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COMPARATIVE EFFECTS OF MANGANESE AND IRON STRESS ON SEED GERMINATION AND VARIOUS GROWTH PARAMETERS OF *PHASEOLUS LUNATUS* L.

SHER WALI[1*], LEENA SYED[1], FAZLI RAHIM[2], FAHEEM TARIQ[1] AND KHUSHNOOD UR RAHMAN[1]

[1]*Department of Botany, Islamia College, Peshawar, Pakistan*
[2]*Department of Botany, Bacha Khan University, Charsadda, Pakistan*
[*]*Corresponding author's email: sherwali@icp.edu.pk*

Abstract

In the current research work, the effects of different concentrations of Manganese and Iron on various growth parameters of *Phaseolus lunatus* L. were studied. For this purpose pots experiments were performed in three replicates. Three concentrations each of Manganese and Iron (2millimolar (mM), 10mM and 20mM) were used as metal stress treatments. A single series of 3 pots with no treatment was designated as the control. The results showed that number of days to germination were more in treated plants as compared to control. Hence, seed germination was negatively affected by the applied metal stresses. Similarly number of seeds germinated per treatment was also decreased with the increasing concentrations of the treatments. All the other growth parameters including plant height, number of leaves, shoot length and root length significantly reduced with the increase in concentration as compared to control. While fresh and dry weights increased at the lower (2mM) concentration while decreased significantly with the higher (10mM and 20mM) concentrations.

Key words: Manganese, Iron, Germination, Growth, *Phaseolus lunatus* L.

Introduction

When organism comes under any danger, it shows its natural defense usually called as stress. To cope with various abiotic environmental stresses, plants adopt various strategies i.e. heat, cold, osmotic stress, high salt, dehydration, which affect plant growth and various physiological processes (Epstein *et al*., 1980; Shu *et al*., 2004). In rice crop the productivity reduced more than 50% due to high salt concentration in soil and water deficit (Mahajan & Tuteja, 2005). Some heavy metals are very lethal, even when they are present in trace amounts i.e. Hg, Cd, Pb, Cr and Ag, theses metalloids are spread indirectly (Qadir *et al*., 2013). Contamination caused as a result of Heavy metal is one the major environmental problem across the globe. Manganese (Mn) role in redox reaction as different enzymes cofactor make it one of vital element for plant growth (Millaleo *et al*., 2010). Plant growing in an environment contaminated with Mn can suffer Mn toxicity via two major pathways i.e., adaptive (intolerant plants) or constitutive (in sensitive plants) (Mou *et al*., 2011). Iron is one of the most vital elements in enzymes, myoglobin, hemoglobin, digestive action and in maintaining a healthy immune system (Ahmed & Chaudhary, 2009). In *Eucalyptus* leaves higher concentrations of heavy metals are in function. This study provides us basis to establish the application of tree leaves as a tool for rapid reduction of pollution by analyzing heavy metal concentration in tree leaves (Khattak & Jabeen, 2012). In rice plant on root surface iron plaque seize Cd; Cd absorption on root surface can be reduced by Fe_2O_3. However, Fe_2O_3 does not block Cd uptake and translocation in rice seedlings. The iron plaque, retreat Cd toxicity under low concentration of Cd in rice seedlings, tissues of root usually play a more important role in reducing Cd translocation to shoot (Lai *et al*., 2012). The current research work was carried out to find the effect of heavy metals, Iron (Fe) and Manganese (Mn) on various growth parameters of bean (*Phaseolus lunatus* L.).

In genus Phaseolus, Lime bean (*Phaseolus lunatus* L.) is one of the major cultivated specie. According to seeds characteristics, there are 3 different commercial types are recognized: flat and big seeds, big lima type, sieve type, with flat medium size flat seeds and potato type, small rounded seeds (Esquivel *et al*., 1990). These are cultivated in tropical and subtropical region due to its edible seeds. *P. lunatus* var. Silverster nadudet and *P. lunatus* var. *lunatus* are the wild and cultivated type of *P. lunatus* respectively. Lima bean is herbaceous plant with growing habit of two types. In perennial form, Lima bean is dynamic, climbing, indeterminate and irregular; reaches up to 2-6 meter (m) tall, having axillary flowers. Based on seeds difference cultivars have been distinguished (Baudoin, 2006). During sprouts young pods, green seeds and leaves are edible and usually eaten as vegetables. The dry seeds are often boiled, fried, powdered and used in stews and soups. Lima bean is one of the few cultivars which might be worthy in intercropping system. Increased phytase activity during germination cause decrease in phytase content that have a positive impact on ruminant nutrition and poultry including whole environment (Azeke *et al*. 2011).

Materials and Methods

The current research work was conducted in Botanical Garden of Islamia College, Peshawar. This research work was designed to find out the effect of heavy metals (Fe and Mn) on various growth parameters of *Phaseolus lunatus* L.

Germination of seeds: The pots of same size were used filled with clay and sand in a 3:1 proportion. Seeds were collected from the local market. The seeds were geminated in such a way that each pot containing 8 seeds. The plants were regularly watered. After ten days of interval heavy metals (Mn, Fe) were applied to the plants. The plants were grown in optimum temperature. The pots were labeled with various concentrations of Mn and Fe as row number 1 for control, 3 rows for the different concentrations (2 mM, 10 mM and 20 mM) of each metal.

$FeCl_3$ and $MnCl_2$ treatments: $FeCl_3$ and $MnCl_2$ solution of 2 mM, 10 mM and 20 mM were prepared using graduated cylinder. The solutions were then transferred to conical flasks (500 ml). Heavy metals stress was applied in pots.

Chemical used: The chemicals used were Fe (BDH, England), Mn (Sigma-Aldrich, USA).

Data collection: After 3-time treatment of *Phaseolus lunatus* L. the plants were harvested. Various growth parameters of *Phaseolus lunatus* L. such as days to germination**,** Number of seeds germinated**,** Number of leaves**,** Height of plant**,** Root length**,** shoot length**,** Fresh weight of plant**,** Dry weight of plant of *Phaseolus lunatus* were studied.

Statistical analysis: The Completely Randomized Block Design (CRBD) was used in whole experiment. For statistical analysis MS Excel 365 was used, and standard deviation of the data along graphs was built.

Results

Germination of seeds

a) Number of days: The effect of different concentrations of Mn and Fe on germination of *Phaseolus lunatus* was recorded. In control treatments the plants germinated after 14 days of sowing. In comparison to that different concentrations of Mn i.e. 2 and 10 mM treated seeds showed the same effect on the number of days to germinate and were 17 days. While 20mM (Mn) concentrations showed germination on average of 15 days (Fig. 1). The maximum days in Fe treated plants were taken by 20 mM with 19.33 days.

b) Number of seeds germinated: Effect of Mn and Fe various concentrations on number of seeds germinated. The average 5.7 seeds were germinated in control treatments. In comparison to control 6.3 seeds were germinated in 2mM Mn, 10mM Mn concentration showed 5.3 and 20 mM showed 4.9 germinated seeds respectively (Fig. 2). In case of Fe 2mM and 10mM concentrations only 5 seeds were germinated. Whereas, 4. 7 seeds were germinated, when provided with Fe 20mM.

Plant height (cm): The Statistical analysis of data revealed that, under control condition the plant height was recorded i.e. 9.3 cm. In comparison to that when 20 mM of Mn showed minimum plant height with 6.0 cm. Fe stress showed minimum plant height in 20 mM with 6.2 cm (Fig. 3). The maximum plant height was recorded in 2 mM of Mn i.e., 7.5.

Number of leaves: The effect of different concentrations of Mn and Fe on number of leaves of *Phaseolus lunatus* was recorded. It was seen that in control medium plants showed a total average of 14.7 numbers of leaves. As compared to control, 13.7, average number of leaves was recorded in 2mM of Mn i.e. maximum amongst all treatment plants. However, minimum number of leaves was recorded in plant treated with 20mM of Fe i.e., 7 (Fig. 4).

Shoot length (Cm): When different concentrations of Mn and Fe treatment was applied, the results showed that the maximum shoot length was appeared in plants treated with 2 mM of Mn i.e., 29.73 cm. whereas, in case of Mn the minimum shoot growth was recorded in plants provided with 20 mM of Mn, i.e., 24.96 cm. As shown in Fig. 5, Fe treated plants the minimum shoot length was recorded in 20 mM Fe treated plants with shoot length of 16.33 cm.

Root Length (cm): The results showed that root length of *Phaseolus lunatus* L. decreases with increasing concentration of Mn and Fe. When 20mM of Mn was applied it was seen that plant showed a root length of 7.2cm i.e., the minimum root length among all treatments of Mn. Whereas, the maximum root length was present in 2mM of Mn with 10.1 cm, in comparison to that Fe at 2 mM showed a root length of 9.81cm (Fig. 6).

Fresh weight (grams): The fresh weight of *Phaseolus lunatus* in control medium showed an average weight of 4.7 gm. As compared to control, Mn 2mM and 10 mM concentrations showed positive effect on fresh weight. While 20mM showed negative effect i.e., 4.2 gm. While in Fe, all concentrations showed negative effect on fresh weight of the plant as compared to control (Fig. 7).

Dry weight: The effect of different concentrations of Mn and Fe on dry weight of *Phaseolus lunatus* showed that control plant has an average dry weight of 2.1gm. As compared to control, Mn 10mM and 20mM concentrations showed negative effect on dry weight while 2mM showed the enhancement of dry weight which was 2.9gm (Fig. 8). While in Fe, all concentration showed negative effect on dry weight as compared to control.

Discussion

Some of heavy metals are required for various biological processes in very trace amount. Depending upon heavy metal and its concentration, it can either enhance the plant growth or affect the growth of plant negatively. Iron is one of the most vital elements and it is involved in numerous biological processes. It is most important transition metal in all living organism. Both prokaryotic and eukaryotic organisms have iron containing proteins. Iron containing protein usually transport and store oxygen, and transfer electrons. Manganese (II) ion is used as cofactor for large variety of enzymes. Superoxide free radicals' detoxification is carried out by enzymes containing Manganese (Mn). Manganese plays an important role in chloroplast photosynthetic oxygen evolution. During light reaction of photosynthesis Oxygen Evolving Complex (OEC) present in thylakoid membrane of chloroplast, it is responsible for terminal photo oxidation of water.

Results showed that with increasing concentration of Mn and Fe i.e., 10mM and 20mM produced negative effect on seed germination of *Phaseolus lunatus* compared to control plants. Similar result was reported as Cd stress decreases seed germination (Raziuddin, 2011). This reduction in seed germination might be due to increase breakdown of reserved food material in embryo of seed. Zinc increases the seed germination, which indicates Zinc is very essential element for plants.

Plant height was drastically affected by increasing concentration of Mn and Fe stress. The plant height was mainly affected at 10mM and 20mM of Mn and Fe comparison to control. These results showed accordance with the results shown by Kibra (2008). According to Kibra (2008) reduction in height of plant occurs when present in a soil contaminated with 1mg/Kg. The reduction in plant height might be due to reduction in root growth, with causes less nutrients and water transport to upper part of plant (Kibra, 2008). Similarly, for number of leave in *Phaseolus lunatus*, was greatly affected by Mn and Fe stress. When 10mM and 20mM treatment of Mn and Fe reduce number of leaves, morover the number was least in Fe at 20mM. Similar results were shown by the application of Cadmium and Sodium chloride strees on *Brassica* species. According to Shah *et al*., (2008) the shoot length significantly reduced, and it was more evident when 20, 40 mg/L dose of Cd, Cr and Cd + Cr was applied. Our results were in agrrement with Shah *et al*. (2008). Shoot length decreased with increasing concentration of Mn and Fe stress. This decrease in shoot length is due to reduction in chlorophyll content in turn decrease photosynthesis.

Root is one of the most important parts of plant that is directly exposed or in contact to the heavy metal stress. Due to increased concentration of Mn and Fe stress the root length decreases. The reduction in root length is more prominent in 20mM and 10mM of Fe and Mn. Similar type of result was shown by Azmat & Akhtar (2010). This reduction in root length is due to break down root cell membrane, cell division, cell elongation and at last cell elongation (Ismail & Iqbal, 2013). As various parameters of plant showed reduction, hence reduction in the overall growth of plant occurred. Reduction in all these parameters due to Mn and Fe stress, also reduce the plant fresh and dry weight. The decrease in fresh and dry weight was more prominent in 10mM and 20mM of Mn and Fe. Marschner (1995) reported similar results in their experiment on *Vigna radiata.*

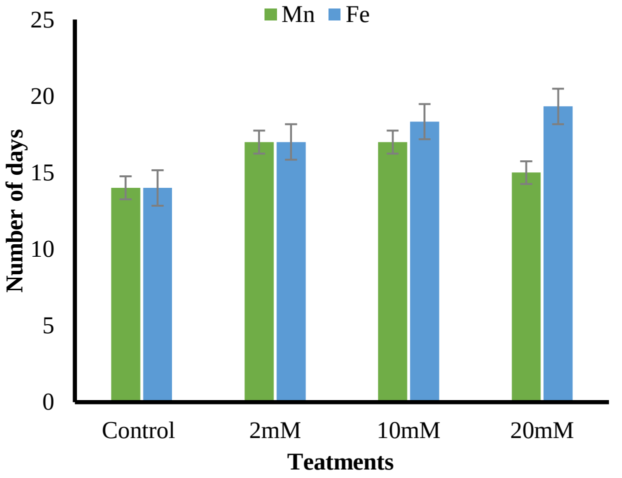

Fig. 1. Effects of different concentrations of Mn and Fe on number of days to germinate *Phaseolus lunatus* seeds.

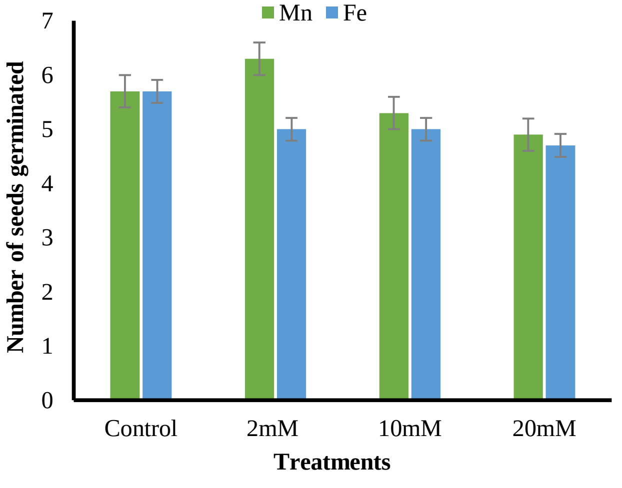

Fig. 2. Effect of different concentrations of Mn and Fe on germination of *Phaseolus lunatus.*

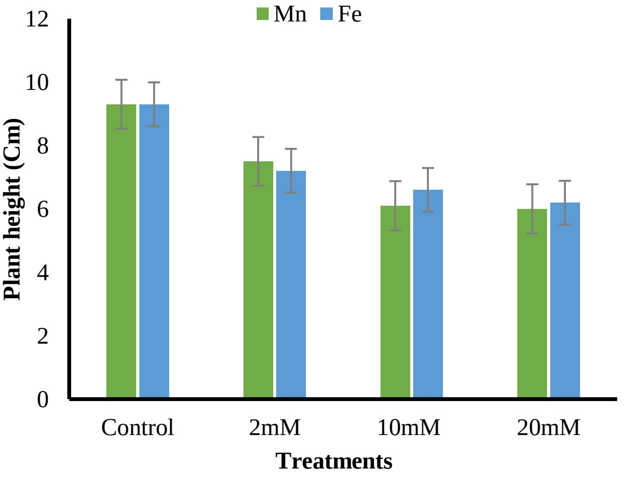

Fig. 3. Effect of different concentrations of Mn and Fe on plant height of *Phaseolus lunatus*.

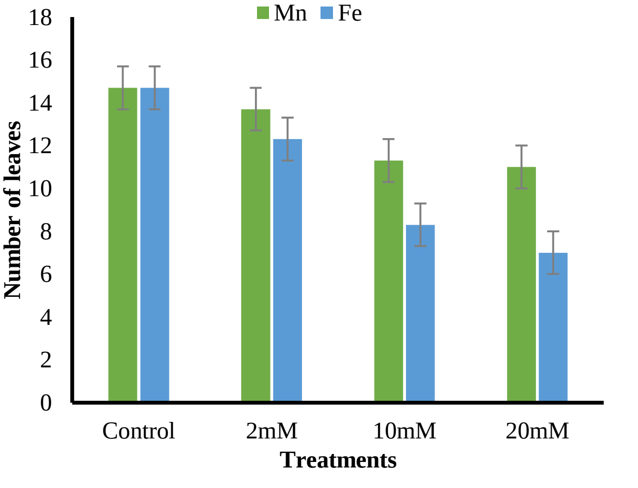

Fig. 4. Effect of different concentrations of Mn and Fe on No. of leaves of *Phaseolus lunatus*.

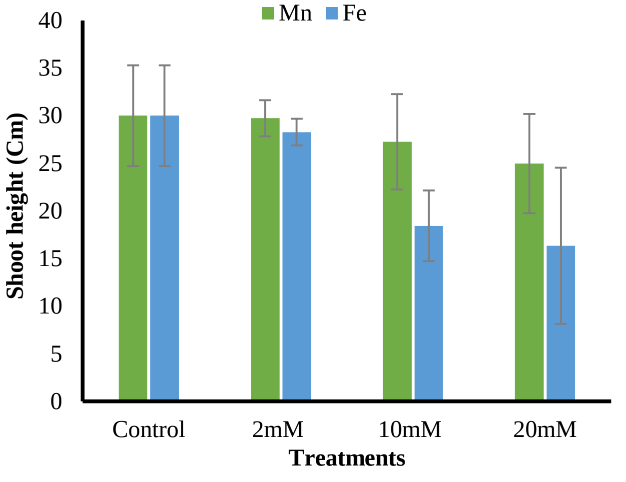

Fig. 5. Effect of different concentrations of Mn and Fe on shoot length (cm) of *Phaseolus lunatus*

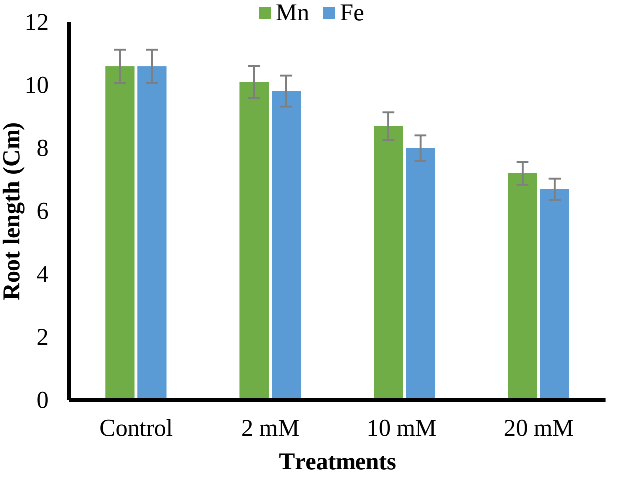

Fig. 6. Effect of different concentrations of Mn and Fe on root length (cm) of *Phaseolus lunatus*.

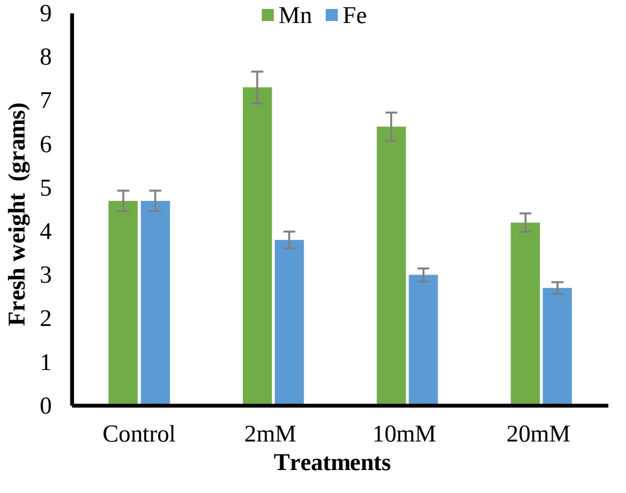

Fig. 7. Effect of different concentrations of Mn and Fe on fresh weight (gm) of *Phaseolus lunatus.*

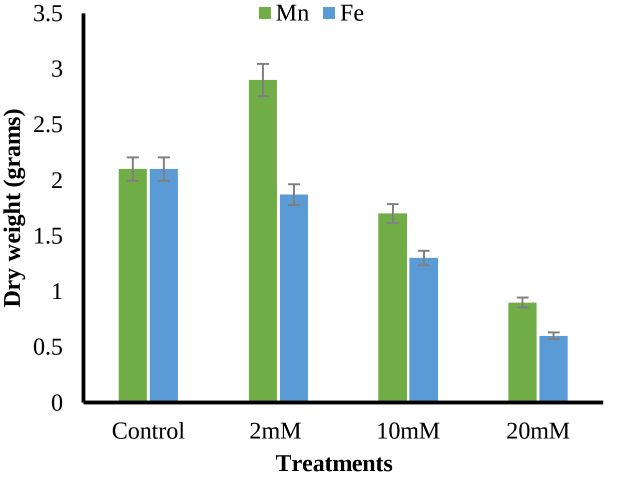

Fig. 8. Effect of different concentrations of Mn and Fe on dry weight (gm) of *Phaseolus lunatus.*

Conclusion

The present study was carried out to understand the effect of various concentrations of Manganese and Iron stress on *Phaseolus lunatus*. Our results revealed that Iron have a highly negative effect with increasing concentration. As above, the effect of Manganese being significantly negative is relatively less pronounced as compared to Iron.

References

Ahmed, D. and M.A. Chaudhary. 2009. Medicinal and nutritional aspects of various trace metals determined in Ajuga bracteosa. *J. App. Sci. Res*., 5(7): 864-869.

Azeke, M.A., R.M. Elsanhoty, S.J. Egielewa and M.U. Eigbogbo. 2011. The effect of germination on the phytase activity, phytate and total phosphorus contents of some Nigerian-grown grain legumes. *J. Sci. Food & Agri.*, 91(1): 75-79.

Azmat, R. and H. Akhter. 2010. Changes in some biophysical and biochemical parameters of mungbean [*Vigna radiata* (L.) wilczek] grown on chromium-contaminated soils treated with solid tea wastage. *Pak. J. Bot*., 42(5): 3065-3071.

Baudoin, J.P. 2006. *Phaseolus lunatus* L. Record from Protabase. (Eds.): Brink, M. & G. Belay. PROTA (Plant Resources of Tropical Africa / Ressources végétales de l'Afrique tropicale), Wageningen, Netherlands.

Epstein, E., J.D. Rush, R.W. Kingsbury, D.B. Kelley, G.A. Cinnigham and A.F. Wrono. 1980. Saline culture of crops: A genetic approach. *Science*, 28: 89-121.

Esquivel, M., L. Castiñeiras and K. Hammer. 1990. Origin, classification, variation and distribution of Lima bean (*Phaseolus lunatus* L.) in the light of Cuban material. *Euphytica*, 49(2): 89-97.

Ismail, S.F.K. and M.Z. Iqbal. 2013. Phytoremediation: assessing tolerance of tree species against heavy metal (Pb and Cd) toxicity. *Pak. J. Bot.*, 45(6): 2181-2186.

Khattak, M.I. and R. Jabeen. 2012. Detection of heavy metals in leaves of Melia azedarach and Eucalyptus Citriodora az biomonitoring tools in the region of Quetta valley. *Pak. J. Bot.*, 44(2): 675-681.

Kibra, M.G. 2008. Effects of mercury on some growth parameters of rice (*Oryza sativa* L.). *Soil & Environ*., 27(1): 23-28.

Lai, Y., B. Xu, L. He, M. Lin, L. Cao, Y. Wu and S. He. 2012. Cadmium uptake by and translocation within rice (*Oryza sativa* L.) seedlings as affected by iron plaque and Fe_2O_3. *Pak. J. Bot.*, 44: 1557-1561.

Mahajan, S. and N. Tuteja. 2005. Cold, salinity and drought stresses: An overview. *Arch. Biochem. Biophys.,* 444: 139-158.

Marschner, H. 1995. Mineral Nutrition of Higher Plants, second Ed. Academic Press, San Diego, USA.

Millaleo, R., M. Reyes-Díaz, A.G. Ivanov, M.L. Mora and M. Alberdi. 2010. Manganese as essential and toxic element for plants: transport, accumulation and resistance mechanisms. *J. Soil Sci. Plant Nutr*., 10(4): 476-494.

Mou, D., Y. Yao, Y. Yang, Y. Zhang, C. Tian and V. Achal. 2011. Plant high tolerance to excess manganese related with root growth, manganese distribution and antioxidative enzyme activity in three grape cultivars. *Ecotox. Environ. Safe,* 74: 776-786.

Qadir, A., R.F. Malik, A. Feroz, N. Jamil and K. Mukhtar. 2013. Spatiotemporal distribution of contaminants in Nullah Palkhu-highly polluted stream of Pakistan. *J. Environ. Sci. Water Resou.*, 2(10): 342-353.

Raziuddin, Farhatullah, G. Hassan, M. Akmal, S.S. Shah, F. Mohammad, M. Shafi, J. Bakht and W. Zhou. 2011. Effects of cadmium and salinity on growth and photosynthesis parameters of *Brassica* species. *Pak. J. Bot*., 43(1): 333-340.

Shah, F.R., N. Ahmad, K.R. Masood and D.M. Zahid. 2008. The influence of cadmium and chromium on the biomass production of shisham (*Dalbergia sissoo* ROXB.) seedlings. *Pak. J. Bot*., 40(4): 1341-1348.

Shu, H.L., K. Chang, C.H. Yau, C.H. Hong and C.H. Wen. 2004. Osmotic sucrose enhancements of single cell embryogenesis and transformation efficiency in Oncidium. *Plant Cell Tiss. Org. Cult.,* 81: 183-192.

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