LR-4820
[1-5]

RESEARCH ARTICLE | Legume Research- An International Journal, Volume Issue : ()

Assessment of Physiological and Biochemical Traits of Ricebean [*Vigna umbellata* (Thunb.) Ohwi and Ohashi] Seedling in Response to Equimolar Concentration of Copper and Lead Stress

Kousik Atta[1], Apurba Pal[2], Snehashis Karmakar[1], Debjani Dutta[1], Kalyan Jana[3], Anjan Kumar Pal[1]
10.18805/LR-4820

ABSTRACT

Background: Heavy metal stress has an adverse effect on crop growth and productivity. The present experiment was undertaken to study the physiological and biochemical effects of two heavy metals, *viz.*, copper and lead, at their equimolar concentrations, on seedling growth of ricebean [*Vigna umbellata* (Thunb.) Ohwi and Ohashi] cv. Bidhan 1.

Methods: The seedlings grown in sand culture were subjected to 50, 100 and 200 µM of Cu and Pb-. The experiment was done in the laboratory of Department of Plant Physiology, Bidhan Chandra Krishi Viswavidyalaya (BCKV), Mohanpur, Nadia, West Bengal in the year 2017-18 and 2018-19.

Result: The total dry weight, chlorophyll, relative water as well as proline content in leaf significantly decreased in most of the cases of metal stress, but the lipid peroxidation increased. The mild and medium stress induced substantial increase in the activities of APX, GPOX and SOD. In general, lead was found to be more detrimental in comparison to copper.

Key words: Anti-oxidative enzymes, Heavy metal stress, Lipid peroxidation, Reactive oxygen species (ROS), Ricebean.

INTRODUCTION

The exposure of plants to cytotoxic levels of heavy metals triggers a range of physiological and metabolic alterations. The most widespread visual proof of significant heavy metal toxicity could be a reduction in plant growth, leaf chlorosis, necrosis, turgor loss, seed germination rate and a crippled photosynthetic apparatus, which consequently promote PCD and accelerate plant death (Dalcarso *et al.,* 2010, Pireh *et al.*, 2016).

Lead (Pb) is ubiquitously distributed and most abundant toxic components in the soil. It exerts adverse impact on plant morphology, germination behaviour and growth pattern as well as photosynthetic processes by hampering the activity of carboxylating enzymes (Stiborova *et al.*, 1987). Lead in high concentration causes inhibition enzyme activities, alteration in membrane permeability, water imbalance and disturbance in mineral nutrition and enhances lipid peroxidation, oxidative stress and DNA damage (Saleem *et al.*, 2021). Copper (Cu), an important component of cytochrome oxidase of respiratory electron transport chain and plastocyanin of photosynthetic system, plays significant role in ATP synthesis and CO_2 assimilation (Gang *et al.,* 2013). High amount of Cu in soil causes cytotoxicity, which results in leaf chlorosis, plant growth retardation and generation of oxidative stress (Lewis *et al.*, 2001).

Ricebean [*Vigna umbellata* (Thunb.) Ohwi and Ohashi] a potential under-utilized leguminous crop, has wider adaptability, along with its inherent tolerance to several biotic and abiotic stresses. Limited research works so far has been conducted in response to abiotic stress in ricebean (Rai, 2013; Pal *et al.* 2009; Atta *et al.* 2020; Atta *et al.* 2021; Subrahmanyam, 1998). However, information on its ability to tolerate heavy metal stress is very limited (Chhetri *et al.* 2004). The present experiment was envisaged to study the effect of equimolar concentration of copper and lead on physiological and biochemical traits of ricebean seedling.

[1]Department of Plant Physiology, Faculty of Agriculture, Bidhan Chandra Krishi Viswavidyalaya, Nadia-741 252, West Bengal, India.
[2]Horticulture College, Khuntpani, Chaibasa Birsa Agricultural University, Ranchi-834 006, Jharkhand, India.
[3]Department of Agronomy, Faculty of Agriculture, Bidhan Chandra Krishi Viswavidyalaya, Nadia-741 252, West Bengal, India.

Corresponding Author: Kousik Atta, Department of Plant Physiology, Faculty of Agriculture, Bidhan Chandra Krishi Viswavidyalaya, Nadia-741 252, West Bengal, India.
Email: kousikatta1995@gmail.com

How to cite this article: Atta, K., Pal, A., Karmakar, S., Dutta, D., Jana, K. and Pal, A.K. (2022). Assessment of Physiological and Biochemical Traits of Ricebean [*Vigna umbellata* (Thunb.) Ohwi and Ohashi] Seedling in Response to Equimolar Concentration of Copper and Lead Stress. Legume Research. DOI: 10.18805/LR-4820.

Submitted: 25-10-2021 **Accepted:** 12-05-2022 **Online:** 28-06-2022

MATERIALS AND METHODS

Plant material

The experiment was conducted on ricebean [*Vigna umbellata* (Thunb) Ohwi and Ohashi] cv. Bidhan 1. Seeds were collected from AICRP on Forage Crops, Kalyani Centre.

Experimental site

The experiment was done in the laboratory of Department of Plant Physiology, Bidhan Chandra Krishi Viswavidyalaya (BCKV), Mohanpur, Nadia, West Bengal in the year 2017-18 and 2018-19.

Plant culture

The experiment was conducted in sand culture using modified Hoagland solution (Epstein, 1972) maintained at pH 6.5 under diffused light, at around 80±1% relative humidity (R.H.) and at a temperature of 28±1°C. The pre-germinated seeds of ricebean cultivar Bidhan 1 were placed in plastic beakers of capacity one litre containing neutral sand supplemented with the nutrient solution. Fresh solution was added at three days' interval for the experimental period. In each beaker five seedlings were maintained in each case.

Treatment application

Fourteen days old seedlings were subjected to lead (Pb) and copper (Cu) stress supplemented in the form of $CuSO_4 \cdot 5H_2O$ and $Pb(NO_3)_2$ respectively, with the concentrations of 50, 100 and 200 µM in each case. A control set containing only the nutrient solution was used for comparison of data. Observations on different growth and biochemical parameters were recorded after nine days of treatment (DAT).

Biochemical estimation

The chlorophyll content in the leaf was estimated as per Arnon (1949) using 80% acetone for extraction.

The proline content in leaf was estimated using the method of Mohanty and Sridhar (1982). The absorbance was recorded at 520 nm.

RLWC was estimated in leaves as per Perez *et al.* (2002) and was expressed as:

$$\text{RLWC (\%)} = \frac{\text{FW} - \text{DW}}{\text{FW} - \text{DW}} \times 100$$

The lipid peroxidation was determined following the protocol of Heath and Packer (1968). The TBARS content was calculated using its extinction coefficient of 155 mM^{-1} cm^{-1} at a wavelength of 532 nm.

The activity of ascorbate peroxidase (APX) enzyme in the leaves was calculated as per Nakano and Asada (1981). The activity was finally calculated using the extinction coefficient (2.8 mM^{-1} cm^{-1} at 290 nm) for ascorbate.

The determination of the activity of guaiacol peroxidase (GPOX) activity was calculated as per Siegel and Galston (1967) and was expressed as the increase in absorbance at 470 nm.

The activity of superoxide dismutase (SOD) was estimated as per the method of Giannopolitis and Ries (1977). One unit of SOD activity caused 50% inhibition of the initial rate of reaction in absence of enzyme.

For the determination of the activity of catalase (CAT), the method described by Cakmak *et al.* (1993) was followed and the decrease in absorbance at 240 nm for 1 minute was recorded using the extinction coefficient (40 mM^{-1} cm^{-1}) for H_2O_2.

Statistical analysis

The mean data was analysed following completely randomized block design using the software SPSS, version 24.0. The comparison of mean values was performed using DMRT.

RESULTS AND DISCUSSION

The analysis of variance showed significant differences among the treatments for all the characters under study (Table 1). The total dry weight of the seedling decreased under both the metal treatments, except for Cu 50µM (Table 2) where the seedling dry weight remained at par with the unstressed control. However, the extent of decrease was more as the molar concentration of the metals increased in the growing medium. Such decrease can be attributed to the decrease in number of cells and reduction in the elongation rate of developing root and shoot. However, lead, in general, was found to be more detrimental for seedling growth in comparison to copper as it was also reported earlier by Ilic *et al.* (2015) and Monalisa *et al.* (2017). In the present study, the highest intensity (200 µM) of copper and lead stress led to a reduction of seedling dry weight by 32.50% and 52.50%, respectively over that of unstressed control.

Heavy metals at higher concentration decrease the leaf chlorophyll content (Thapar *et al.* 2008). Copper-induced chlorosis may result from the inhibition of biosynthesis and damage in chlorophyll structure (Kupper *et al.* 2003). In general, the total chlorophyll content of leaf in the present experiment decreased (Table 2) under both the metal treatments, except for copper at 50 µM where, it recorded 5.60% increase over control. Copper, being an essential element for plant, might attribute for higher rate of pigment biosynthesis at lower concentration. In contrast, lead caused reduction in leaf chlorophyll with the effect being more drastic as compared to corresponding concentrations of copper. This decrease in pigment content under Pb stress, might lead to subsequent reduction in photosynthetic capacity (Zhou *et al.* 2018). The highest intensity of copper and lead

Table 1: Analysis of variance (mean squares) for different parameters in ricebean cv. Bidhan 1 in heavy metal treatments.

Sources of variation	df	Total dry weight	Chlorophyll	Proline	RLWC	Lipid peroxidation	APX	GPOX	SOD	Catalase
Treatments	6	0.00018**	0.3051*	36054.73***	401.40***	2160.05***	0.5702***	11927.08***	47.128***	0.090***
Error	14	0.00003	0.0844	128.889	18.990	54.090	0.0057	52.750	5.250	0.005

* = $p \leq 0.05$; ** = $p \leq 0.01$ *** = $p \leq 0.001$.

stress led to a reduction in total chlorophyll content by 22.93% and 24.17%, respectively, over that of unstressed control.

Proline, under abiotic stress acts as a compatible solute, osmo-protectant, free radical scavenger, as well as antioxidant (Zhang *et al.* 2008; Hayat *et al.* 2012). However, different abiotic stresses may have varying effects on leaf proline depending upon the intensity of stress. In the present experiment, the leaf proline content decreased under moderate to high concentrations of both the metals with the effects being more as the molar concentration increased (Table 2). On the contrary, mild metal stress (50 µM) induced increase in leaf proline over that of control indicating an attempt of the plant to combat dehydration shock. However, lead, at its highest concentration, was found to be more detrimental for proline content in comparison with copper for this variety of ricebean, a finding corroborating the early observation of (Ilic *et al.* 2015). The leaves recorded 16.51% and 27.36% decrease in proline content at an equimolar concentration (200 µM) of copper and lead stress, respectively, over that of control.

The relative leaf water content (RLWC) is one of the reliable parameters to know the water status in plants. The RLWC in the present experiment also decreased under both the metal treatments with the effects being more as the molar concentration increased (Table 2). The observed decrease in RLWC under metal stress might be a consequence of impairment of root growth resulting from mitotic disturbance. The RLWC registered more adverse effects of copper stress in comparison with lead stress at the highest concentration, although a reverse trend was noted at the lower levels. Excessive concentration of copper generally causes low biomass accumulation, chlorosis, inhibition of growth and photosynthesis, altered water balance and nutrient assimilation and senescence, which ultimately lead to death (Ozdener and Kutbay 2009; Ling and Jun 2010).

The extent of leaf membrane damage was measured by determining the level of lipid peroxidation which in turn, was estimated as the content of thiobarbituric acid reactive substances (TBARS). The increased values (Table 2) of TBARS under copper and lead toxicity indicated enhanced generation of free radicals under metal toxicity in the present experiment. It might be further noted that the lead stress was found to be more damaging for leaf membrane than copper stress at all concentrations. The increased level of TBARS indicated the enhanced production and accumulation of reactive oxygen species (ROS) due to Pb toxicity (Thakur *et al.* 2017; Vasavi *et al.* 2012). The variety

Table 2: Effect of heavy metal stress on seedling growth and biochemical parameters in ricebean cv. Bidhan 1.

Treatments	Total dry weight (gm)	Chlorophyll[I]	Proline[II]	RLWC(%)	Lipid peroxidation[III]
Control	0.040[a]	2.77[ab]	789.13[c]	87.62[a]	94.38[d]
Cu 50 µM	0.040[a] (±0.00)	2.92[a] (+5.60)	821.06[b] (+4.05)	81.05[ab] (-7.50)	102.46[d] (+8.56)
Cu 100 µM	0.033[ab] (-17.50)	2.42[abc] (-12.39)	658.84[e] (-18.78)	73.52[bc] (-16.09)	122.64[c] (+29.95)
Cu 200 µM	0.027[bc] (-32.50)	2.13[c] (-22.93)	640.96[e] (-16.51)	52.43[e] (-40.17)	144.85[b] (+53.48)
Pb 50 µM	0.030[ab] (-25.00)	2.61[abc] (-5.46)	875.99[a] (+11.01)	75.31[b] (-14.05)	118.35[c] (+25.40)
Pb 100 µM	0.029[b] (-27.50)	2.26[bc] (-18.25)	684.39[d] (-13.27)	66.35[cd] (-24.27)	151.92[b] (+60.96)
Pb 200 µM	0.019[c] (-52.50)	2.10[c] (-24.17)	573.26[f] (-27.36)	64.94[d] (-25.88)	167.56[a] (+77.54)

[I]Data expressed as mg gm^{-1} fresh weight.

[II]Data expressed as µM g^{-1} fresh weight.

[III]Data expressed as µM of TBARS content g^{-1} fresh weight.

Values with different letters are significantly different at 5% level of significance.

*Data in parentheses indicate percentage increase (+) or decrease (-) over control.

Table 3: Effect of heavy metal stress on anti-oxidative enzyme activities in the leaves of ricebean cv. Bidhan 1.

Treatments	APX[IV]	GPOX[V]	SOD[VI]	CAT[VII]
Control	0.49[cd]	169.20[e]	6.08[cd]	1.55[bc]
Cu 50 µM	1.36[a] (+177.55)	340.20[a] (+101.06)	16.33[a] (+168.59)	1.80[a] (+16.13)
Cu 100 µM	1.06[b] (+116.33)	208.20[d] (+23.05)	12.82[ab] (+110.86)	1.90[a] (+22.58)
Cu 200 µM	0.50[c] (+2.04)	200.60[d] (+18.56)	10.29[bc] (+69.24)	1.49[cd] (-3.87)
Pb 50 µM	0.99[b] (+102.04)	284.20[b] (+67.97)	10.13[bc] (+66.61)	1.65[b] (+6.45)
Pb 100 µM	0.36[d] (-26.53)	239.40[c] (+41.49)	8.76[bcd] (+44.08)	1.60[bc] (+3.23)
Pb 200 µM	0.16[e] (-67.35)	169.60[e] (+0.24)	4.58d (-24.67)	1.40[d] (-9.68)

[IV]Data expressed as Unit min^{-1} g^{-1} fresh weight.

[V]Data expressed as ΔA470 min^{-1} g^{-1} fresh weight.

[VI]Data expressed as Unit min^{-1} g^{-1} fresh weight.

[VII]Data expressed as Unit min^{-1} g^{-1} fresh weight.

Values with different letters significantly different at 5% level of significance.

*Data in parentheses indicate percentage increase (+) or decrease (-) over control.

Bidhan-1 recorded a 53.48% and 77.54% increase in lipid peroxidation over control at the highest concentration of copper and lead respectively.

For protection against ROS, plant cells contain both enzymatic and non-enzymatic components. Out of the enzymatic components, superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and guaiacol peroxidase (GPOX) are found to be very important (Atta *et al.,* 2021b). The leaf APX activity showed the same pattern of change in copper and lead stress, a remarkable enhancement over control under mild stress followed by a decreasing trend as the intensity of stress increased (Table 3). The higher doses of lead caused decrease in the activity of this enzyme over that of control. High amount of copper Cu caused oxidative damage and induced anti-oxidative defence by enhancing activities of ascorbate peroxidase (Ali *et al.,* 2006).) However, lead, in general, was found to be more detrimental for APX activity in comparison with copper for this variety of ricebean as was also observed in other crops (Singh *et al.,* 2011, Ilic *et al.,* 2015).

Like APX, the GPOX activity also revealed the same trend (Table 3) under both the metal treatments where the mild and medium stress-induced much higher activity which then decreased somewhat as the stress increased. Earlier Wang *et al.* (2004) reported alleviation of oxidative damage under copper stress by enzymatic system involving GPOX, SOD, CAT and APX. Whereas lower induction of GPOX under lead stress as compared to copper was noted earlier by Singh *et al.* (2011) and Ilic *et al. (*2015). In the present experiment, the variety Bidhan 1 recorded 18.56% increase in the content of leaf GPOX activity under copper toxicity at 200 ìM concentration and there was almost no change under lead stress as compared to that of control.

The leaf SOD activity increased significantly under both the metal treatments at all concentrations except for Pb 200 µM which registered 24.67% decrease over control (Table 3). From the present study, it was found that SOD recorded much higher activity under all concentrations of Cu than Pb. This was expected for copper as this metal, by itself, is a cofactor of SOD. In case of Pb stress a significant increase in the SOD activity than control was seen for low and medium concentration but at the highest concentration of Pb it decreased. The observed increase in SOD activity under lead stress might be a consequence of upregulation via an increase in levels of O_2- (Chongpraditnum *et al.* 1992).

The leaf CAT activity registered a sigmoidal pattern of change under varying concentrations of Cu, an increase over control under 50 µM, reaching the peak at 100 mM followed by a decline (3.87%) at the highest concentration of Cu (Table 3). In contrast, the enzyme showed a decreasing trend concomitant with a rise in the concentration of lead with the highest concentration registering 9.68% decrease in enzyme activity. However, such decrease in the activity of antioxidative enzymes along with the rise in heavy metal concentration might be caused by either the direct action of ROS on the enzymatic proteins or on the inhibition of protein synthesis (Mazhoudi *et al.* 1997).

Conclusion

In the present experiment, the seedlings of ricebean cv. Bidhan 1 mostly showed reduction in biomass, photosynthetic pigment as well as relative water and proline content in leaf, whereas the leaf membrane injury increased under different concentrations of lead and copper. Under such stresses the seedlings were found to induce their own antioxidant defence system to reduce the harmful effects of oxidative stress. From the present study, it can be concluded that the lead stress was found to register more drastic effects on different physiological and biochemical parameters of the seedling as compared to copper.

Acknowledgement

The authors acknowledge the assistance extended by AICRP on Forage Crops, Kalyani Centre, for supplying plant materials. The authors are thankful to the Department of Plant Physiology, Faculty of Agriculture, BCKV, Mohanpur, Nadia, West Bengal, for extending the experimental facilities.

Conflict of interest: None.

References

Ali, M.M., Singh, N., Shohael, A.M., Hahn, E.J. and Paek, K.Y. (2006). Phenolics metabolism and lignin synthesis in root suspension cultures of Panax ginseng in response to copper stress. Chemosphere. 171: 147-154.

Arnon, D.I. (1949) Copper enzyme in isolated chloroplast polyphenol oxidase in *Beta vulgaris*. Plant Physiol. 24: 1-15. DOI: https://doi.org/10.1104/pp.24.1.1.

Atta, K., Chettri, P. and Pal, A.K. (2020). Physiological and Biochemical Changes under Salinity and Drought Stress in Ricebean [*Vigna umbellata* (Thunb.) Ohwi and Ohashi] Seedlings. International Journal of Environment and Climate Change. 10(8): 58-64. https://doi.org/10.9734/ijecc/2020/v10i830218.

Atta, K., Pal, A.K. and Jana, K. (2021). Effects of salinity, drought and heavy metal stress during seed germination stage in ricebean [*Vigna umbellata* (Thunb.) Ohwi and Ohashi]. Plant Physiol. Rep. 26: 109-115. https://doi.org/10.1007/s40502-020-00542-4.

Atta, K., Sen, J., Chettri, P. and Pal, A.K. (2021b). Antioxidant responses of ricebean [*Vigna umbellata* (Thunb.) Ohwi and Ohashi] seedling under iso-osmotic potential of salinity and drought stress. Legume Research. DOI: 10.18805/LR-4551.

Cakmak, I., Strbac, D. and Marschner, H. (1993). Activities of hydrogen peroxide scavenging enzymes in germinated wheat seeds. J. Exp. Bot. 44:127-132. https://doi.org/10.1093/jxb/44.1.127.

Chhetri, D.R., Modak, S. and Ahmed, S. (2004). Physiological and biochemical responses of two ricebean (*Vigna umbellata* T.) cultivers to heavy metal stress. Environment and Ecology. 22(1): 27-33.

Chongpraditnum, P., Mori, S. and Chino, M. (1992). Excess copper induces a cytosolic Cu, Zn-superoxide dismutase in soybean root. Plant Cell Physiol. 33: 239-244. https://doi.org/10.1093/oxfordjournals.pcp.a078246.

Dalcorso, G., Farinati, S. and Furini, A. (2010). Regulatory networks of cadmium stress in plants. Plant Signalling and Behavior. 5: 1-5. https://dx.doi.org/10.4161/psb.5. 6.11425.

Epstein, E. (1972). Mineral nutrition of plants: Principles and Perspectives. John Wiley and Sons, New York.

Gang, A., Vyar, A. and Vgas, H. (2013). Toxic effect of heavy metals on germination and seedling growth of wheat. Journal of Environmental Research and Development. 8: 206-213.

Giannopolitis, C.N. and Ries, S.K. (1977). Superoxide dismutase. I. occurrence in higher plants. Pl. Physiol. 59: 309-14.

Hayat, S., Hayat, Q., Alyemeni, M.N., Wani, A.S., Pichtel, J. and Ahmad, A. (2012). Role of proline under changing environments: A review. Plant Signal Behaviour. 7: 1456-1466. https://dx.doi.org/10.4161%2Fpsb.21949.

Heath, R.L. and Packer, L. (1968). Photoperoxidaton in isolated chloroplast. 1. Kinetics and stoichiometry of fatty acid peroxidation. Archives of Biochemistry and Biophysics. 12: 189-198. https://doi.org/10.1016/0003-9861(68)90 654-1.

Ilic, S.Z., Mirecki, N., Trajković, R., Kapoulas, N., Milenković, L. and Šunić, L. (2015). Effect of Pb on germination of different seed and his translocation in bean seed tissues during sprouting. Fresenius Environmental Bulletin. 24: 670-675.

Kupper, H., Setlik, I., Setlikova, E., Ferimazova, N., Spiller, M. and Kupper, F.C. (2003). Copper induced inhibition of photosynthesis: Limiting steps of *in vivo* copper chlorophyll formation in *Scenedesmus quadricauda*. Functional Plant Biology. 30: 1187-1196. https://doi.org/10.1071/fp03129.

Lewis, S., Donkin, M.E. and Depledge, M.H. (2001). Hsp 70 expression in *Enteromorpha intestinalis* (Chlorophyta) exposed to environmental stressors. Aqua Toxicol. 51: 277-91.

Ling, T. and Jun, R. (2010). Effect of Hg on seed germination, coleoptile growth and root elongation in seven pulses. Fresenius Environmental Bulletin. 19: 1144-1150.

Mazhoudi, S.A., Chaoui, M.H., Ghorbal and Ferjani, E.E.I. (1997). Response of antioxidant enzymes to excess copper in tomato (*Lycopersicon esculentum* Mill). Plant Science. 127: 129-137. https://doi.org/10.1016/S0168-9452(97)00 116-7.

Mohanty, S.K. and Sridhar, R. (1982). Physiology of rice tungro virus disease: Proline accumulations due to infection. Physiologia Plantarum. 56: 89-93. https://doi.org/10.1111/j.1399-3054.1982.tb04904.x.

Monalisa, Mohil, P., Saini, R. and Sapna (2017). Effects of heavy metals on seed germination and seedling growth of *Phaseolous aconitifolius* jacq. Cv. Rmo 40. Indian Journal of Plant Sciences. 6: 50-62.

Nakano, Y. and Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. Plant Cell Physiol. 22: 867-880.

Ozdener, Y. and Kutbay, H.G. (2009). Toxicity of copper, cadium, nickel, lead and zinc on seed germination and seedling growth in *Eruca sativa*. Fresenius Environmental Bulletin. 18: 26-31.

Pal, A.K., Rai, D. and De, D.K. (2009). Evaluation of ricebean genotypes for salt tolerance during early seedling growth and physiological basis of tolerance. Forage Research. 35: 73-79.

Perez, N.C.M., Espinosa, R.G., Castaneda, C.L., Gallegos, J.A.A. and Simpson, J. (2002). Water relation, histopathology and growth of common bean (*Phaseolus vulgaris* L.) during pathogenesis of *Macrophomina Phoseolina* under drought stress. Physiological and Molecular Plant Pathology. 60: 185-195.

Pireh, P., Yadavi, A., Balouchi, H. (2016). Effect of cadmium chloride on soybean in presence of arbuscular mycorrhiza and vermicompost. Legume Research. 40: 63-68.

Rai, D. (2013). Changes in growth, enzyme activities and osmolytes of ricebean [*Vigna umbellata* (*Thunb.)* Ohwi and Ohashi] seedlings during abiotic stress and recovery. Ph.D. Thesis, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia.

Saleem, S., Yasin, G., Haq, I.U., Altaf, A., Hussain, K. and Nawaz, K. (2021). Indole Acetic Acid (IAA) Mediated Amelioration of Lead (Pb) Stress-Physiological Indices of Mung Bean [*Vigna radiata* (L.) Wilczek]. Legume Research. 44(10): 1152-1158. DOI: 10.18805/LR-630.

Singh, H.P., Kaur, G., Batish, D.R. and Kohli, R.K. (2011). Lead (Pb) inhibited radicle emergence in *Brassica campestris* involves alterations in starch-metabolizing enzymes. Biological Trace Element Research. 144:1295-1301. https://doi.org/10.1007/s12011-011-9129-3.

Siegel, B.Z. and Galston, A.W. (1967). The isoperoxidases of *Pisum sativum*. Physiol. Plant. 42(2): 221-226. https://dx.doi.org/10.1104%2Fpp.42.2.221.

Stiborova, M., Pitrichova, M. and Brezinova, A. (1987). Effect of heavy metal ions in growth and biochemical characteristic of photosynthesis of barley and maize seedlings. Biol. Plant. 29: 453-467.

Subrahmanyam, D. (1998). Effect of aluminium on growth, lipid peroxidation, superoxide dismutase and peroxidase activities in rice and French bean seedlings. Indian Journal of Plant Physiology. 3: 240-242.

Thakur, S., Singh, L., Zularisam, A.W. *et al.* (2017). Lead induced oxidative stress and alteration in the activities of antioxidative enzymes in rice shoots. Biol. Plant. 61: 595-598. https://doi.org/10.1007/s10535-016-0680-9.

Thapar, R., Srivastava, A.K., Bhargava, P., Mishra, Y. and Rai, L.C. (2008). Impact of different abiotic stress on growth, photosynthetic electron transport chain, nutrient uptake and enzyme activities of Cu-acclimated *Anabaena doliolum*. Journal of Plant Physiology. 165: 306-316. https://doi.org/10.1016/j.jplph.2007.05.002.

Vasavi, A., Sudha Madhavi, K. and Usha, R. (2012). Effect of lead toxicity on the growth and antioxidant enzymes in *Helianthus annuus*. L seedlings. Journal of Pharmacy Research. 5: 2395-2401.

Wang, H., Shan, X.Q., Wen, B., Zhang, S. and Wang, Z.J. (2004). Responses of antioxidative enzymes to accumulation of copper in a copper hyper accumulator of *Commoelina communis*. Arch. Environ. Contamination Toxicology. 47: 185-192.

Zhang, L.P., Mehta, S.K., Liu, Z.P. and Yang, Z.M. (2008). Copper-induced proline synthesis is associated with nitric oxide generation in *Chlamydomonas reinhardtii*. Plant and Cell Physiology. 49: 411-419. https://doi.org/10.1093/pcp/pcn017.

Zhou, J., Zhang, Z., Zhang, Y., Wei, Y. and Jiang, Z. (2018). Effects of lead stress on the growth, physiology and cellular structure of privet seedlings. PLOS ONE. 13(3): e0191139. https://doi.org/10.1371/journal.pone.019113.