Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.6274

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **6 (3):** 146-153 (2018)

Research Article

International Journal of Pure & Applied Bioscience

OPEN ACCESS

Effects of Different Heavy Metals and Mycorrhizal Treatments on Various Physiological Processes in Cotton Genotypes

Manohar Lal*, Ajeev Kumar, Manish Jangra, Mahesh Kumar and Sunita Sheokand

Department of Botany and Plant Physiology, CCS HAU, Hisar (India) - 125004

*Corresponding Author E-mail: manohar.hau@gmail.com

Received: 21.02.2018 | Revised: 28.03.2018 | Accepted: 6.04.2018

ABSTRACT

Soil pollution by heavy metals is the most important problem nowadays because toxic contaminants like heavy metals are released in the natural ecosystem due to anthropogenic activities and affect the entire ecosystem and pose harmful health consequences in all life forms. The present study was carried out to study the effects of Cd and Pb and mycorrhizal inoculation on two cotton genotypes at vegetative and flowering stage. These metals delayed and reduce the germination of cotton genotypes and induced oxidative stress. The oxidative stress caused the peroxidation of membrane lipid and disturb the membrane structure, which resulted in electrolyte leakage from the cells. These metals also reduced the chlorophyll content and chlorophyll fluorescence which affect the photosynthetic efficiency of plants and reduced the growth and yield of plants. The mycorrhizal inoculation improve the plant growth under heavy metals stress by reducing the toxic effects of heavy metals and improving anti-oxidative system of plants. The effect of Cd was more toxic than Pb. The effects of these heavy metals was relatively more in Bt cotton as compared to Desi cotton genotype.

Key words: *Heavy metals, Cadmium, Lead, Cotton, Membrane damage*

INTRODUCTION

Metal pollution has become one of the most severe environmental problems nowadays because of increasing environmental pollution from human activities such as mining, electroplating, energy smelting, sludge dumping, military operations, fuel production, power transmission and intensive agriculture[12]. Heavy metals are noxious and non-biodegradable that persist in the environment for long time. Cd and Pb are the most hazardous heavy metals because these are non essential for plants and causes toxic effects even at low concentrations. When plants take up these metals from the soil, they adversely affect the plant both directly and indirectly and some of the direct toxic effects caused by high metal concentration include inhibition of cytoplasmic enzymes and damage to cell structures due to oxidative stress[2,8]. The plasma membrane of a plant cell is a highly organized system that mediates the exchange of information and materials between the cell interior and the extracellular environment. The oxidative stress leads to peroxidation of lipids in the plasma membrane and loose its permeability.

Cite this article: Lal, M., Kumar, A., Jangra, M., Kumar, M. and Sheokand, S., Effects of Different Heavy Metals and Mycorrhizal Treatments on Various Physiological Processes in Cotton Genotypes, *Int. J. Pure App. Biosci.* **6(3):** 146-153 (2018). doi: http://dx.doi.org/10.18782/2320-7051.6274

MDA and electrolyte leakage increased gradually with increase in concentration of Cd and Pb in roots and leaves of pigeonpea genotypes but Pb plants had less MDA content and lesser membrane damage as compared to Cd treatments[4]. Yang *et al.*[17], reported that the MDA content in the leaves of *R. pseudoacacia* increased linearly with increasing Pb concentration in the soil. The chlorophyll content in the leaves is an important index to evaluate the photosynthesis capability and plant tolerance to environmental stress[16]. 10 μM of Cd treatment for one week caused significant reduction in the chlorophyll content in rice seedlings. The ratio of Fv/Fm and ion leakage is a measurement of the photosynthetic capability and the degree of membrane damage, respectively. Cd treatment decreased Fv/Fm and increased the ion leakage.

Uptake of metal by plants can be influenced by soil microorganisms closely associated with plant roots to form a rhizosphere community. AM fungi are one of the major components of rhizosphere and form symbiotic associations with most plant species[11]. Mycorrhizal application to crops is found to be promising and useful in reducing heavy metal toxicity and enhancing nutrient uptake. Mycorrhizal fungi plays an important role in heavy metal detoxification and the establishment of plants in strongly polluted areas[13]. Cotton is a major fibre crop of global importance and having high commercial value. It is a non-edible industrial plant species with large biomass and grown commercially in the temperate and tropical regions. The present investigation was carried out to observe the effects of Cd and Pb, with and without mycorrhizal treatments on physiological processes of cotton genotypes.

MATERIAL AND METHODS

Two cotton genotypes (*Desi* cotton- HD432 and *Bt* cotton- Bio6588 BGII) were procured from the Cotton Section, Department of Genetics and Plant Breeding, CCS HAU, Hisar (India). The mycorrhizal culture strain '*Glomus hoi*' was obtained from TERI, New Delhi (India). The experiment was carried out in 'Screen House' of the Department of Botany and Plant Physiology CCS HAU, Hisar (India).

The surface sterilized seeds of *Desi* and *Bt* cotton were sown in the polythene lined earthen pots filled with 5 kg soil. Six treatment of heavy metals- Control without mycorrhiza, Control with Mycorrhiza, Cd (10 ppm) without mycorrhiza, Cd (10 ppm) with Mycorrhiza, Pb (100 ppm) without mycorrhiza, Pb (100 ppm) with Mycorrhiza were given in the soil. The normal water and nutrients solution (Hoagland and Arnon, 1950) was supplied to the plants at regular intervals. The percent germination was recorded 15 days after sowing (DAS). At vegetative (35 DAS) and Flowering (65 DAS) stage following observations were recorded in leave of *Desi* and *Bt* cotton plants-

Electrolyte leakage

Membrane injury was calculated as described by Zhang *et al.*[18]. 100 mg of fresh leaf tissue was taken separately in 20 ml test tubes containing 10 ml of de-ionized water. The tubes were placed at 4^0C for 24 hours. The initial electrical conductivity of the medium (EC1) was assessed. The samples were then subjected to heating at 100 ^{0}C in a water bath for 30 minutes to expel all electrolytes. Samples were cooled at 25 ^{0}C and second electrical conductivity (EC2) was measured. The electrolyte leakage was expressed by the following formula:

Electrolyte leakage (%) = (EC1/ EC2) x100

Malondialdehyde (MDA) content

The level of lipid peroxidation in the leaf tissue was measured in terms of malondialdehyde (MDA, a product of lipid peroxidation) content determined by the thiobarbituric acid (TBA) reaction using the method of Heath and Packer[5], with minor modifications.

Chlorophyll content

Chlorophyll content in the fresh leaves (100 mg) of the plants was measured in 10 ml dimethylsulfoxide (DMSO) by using the method of Hiscox and Israelstam[6].

Chlorophyll fluorescence

Chlorophyll fluorescence was recorded using CIP chlorophyll fluorescence Os-30P meter at midday (between 10.00 AM to 12:00 AM). The fully expended leaf was first acclimated to dark for minimum five minutes by fixing clip on it. The dark adapted leaf was then continuously irradiated for one second (1500 µmol $m^{-2}s^{-1}$) provided by an array of three light emitted diodes in the sensor. The Fv/Fm ratio was recorded.

Statistical analysis

All values reported in this experiment are mean of three replicates. Data analysis was done by using online statistical software, O. P. Stat available on http://www.hau.ernet.in.

RESULTS AND DISCUSSION

Percent Germination

In the present investigation the germination of cotton seeds was delayed as well as decreased non-significantly with the treatment of Cd and Pb. In *Desi* cotton 26 to 30% decline and in *Bt* cotton 19 to 22% decline was observed. The mycorrhizal treatment improves the seed germination in both genotypes from 10 to 20% as compared to non-mycorrhizal treatments as shown in table 1. Similar results were obtained by Abraham *et al.*[1],in *Arachis hypogeae* seeds with Cd, Pb and Cu stress. Lower doses of Zn improved seed germination and young seedling growth, while higher doses of Zn inhibited germination and decreased growth of *Dorycnium pentaphyllum*[10]. The seed germination of wheat and bean was significantly reduced with increase in concentration of Cd[3].

Table 1: Effects of heavy metals (Cd & Pb) and mycorrhizal treatments on percent seed germination of cotton genotypes

Genotypes	Genotypes					
	***Desi* Cotton (HD 432)**			***Bt* Cotton (Bio 6588 BGII)**		
	NM	**M**	**MEAN (T)**	**NM**	**M**	**MEAN (T)**
Control	59.97	66.63	**63.30**	91.10	93.30	**92.20**
Cd (10ppm)	42.23	46.67	**44.45**	71.10	73.33	**72.22**
Pb (100ppm)	44.43	48.83	**46.63**	71.10	75.53	**73.32**
MEAN (E)	**48.88**	**54.04**		**77.77**	**80.72**	
MEAN (G)	**51.46**			**79.24**		
CD (P≤5)	G^* = 3.58		G x T= N.S.			
	T^* = 4.38		G x E= N.S.		G x T x E = N.S.	
	E^* = 3.58		T x E= N.S.			

G*= Genotypes, T*= Treatments, E*= Non-Mycorrhiza (NM) and Mycorrhiza (M)

Electrolyte Leakage

The membrane damage increased due to the effect of heavy metals (Table 2 & 3). The increased in electrolyte leakage (EL) was more in *Bt* cotton as compared to the *Desi* cotton with both metals treatments. In *Desi* cotton 85-119% and in *Bt* cotton 105-125% increase in EL was observed with respect to control plants. The mycorrhizal inoculation reduced the toxicity of heavy metals 10-20% in both genotypes. At flowering stage the severity of heavy metals stress was more than vegetative stage. The interaction between genotype x treatment and treatment x environment was significant. Garg and Agarwal[4], also reported that MDA and electrolyte leakage increased gradually with increase in concentration of Cd and Pb in roots and leaves of pigeonpea

genotypes and presence of *G. mosseae* significantly controlled the stress induced membrane damage. Pb induced the loss of membrane permeability coupled with the increasing electrolyte leakage and MDA production observed in *Vallisneria natans*[15].

Malondialdehyde (MDA) content

The MDA is product of oxidation of membrane lipid. The reactive oxygen species produced due to the toxic effects of heavy metals caused the peroxidation of membrane lipid. The MDA level increased from 41 to 66% in *Desi* cotton and 59 to 73% in *Bt* cotton with the effects of both metals (Table 4 & 5). The Cd treatment was relatively more toxic at both vegetative and flowering stage. The severity of stress was more at flowering stage than the vegetative stage. The mycorrhizal treatments reduced the toxic effects of both metals from 7 to 15% at vegetative and 9 to 19% at flowering stage. The lipid peroxidation level was more in *Bt* cotton genotype as compared to *Desi* cotton. The interaction between genotype x treatment and treatment x environment was significant at both vegetative and flowering stage. Similar results were obtained by Vassilev *et al.*[14],in barley, Garg and Agarwal[4],in pigeonpea, Yang et al[16]. in the leaves of *R. pseudoacacia*, where the increases in the MDA content of mycorrhizal plants were significantly lower than those of non-mycorrhizal ones.

Chlorophyll content and chlorophyll fluorescence

The effect of heavy metals and mycorrhizal treatments on chlorophyll content and chlorophyll fluorescence were shown in table 6,7,8 &9. The heavy metals stress adversely affect the photosynthetic machinery of the cells. The chlorophyll content was reduced non-significantly from 6-13% in *Desi* cotton and 8-14% in *Bt* cotton at vegetative stage while 8-15% in *Desi* and 10-16% in *Bt* cotton at flowering stage. The toxic effects of Cd was more as compared to Pb. The mycorrhizal inoculation improved the chlorophyll content in both genotypes at vegetative and flowering stage. The chlorophyll fluorescence (Fv/Fm) was also reduced due to the toxicity of both metals and mycorrhizal treatment reduced the toxicity as shown in Table 8 and 9. The interaction between genotypes x treatment and genotypes x environment were significant at both vegetative and flowering stage.

Table 2: Effect of heavy metals (Cd & Pb) and mycorrhizal treatments on electrolyte leakage (%) in leaves of cotton genotypes at vegetative stage

Genotypes	**Genotypes**					
	***Desi* Cotton (HD 432)**			***Bt* Cotton (Bio 6588 BGII)**		
	NM	**M**	**MEAN (T)**	**NM**	**M**	**MEAN (T)**
Control	18.87	16.55	**17.71**	20.60	18.35	**19.47**
Cd (10 ppm)	41.31	33.19	**37.25**	46.25	41.56	**43.90**
Pb (100 ppm)	38.33	30.61	**34.47**	43.85	37.69	**40.77**
MEAN (E)	**32.84**	**26.78**		**36.90**	**32.53**	
MEAN (G)	**29.81**			**34.72**		
CD (P≤5)	G = 1.05		G x T= 1.82			
	T = 1.29		G x E= N.S.		G x T x E = N.S.	
	E = 1.05		T x E= 1.82			

G*= Genotypes, T*= Treatments, E*= Non-Mycorrhiza (NM) and Mycorrhiza (M)

Table 3: Effect of heavy metals (Cd & Pb) and mycorrhizal treatments on electrolyte leakage (%) in leaves of cotton genotypes at flowering stage

Genotypes	Genotypes					
	***Desi* Cotton (HD 432)**			***Bt* Cotton (Bio 6588 BGII)**		
	NM	**M**	**MEAN (T)**	**NM**	**M**	**MEAN (T)**
Control	22.25	18.20	**20.23**	23.71	21.01	**22.36**
Cd (10 ppm)	50.42	38.61	**44.52**	56.38	48.08	**52.23**
Pb (100 ppm)	44.65	35.67	**40.16**	52.97	44.85	**48.91**
MEAN (E)	**39.11**	**30.83**		**44.35**	**37.98**	
MEAN (G)	**34.97**			**41.17**		
CD (P≤5)	G = 1.18 T = 1.45 E = 1.18		G x T= 2.05 G x E= N.S. T x E= 2.05	G x T x E = N.S.		

G*= Genotypes, T*= Treatments, E*= Non-Mycorrhiza (NM) and Mycorrhiza (M)

Table 4: Effect of heavy metals (Cd & Pb) and mycorrhizal treatments on MDA content (µmol/g FW) in leaves of cotton genotypes at vegetative stage

Genotypes	Genotypes					
	***Desi* Cotton (HD 432)**			***Bt* Cotton (Bio 6588 BGII)**		
	NM	**M**	**MEAN (T)**	**NM**	**M**	**MEAN (T)**
Control	2.31	2.09	**2.20**	2.56	2.37	**2.46**
Cd (10 ppm)	3.84	3.33	**3.59**	4.42	3.89	**4.15**
Pb (100 ppm)	3.47	2.95	**3.21**	4.34	3.76	**4.05**
MEAN (E)	**3.21**	**2.79**		**3.77**	**3.34**	
MEAN (G)	**3.00**			**3.56**		
CD (P≤5)	G = 0.10 T = 0.12 E = 0.10		G x T= 0.17 G x E= N.S. T x E= 0.17	G x T x E = N.S.		

G*= Genotypes, T*= Treatments, E*= Non-Mycorrhiza (NM) and Mycorrhiza (M)

Table 5: Effect of heavy metals (Cd & Pb) and mycorrhizal treatments on MDA content (µmol/g FW) in leaves of cotton genotypes at flowering stage

Genotypes	Genotypes					
	***Desi* Cotton (HD 432)**			***Bt* Cotton (Bio 6588 BGII)**		
	NM	**M**	**MEAN (T)**	**NM**	**M**	**MEAN (T)**
Control	2.50	2.27	**2.39**	2.80	2.54	**2.67**
Cd	4.58	3.86	**4.22**	5.37	4.71	**5.04**
Pb	4.17	3.38	**3.78**	4.93	4.22	**4.58**
MEAN (E)	**3.75**	**3.17**		**4.37**	**3.82**	
MEAN (G)	**3.46**			**4.10**		
CD (P≤5)	G = 0.15 T = 0.18 E = 0.15		G x T= 0.25 G x E= N.S. T x E= 0.25	G x T x E = N.S.		

G*= Genotypes, T*= Treatments, E*= Non-Mycorrhiza (NM) and Mycorrhiza (M)

Yang et al[17]. observed that the leaf chlorophyll content and Fv/Fm of both mycorrhizal and non-mycorrhizal *R. pseudoacacia* plants decreased with increasing Pb stress level. Mycorrhizal plants had much higher chlorophyll content and Fv/Fm than those of non-mycorrhizal plants. John *et al*.[9], found that the prolonged exposure to high concentration of Cd (40 mg/l) reduced chl *a* significantly to about 82% of the control and Pb showed the decline of 77% of the control after 30 days. Similarly, chl *b* decreased from 0.36 to 0.01 and 0.04 mg/g fw after 24 days of exposure of Cd and Pb, respectively.

Table 6: Effect of heavy metals (Cd & Pb) and mycorrhizal treatments on chlorophyll content (mg/g FW) in leaves of cotton genotypes at vegetative stage

Genotypes	**Genotypes**					
	***Desi* Cotton (HD 432)**			***Bt* Cotton (Bio 6588 BGII)**		
	NM	**M**	**MEAN (T)**	**NM**	**M**	**MEAN (T)**
Control	1.38	1.46	**1.42**	1.28	1.33	**1.30**
Cd (10 ppm)	1.20	1.33	**1.27**	1.10	1.18	**1.14**
Pb (100 ppm)	1.23	1.37	**1.30**	1.11	1.22	**1.16**
MEAN (E)	**1.27**	**1.39**		**1.16**	**1.24**	
MEAN (G)	**1.33**			**1.20**		
CD (P≤5)	G = 0.05 T = 0.06 E = 0.05		G x T= N.S G x E= N.S. T x E= N.S.	G x T x E = N.S.		

G*= Genotypes, T*= Treatments, E*= Non-Mycorrhiza (NM) and Mycorrhiza (M)

Table 7: Effect of heavy metals (Cd & Pb) and mycorrhizal treatments on chlorophyll content (mg/g FW) in leaves of cotton genotypes at flowering stage

Genotypes	**Genotypes**					
	***Desi* Cotton (HD 432)**			***Bt* Cotton (Bio 6588 BGII)**		
	NM	**M**	**MEAN (T)**	**NM**	**M**	**MEAN (T)**
Control	1.44	1.54	**1.49**	1.36	1.44	**1.40**
Cd (10 ppm)	1.23	1.37	**1.30**	1.15	1.26	**1.21**
Pb (100 ppm)	1.25	1.41	**1.33**	1.16	1.29	**1.23**
MEAN (E)	**1.31**	**1.44**		**1.23**	**1.33**	
MEAN (G)	**1.37**			**1.28**		
CD (P≤5)	G = 0.06 T = 0.07 E = 0.06		G x T= N.S G x E= N.S. T x E= N.S.	G x T x E = N.S.		

G*= Genotypes, T*= Treatments, E*= Non-Mycorrhiza (NM) and Mycorrhiza (M)

Table 8: Effect of heavy metals (Cd & Pb) and mycorrhizal treatments on chlorophyll fluorescence (Fv/Fm) in leaves of cotton genotypes at vegetative stage

Genotypes	Genotypes					
	***Desi* Cotton (HD 432)**			***Bt* Cotton (Bio 6588 BGII)**		
	NM	**M**	**MEAN (T)**	**NM**	**M**	**MEAN (T)**
Control	0.72	0.75	**0.74**	0.66	0.69	**0.67**
Cd (10 ppm)	0.63	0.66	**0.65**	0.59	0.63	**0.61**
Pb (100 ppm)	0.67	0.70	**0.69**	0.61	0.64	**0.63**
MEAN (E)	**0.67**	**0.71**		**0.62**	**0.65**	
MEAN (G)	**0.69**			**0.64**		
CD (P≤5)	G = 0.01 T = 0.01 E = 0.01		G x T= 0.01 G x E= 0.01 T x E= N.S.		G x T x E = N.S.	

G*= Genotypes, T*= Treatments, E*= Non-Mycorrhiza (NM) and Mycorrhiza (M)

Table 8: Effect of heavy metals (Cd & Pb) and mycorrhizal treatments on chlorophyll fluorescence (Fv/Fm) in leaves of cotton genotypes at flowering stage

Genotypes	Genotypes					
	***Desi* Cotton (HD 432)**			***Bt* Cotton (Bio 6588 BGII)**		
	NM	**M**	**MEAN (T)**	**NM**	**M**	**MEAN (T)**
Control	0.74	0.76	**0.75**	0.69	0.71	**0.70**
Cd (10 ppm)	0.63	0.66	**0.65**	0.61	0.64	**0.62**
Pb (100 ppm)	0.67	0.69	**0.68**	0.63	0.65	**0.64**
MEAN (E)	**0.68**	**0.70**		**0.64**	**0.67**	
MEAN (G)	**0.69**			**0.65**		
CD (P≤5)	G = 0.01 T = 0.02 E = 0.01		G x T= 0.01 G x E= 0.01 T x E= N.S.		G x T x E = N.S.	

G*= Genotypes, T*= Treatments, E*= Non-Mycorrhiza (NM) and Mycorrhiza (M)

CONCLUSION

Heavy metals affected seed germination of both *Bt* and *Desi* cotton. The germination was delayed and reduced. Heavy metals has deleterious effect on membranes as was evident from increased membrane injury and lipid peroxidation level. The chlorophyll content and chlorophyll fluorescence was also adversely affected by the heavy metals which reduced the photosynthetic efficiency of the plants. Cd was more toxic as compared to Pb in term of membrane damage and chlorophyll reduction. Mycorrhizal treatments could alleviate the toxic effects of heavy metals to some extent. Among the two cotton varieties *Desi* cotton was more tolerant to heavy metal stress and can be further used for phytoremediation of contaminated soil.

Acknowledgement

Thanks to Choudhary Charan Singh Haryana Agricultural University, Hisar (India) for financial support. The results presented in this paper are a part of Ph.D. research of Mr. Manohar Lal.

REFERENCES

1. Abraham, K., Sridevi, R., Suresh, B. and Damodharam, T., Effect of heavy metals (Cd, Pb, Cu) on seed germination of

Arachis hypogeae. L. *Asi J Plant Sci Res.* **3(1):** 10-12 (2013).

2. Assche, F. and Clijsters, H., Effects of metals on enzyme activity in plants. *Plant Cell Environ.* **24:** 1-15 (1990).

3. El Rasafi,T., Nouri, M.,Bouda, S. and Haddioui, A., The effect of Cd, Zn and Fe on seed germination and early seedling growth of wheat and Bean. *Ekológia* (Bratislava). **35(3):** 213-223 (2016).

4. Garg, N. and Agarwal, N., Effect of mycorrhizal inoculations on heavy metal uptake and stress alleviation of *Cajanus cajan* (L.) Millsp. Genotypes grown in cadmium and lead contaminated soils. *Plant Growth Regul.* **66:** 9-26 (2012).

5. Heath, R.L. and Packer, L**.,** Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys*. **125:** 189-198 (1968).

6. Hiscox, J.D. and Israelstam, G.F., A method for the extraction of chlorophyll from leaf tissue without maceration. *Canad J Bot*. **57:** 1332-1334 (1979).

7. Hoagland, D.R. and Arnon, D.I., The water culture method for growing plants without soil. *Calif Agril Exptll Station.* **347:** 1-32 (1950).

8. Jadia, C.D. and Fulekar M.H., Phytoremediation of heavy metals: recent techniques. *Afric J Biotechnol.* **8(6):** 921-928 (1999).

9. John, R., Ahmad, P., Gadgil, K., and Sharma, S., Effect of cadmium and lead on growth, biochemical parameters and uptake in *Lemna polyrrhiza* L. *Plant Soil Environ*. **54 (6):** 262-270 (2008).

10. Lefevre, I., Marchal, G., Correal, E., Zanuzzi, A. and Lutts, S., Variation in response to heavy metals during vegetative growth in *Dorycnium pentaphyllum* Scop. *Plant Growth Regul*. **59:** 1-11 (2009).

11. Miransari, M., Contribution of arbuscular mycorrhizal symbiosis to plant growth under different types of soil stress. *Plant Biol (Stuttg).* **12(4):** 563-569 (2010).

12. Nedelkoska, T.V. and Doran, P.M., Hyperaccumulation of cadmium by hairy roots of *Thlaspi caerulescens*. *Biotechnol. Bioeng*., **67:** 607-615 (2000).

13. Upadhyaya, H., Panda, S.K., Bhattacharjee M.K. and Dutta, S., Role of arbuscular mycorrhiza in heavy metal tolerance in plants: prospects for phytoremidiation. *J Phytol*. **2(7):** 16-27 (2010).

14. Vassilev, A., Lidon, F., Scotti, P., Graca, M. and Yordanov, I., Cadmium-induced changes in chloroplast lipids and photo-system activities in barley plants. *Biologia Plantarum.* **48:** 153-156 (2004).

15. Wang, P., Zhang, S., Wang, C. and Lu, J., Effects of Pb on the oxidative stress and antioxidant response in a Pb bioaccumulator plant *Vallisneria natans. Ecotoxicol Environ Saf.* **78:** 28-34 (2012).

16. Yang, L., Ji, J., Harris-Shultz, K.R., Wang, H., Wang, H., Abd-Allah, E.F., Luo, Y. and Hu. X., The dynamic changes of the plasma membrane proteins and the protective roles of nitric oxide in rice subjected to heavy metal cadmium stress. *Front. Plant Sci.* **7:** 190-218 (2016).

17. Yang, Y.R., Song, Y.Y., Scheller, H.V., Ghosh, A., Ban, Y.H., Chen, H. and Tang, M., Community structure of arbuscular mycorrhizal fungi associated with *Robinia pseudoacacia* in uncontaminated and heavy metal contaminated soils. *Soil Biol Biochem.* **86:** 146-158 (2015).

18. Zhang, Y.Y.; Wang, L.L.; Lui, Y.L.; Zhang, Q.; Wei, Q.P. and Zhang W.L., Nitric oxide enhances salt tolerance in maize seedling through increasing activities of proton-pump and Na^+/H^+ antiport in the tonoplast. *Planta.* **224:** 545-555 (2006).