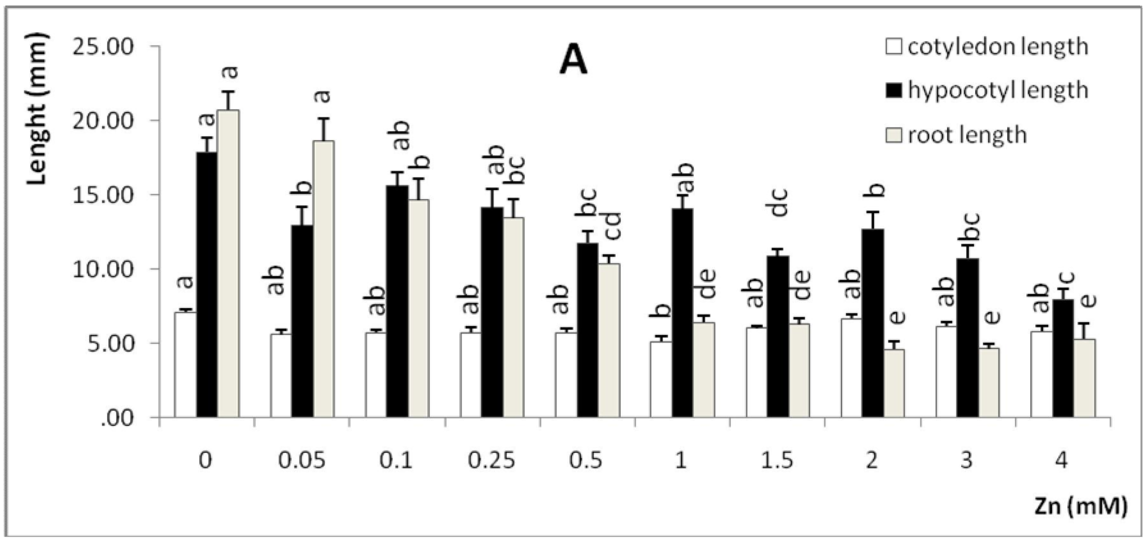
Table 1 Final germination percentage after 6 days, T0 and T50 of *Atriplex halimus subsp. Schweinfurthii* seeds sown in different concentrations of Zn, Cu and Cd.

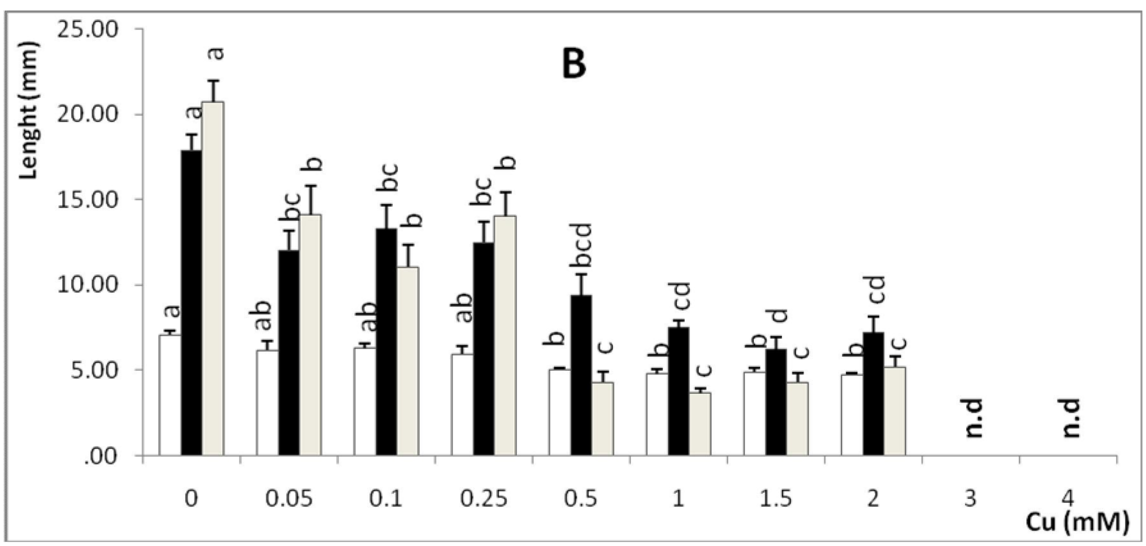
Treatment	Concentration (µM)	Final germination (%)	T0	T50
Control	0	99.2 ± 1.4 a	1.0 ± 0.0 a	1.0 ± 0.0 a
ZnSO4	50	98.0 ± 2.0 a	1.0 ± 0.0 a	1.0 ± 0.0 a
	100	99.0 ± 1.0 a	1.0 ± 0.0 a	1.0 ± 0.0 a
	250	97.7 ± 2.3 a	1.0 ± 0.0 a	1.0 ± 0.0 a
	500	98.3 ± 0.6 a	1.0 ± 0.0 a	1.0 ± 0.0 a
	1000	99.0 ± 1.0 a	1.0 ± 0.0 a	1.0 ± 0.0 a
	1500	97.7 ± 1.2 a	1.0 ± 0.0 a	1.0 ± 0.0 a
	2000	98.7 ± 1.5 a	1.0 ± 0.0 a	1.0 ± 0.0 a
	3000	98.0 ± 2.0 a	1.0 ± 0.0 a	1.0 ± 0.0 a
	4000	99.0 ± 1.0 a	1.0 ± 0.0 a	1.0 ± 0.0 a
CuSO4	50	100.0 ± 0.0 a	1.0 ± 0.0 a	1.0 ± 0.0 a
	100	100.0 ± 0.0 a	1.0 ± 0.0 a	1.0 ± 0.0 a
	250	100.0 ± 0.0 a	1.0 ± 0.0 a	1.0 ± 0.0 a
	500	99.3 ± 1.2 a	1.0 ± 0.0 a	1.0 ± 0.0 a
	1000	100.0 ± 0.0 a	1.0 ± 0.0 a	1.0 ± 0.0 a
	1500	99.0 ± 1.8 a	1.0 ± 0.0 a	1.0 ± 0.0 a
	2000	100.0 ± 0.0 a	1.0 ± 0.0 a	1.0 ± 0.0 a
	3000	98.7 ± 2.3 a	1.0 ± 0.0 a	1.0 ± 0.0 a
	4000	98.7 ± 2.3 a	1.0 ± 0.0 a	1.0 ± 0.0 a
CdSO4	50	99.2 ± 1.4 a	1.0 ± 0.0 a	1.0 ± 0.0 a
	100	100.0 ± 0 a	1.0 ± 0.0 a	1.0 ± 0.0 a
	250	97.7 ± 3.9 a	1.0 ± 0.0 a	1.0 ± 0.0 a
	500	98.6 ± 2.4 a	1.0 ± 0.0 a	1.0 ± 0.0 a
	1000	98.6 ± 2.4 a	1.0 ± 0.0 a	1.0 ± 0.0 a
	1500	89.0± 4.6 abc	1.0 ± 0.0 a	1.0 ± 0.0 a
	2000	91.4 ± 2.5 ab	1.0 ± 0.0 a	2.0 ± 0.0 b
	3000	79.7 ± 4.8 c	1.0 ± 0.0 a	2.0 ± 0.0 b
	4000	83.1 ± 3.8 bc	1.0 ± 0.0 a	2.0 ± 0.0 b

The data show mean ± SD of three different replicates (n = 3). Different letters indicate significant differences between each metal and the control, following newman keuls post hoc test ($p < 0.01$) for final germination percentages and Kruskal Wallis and Mann Whitney U tests ($p < 0.01$) for the T0 and T50 values

Table 2 Effect of Cd, Cu and Zn on seedling fresh weight of *A. halimus subsp. Schweinfurthii*.			
Concentration (µM)	Zn	Cu	Cd
0	6.45 ±0.22 a	6.45 ± 0.22 a	6.45 ±0.22 a
50	5.26 ±0.18 abc	5.23 ± 0.46 b	5.57 ±0.27 a
100	5.52 ±0.41 ab	3.73 ± 0.08 c	3.18 ±0.15 b
250	4.56 ±0.38 bc	3.40 ±0.28 cd	2.60 ±0.14 b
500	4.71 ±0.23 bc	3.07 ±0.21 cde	n.d
1000	4.48 ±0.30 bc	2.87 ±0.21 cde	n.d
1500	4.83 ±0.24 bc	1.88 ± 0.10 e	n.d
2000	3.86 ±0.46 cd	2.27 ± 0.29 de	n.d
3000	3.91 ±0.18 cd	n.d	n.d
4000	2.82 ±0.15 d	n.d	n.d
n.d: not determined, if the embryo did not grow further within 6 days after imbibition.			

The data show mean ± SD of three different replicates (n = 6). Different letters indicate significant differences between each metal and the control, following Newman keuls post hoc test ($p < 0.01$)





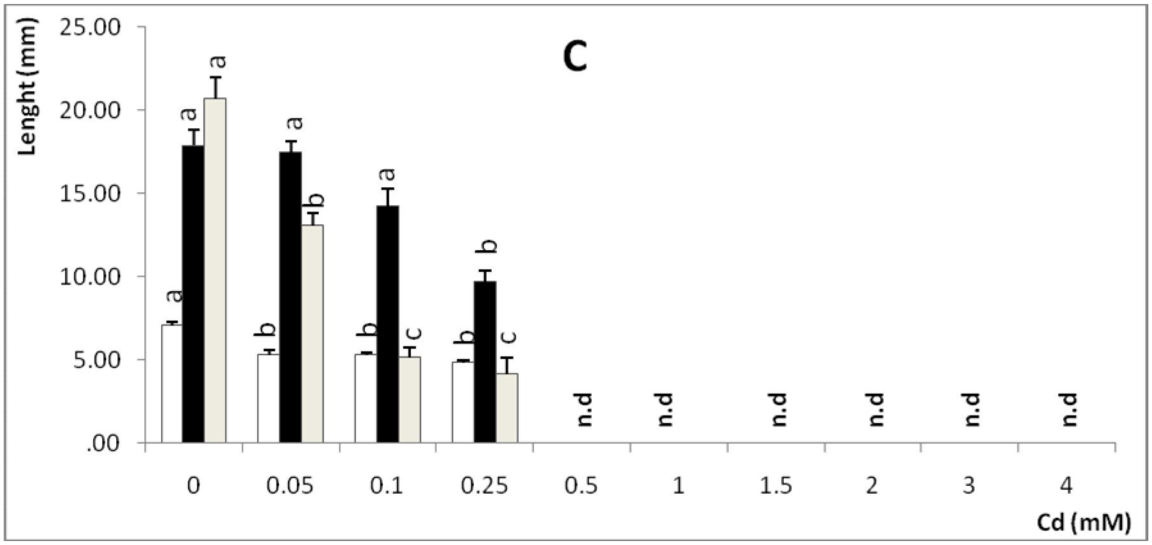


Figure 1 Effect of heavy metal on seedling growth of *A. halimus subsp. Schweinfurthii.* The data show mean ± SD of three different replicates (n = 6). Different letters indicate significant differences between each metal and the control, following Newman keuls post hoc test ($p < 0.01$).
n.d: not determined, if the embryo did not grow further within 6 days after imbibition.

Conclusion

This study concluded that *A. halimus subsp. Schweinfurthii* seedling growth stage (especially root length) was more sensitive than seed germination, and maybe used as good indicator of heavy metal pollutions. Among heavy metals, Cd showed more toxicity to seed germination and seedling growth followed by Cu than Zn. Results of the findings can be useful indicator of metal tolerance to some extent for plantation of this species in metal contaminated area.

Acknowledgments

The authors acknowledge financial support from Scientific and Technical Research Centre for Arid Areas (CRSTRA), Biskra, Algeria.

Short Title:

Effect of Heavy metals on seed germination of *Atriplex halimus.*

Note:

In this paper, I studied the germination rates of seeds, the root elongation, and the growth of hypocotyls and cotyledons in response to different concentrations of Cadmium, Copper and Zinc. The species used here was commonly resistant to harsh conditions such as salinity and drought. A recent study reported that this plant is present on heavy metal contaminated mining sites and exhibited a high level of resistance to Cd and Zn. The importance of the present study is that it has a focus on the possible use of *A. halimus subsp. Schweinfurthii* in rehabilitation of soils affected by excessive salinity and low moisture. The paper should be of interest to readers in the areas of heavy metal toxicity and remediation.

Author Info:

Mohammed MESNOUA
Scientific and Technical Research Centre for Arid Areas (CRSTRA), Biskra, BP 1682 R.P. BISKRA 07000 – Algeria.
Laboratory of Plant Protection, University of Abdelhamid Ben Badis of Mostaganem, Algeria
mohamesnoua@gmail.com.

References

1. Demirevska-Kepova, K., Simova-Stoilova, L., Stoyanova, Z., Holzer, R., and Feller, U. (2004) *Environmental and Experimental Botany* 52, 253-266.
2. Munzuroglu, O., and Geckil, H. (2002) *Archives of Environmental Contamination and Toxicology* 43, 203-213.
3. Li, W. Q., Khan, M. A., Yamaguchi, S., and Kamiya, Y. (2005) *Plant Growth Regulation* 46, 45-50.
4. Bieby, V. T., Siti, R. S., Hassan, B., Mushrifah, I., Nurina, A., and Muhammad, M. (2011) *International Journal of Chemical Engineering* ID 939161, 1-31 pages.
5. Cho, M., Chardonnens, A. N., and Dietz, K. J. (2003) *New Phytologist* 158, 287-293.

6. Kabata-Pendias, A., and Pendias, H. (2001) *CRC Press, London, UK.*
7. Kavuličová, J., Kaduková, J., and Ivánová, D. (2012) *Nova Biotechnologica et Chimica* 11, 101–110.
8. Moosavi, S. A., Gharineh, M. H., Afshari, R. T., and Ebrahimi, A. (2012) *Journal of Agricultural Science* 4, 11-19.
9. Liu, S. J., Yang, C. Y., Xie, W. J., Xia, C. H., and Fan, P. (2012) The effects of cadmium on germination and seedling growth of Suaeda salsa. in *Seventh International Conference on Waste Management and Technology* (Jinhui, L., and Hualong, H. eds.), Elsevier Science Bv, Amsterdam. pp 293-298.
10. Cheng, Y., and Zhou, Q. X. (2002) *Journal of Environmental Sciences-China* 14, 136-140.
11. Kranner, I., and Colville, L. (2011) *Environmental and Experimental Botany* 72, 93-105.
12. Bajji, M., Kinet, J. M., and Lutts, S. (1998) *Plant Science* 137, 131-142.
13. Streb, P., Tel Or, E., and Feierabend, J. (1997) *Functional Ecology* 11, 416-424.
14. Martinez, J. P., Lutts, S., Schanck, A., Bajji, M., and Kinet, J. M. (2004) *Journal of Plant Physiology* 161, 1041-1051.
15. Walker, D. J., Romero, P., de Hoyos, A., and Correal, E. (2008) *Environmental and Experimental Botany* 64, 217-224.
16. Salahas, G., Cormas, E., and Zervoudakis, G. (2002) *Russian Journal of Plant Physiology* 49, 211-215.
17. Lefevre, I., Marchal, G., Meerts, P., Correal, E., and Lutts, S. (2009) *Environmental and Experimental Botany* 65, 142-152.
18. Lutts, S., Lefevre, I., Delperee, C., Kivits, S., Dechamps, C., Robledo, A., and Correal, E. (2004) *Journal of Environmental Quality* 33, 1271-1279.
19. Di Salvatore, M., Carafa, A. M., and Carratu, G. (2008) *Chemosphere* 73, 1461-1464.
20. Marquez-Garcia, B., Marquez, C., Sanjose, I., Nieva, F. J. J., Rodriguez-Rubio, P., and Munoz-Rodriguez, A. F. (2013) *Marine Pollution Bulletin* 70, 119-124.
21. Reichman, S. M. (2002) *Australian Minerals & Energy Environment Foundation, Melbourne*, 22-24.
22. Broadley, M. R., White, P. J., Hammond, J. P., Zelko, I., and Lux, A. (2007) *New Phytologist* 173, 677-702.
23. Solanki, R., Anju, Poonam, and Dhankhar, R. (2011) *Journal of Environmental Biology* 32, 747-751.
24. Peralta, J. R., Gardea-Torresdey, J. L., Tiemann, K. J., Gomez, E., Arteaga, S., Rascon, E., and Parsons, J. G. (2001) *Bulletin of Environmental Contamination and Toxicology* 66, 727-734.
25. Street, R. A., Kulkarni, M. G., Stirk, W. A., Southway, C., and Van Staden, J. (2007) *Bulletin of Environmental Contamination and Toxicology* 79, 371-376.
26. Singh, D., Nath, K., and Sharma, Y. K. (2007) *Journal of Environmental Biology* 28, 409-414.
27. Llorens, N., Arola, L., Blade, C., and Mas, A. (2000) *Plant Science* 160, 159-163.
28. Tran, A. T., and Petrova, L. P. (2013) *Turkish Journal of Botany* 37, 1-13.
29. Pereira, S., Saker, M. L., Vale, M., and Vasconcelos, V. M. (2009) *Bulletin of Environmental Contamination and Toxicology* 83, 81-84.

7/25/2017