Advanced Techniques in Biology & Medicine

Shrivastava et al., Adv Tech Biol Med 2016, 4:2
DOI: 10.4172/2379-1764.1000178

Research Article Open Access

Co-exposure Effects of Selenium and Mercury on *Phaseolus vulgaris* Excised Leaves Segment by Enhancing the NR, Anti-oxidative Enzyme Activity and Detoxification Mechanisms

Shilpa Shrivastava[1]*, Archana Shrivastav[2] and Jot Sharma[3]

[1]Department of Biotechnology, College of Life Sciences, CHRI Campus, Gwalior, Madhya Pradesh, India
[2]Department of Microbiology, College of Life Sciences, CHRI Campus, Gwalior, Madhya Pradesh, India
[3]Department of Biotechnology, Jiwaji University, Gwalior, Madhya Pradesh, India

Abstract

Mercury is known to disrupt the biological function in plants by inhibiting their growth and developmental process, while selenium (Se) is an essential micronutrient within the appropriate amount. This paper is aimed to study co application and interactive effects of selenium (Se) and mercury (Hg) on the Nitrate Reductase NR (such as in-vivo and endogenous) and the antioxidant system through a pot experiment and clarify the possible mechanism how Se alleviates the toxicity of Hg. The observations indicate that when selenium applied after mercury exposure the enzyme activity enhanced hence Se may reduce the toxic level of Hg in *phaseolus vulgaris*.

Keywords: Selenium; Mercury; Nitrate reductase; Endogenous; *Phaseolus vulgaris*

Introduction

Agricultural useful soils in many parts of India and the world are slightly contaminated by presence of heavy metal toxicity. Few as Cd, Cu, Zn, Ni, Co, Cr, Pb, and As. This because long-term use of phosphatic fertilizers, sewage sludge application, dust from smelters, industrial waste and bad watering practices in agricultural lands [1-3]. Toxics Link in India has been involved both at the global and the national level in working on the issues of mercury (Hg). Hg distribution in the environment has been a focus of scientific attention because of the potential health risks posed by Hg exposure [4].

Selenium (Se) is an essential micronutrient and has important benefits for animal and human nutrition, but in trace amount after that limit it causes toxicity in human and plants [5,6]. The presumed protective effect of Se against cadmium and mercury toxicity is through the diversion in their binding from low molecular weight proteins to higher molecular weight ones [7]. The Se distribution pattern was found to be unaffected by the presence of Hg, but the amount of Se assimilated was found to be higher in plants co-exposed to Hg [8].

However, until now the study of interaction of selenium and mercury in *phaseolus vulgaris* plants have not yet been reported. In this study, pot culture method under lab condition was used to study the interaction between (rajmah) selenium and mercury, which was of practical significance.

Materials and Method

Plant growth and metal treatment

Seeds of *phaseolus vulgaris* were rinsed in running water for 2 min. Seeds were surface sterilized with 0.1% $HgCl_2$ for 30 sec. and then washed with three times autoclaved double distill sterilized water. Sterilized seeds were sown on plastic pot containing 1 kg acid washed sand and 20 seeds per pot. Pots were placed in continuous light 30 wm^{-2} intensity supplied by fluorescent tubes at 26 ± 2°C for 7-8 days. Half strength Hoagland solution without nitrogen was used for watering. Mercury Chloride ($HgCl_2$) and Sodium Selenite (Na_2SeO_3) was used as test chemical. Different concentrations of the compounds were prepared (namely 0, 0.001, 0.01, 0.1, and 1 mM) using half strength Hoagland solution as solvent.

Metal treatment: Treatment with metal at two levels in order to correlate uptake, accumulation and comparison of the same with the following treatment schedules.

a) Seeds were treated with Hg metals for 2 hrs and 4 hrs followed by thorough wash and subsequently planted on acid washed sand contained in plastic pots.

b) Different concentration of metal treated acid washed sand for 24 hrs contained in plastic pots.

Leaves floating: For Hg treated soil (for 24 hrs) and seed treated (two and four hour) in continuous light leaf were floated on 1/4th strength Hoagland solution. The young two cotyledons leaves were co treated with selenium (c (without treatment, 0.001, 0.01, 0.1, 1mM) different concentration to see effect on different enzymes and correlate with detoxification mechanism. Incubation in continuous light inside "Indosan growth chamber" BOD. The leaf is then used in estimation of nitrate reductase *in vivo* and endogenous nitrate pool, peroxidase and catalase assay.

Nitrate reductase assay: The activity of nitrate reductase was estimated by *in vivo* nr by Srivastava [9] method with slight modification. Briefly About 0.25 g of leaf material were incubated with 10 ml of incubation medium consisting of 0.1 M sodium phosphate buffer (pH 7.2), 0.2 M KNO_3, and 25% iso-propanol in dark vial of 20 ml capacity. The whole set was incubated in dark for 30 min at 30°C. Nitrite released in the incubation mixture due to enzyme activity was measured by colour development by the formation of diazo compound with sulfanilamide and nitrate coupled with NED to give a red

***Corresponding author:** Shilpa Shrivastava, Department of Biotechnology, College of Life Sciences, CHRI Campus, Gwalior, Madhya Pradesh, India, Tel: 08989735821; E-mail: shilpa_biotech44@yahoo.com

Received April 15, 2016; **Accepted** April 27, 2016; **Published** May 04, 2016

Citation: Shrivastava S, Shrivastav A, Sharma J (2016) Co-exposure Effects of Selenium and Mercury on *Phaseolus vulgaris* Excised Leaves Segment by Enhancing the NR, Anti-oxidative Enzyme Activity and Detoxification Mechanisms Adv Tech Biol Med 4: 178. doi: 10.4172/2379-1764.1000178

dye. The absorbance was read at 540 nm after 20 min by using UV-spectrophotometer.

Endogenous nitrate pool in the leaf segments were estimated according to Aslam [10]. 20 ml incubation buffer containing 0.1 M phosphate buffer and 25% isopropanol. Incubated for one hour at 30°C. It was mixed with sulfanilamide and NED. After 20-25 min the reading is taken at 540 nm.

Antioxidant enzyme: Peroxidase was estimated according to Maehly [11]. The leaf tissue was extracted in Phosphate buffer (pH 6.8) at 0-4°C. The reaction mixtures contained distill water, 0.1 M buffer, 0.22% Guaiacol, 3% Hydrogen peroxide and enzyme. POD catalyses the transformation of guaiacol to tetraguaiacol (brown product). The oxidation of guaiacol was measured by the increase in absorbance at 436 nm for 1 min.

Activities of catalase (EC 1.11.1.6) was assayed in fresh leaf tissue extracts by using a modification of the method of Zhou, 2001 and Zhang, 1990 as mentioned in Cui and Wang, 2006 [12-14] with slight modification. Briefly, the samples were prepared for catalase analysis by homogenization of fresh tissue with a mortar and pestle in a buffer solution containing 0.2M buffer (pH 7.8). After the homogenate was centrifuged at 10,000 rpm for 20 min at 4°C, the supernatant was used immediately to determined catalase activity by monitoring the disappearance of H_2O_2 by measuring the decrease in absorbance at 240 nm of a reaction mixture containing 0.1M H_2O_2, 0.2M buffer solution (pH 7.8), deionized water and enzyme extract. Catalase activity was expressed as µmol of H_2O_2 decomposed per min per gram of fresh weight (µ mol/min/g FW).

Statistical Analysis

Each experiment was repeated at least thrice and data presented are the average value and standard deviation value of findings. Statistical data collected from one-way ANOVA test software.

Results

Sand treated

***In vivo* NR and endogenous NR:** During *in vivo*, Supply of Hg in sand 1mM shows significant (<0.001) inhibition than followed by 0.01, 0.001 mM as compared to control. Whereas when Se supplied with Hg (co-exposure) it enhanced activity with increase in concentration. As compared to control maximum enhanced activity in 1mM with 194 % time was observed (Table 1).

During endogenous NR, Supply of mercury shows significantly decrease in activity at 0.1 mM followed by 1 and 0.001 mM conc. Whereas in compared to Se treatment with Hg maximum activity was found in 0.001mM concentration with 212 % times then followed by 0.01, 0.1 and 1 mM (Table 1).

Antioxidant enzymes: During peroxidase, a concentration dependent activity of peroxidase was observed from 0.001 to 0.1 mM of Hg. The activity slightly inhibited in 0.01 and 0.1 mM in sand treated with Hg. This enzyme activity enhanced in presence of selenium and 0.1 mM concentration with 221% times followed by 1 mM with 169% maximum activity was observed (Table 1).

During catalase, Supply of Hg in sand treatment shows more inhibition in 0.01 mM than followed by 0.1 in compare to control. Se with mercury shows maximum inhibition in 0.01 mM with 159 % times and minimum in 1 mM concentration as compared to control (Table 1).

Seed treatment

***In vivo* NR and endogenous NR:** In two hour 1 mM concentration shows significant inhibition (<0.001) than followed by 0.01 and 0.1 mM whereas in four hour 0.01 mM concentration shows more significantly enzyme inhibition. When Se supplied with mercury, activity enhanced with increase in concentration (Table 2). 252% times increased activity was found in 0.1 mM then 0.01mM with 227% in two hour seed

s.no conc in mM	Nr *in vivo* Hg (µ mol NO_2/hr/g) fresh weight) Mean ± SD	Nr *in vivo* Hg+Se (µ mol NO_2/hr/g) fresh weight) Mean ± SD	Nr Endo Hg (µ mol NO_2/hr/g) fresh weight) Mean ± SD	Nr Endo Hg+Se (µ mol NO_2/hr/g) fresh weight) Mean ± SD	Peroxidase Hg(mg enz./ ml original sol / protein) fresh weight	Peroxidase Hg+Se(mg enz./ ml original sol / protein) fresh weight	Catalase Hg(µ mol/min/gm fresh weight	Catalase (µ mol/min/gm fresh weight Hg+Se
c	68.56 ± 13.84	40.18 ± 0.21	16.18 ± 1.54	42.87 ± 3.76	2.02 ± 0.18	1.3 ± 0.21	49.68 ± 4.56	33.82 ± 12.41
0.001	12.85 ± 15.66**	58.34 ± 10.07**	10.86 ± 1.73**	91.06 ± 6.48**	1.03 ± 0.18**	1.52 ± 0.18	41.66 ± 18.81	44.46 ± 13.22
0.01	64.50 ± 35.59*	65.68 ± 5.73**	15.59 ± 1.33	90.36 ± 6.38**	1.66 ± 0.07**	1.23 ± 0.25	49.34 ± 4.42	53.87 ± 13.04
0.1	35.26 ± 11.47	76.89 ± 3.68**	22.06 ± 0.63**	80.01 ± 7.63**	1.44 ± 0.15**	2.88 ± 0.15**	47.62 ± 4.65	23.54 ± 8.08
1	11.19 ± 4.38**	77.96 ± 0.62**	10.72 ± 1.20**	37.32 ± 4.97	0.99 ± 0.06**	2.2 ± 0.27**	45.13 ± 4.49	12.03 ± 2.17

*=p<0.05 **=p<0.01

Table 1: When mercury concentration supplied to sand and comparisons of this values to when mercury treated sand, excised leaf was treated with selenium different concentration i.e. co-exposure of selenium with mercury during sand treatment.

s.no conc. in Mm	Nr *in vivo* Hg (µ mol NO_2/hr/g) fresh weight) Mean ± SD	Nr *in vivo* Hg+Se (µ mol NO_2/hr/g) fresh weight) Mean ± SD	Nr Endo Hg (µ mol NO_2/hr/g) fresh weight) Mean ± SD	Nr Endo Hg+Se (µ mol NO_2/hr/g) fresh weight) Mean ± SD	Peroxidase hg(mg enz./ml original sol / protein) fresh weight	Peroxidase hg+Se(mg enz./ ml original sol / protein) fresh weight	Catalase Hg(µ mol/min/gm fresh weight	Catalase (µ mol/min/gm fresh weight Hg+Se
C	60.62 ± 11.39	14.13 ± 0.36**	18.58 ± 0.56	28.37 ± 0.92	0.21 ± 0.01	0.76 ± 0.25	38.88 ± 3.33	24.07 ± 0.51
0.001	74.54 ± 13.05	30.77 ± 0.02**	7.99 ± 0.79**	42.60 ± 0.55**	0.21 ± 0.01	2.34 ± 0.27**	35.27 ± 5.46	18.3 ± 0.4**
0.01	24.55 ± 4.18**	32.15 ± 1.75**	4.73 ± 0.60**	17.61 ± 1.13**	0.21 ± 0.01	1.24 ± 0.02	32.81 ± 6.12	31.29 ± 0.88**
0.1	11.46 ± 14.67**	35.71 ± 0.18**	28.57 ± 0.22**	27.56 ± 1.09	0.21 ± 0.01	1.74 ± 0.25**	31.68 ± 6.8	32.95 ± 0.87**
1	30.73 ± 3.81**	21.38 ± 1.07**	0.84 ± 0.36**	22.62 ± 0.93**	0.09 ± 0.05**	0.81 ± 0.12	16.95 ± 0.52**	27.92 ± 0.48**

*=p<0.05 **=p<0.01

Table 2: When mercury concentration supplied to seed two hour and comparisons of this values to when mercury treated seed (two hr.), excised leaf was treated with selenium different concentration i.e. co-exposure of selenium with mercury.

s.no conc. in Mm	Nr *in vivo* Hg (μ mol NO_2/hr/g) fresh weight) Mean ± SD	Nr *in vivo* Hg+Se (μ mol NO_2/hr/g) fresh weight) Mean ± SD	Nr Endo Hg (μ mol NO_2/hr/g) fresh weight) Mean ± SD	Nr Endo Hg+Se (μ mol NO_2/hr/g) fresh weight) Mean ± SD	Peroxidase hg(mg enz./ml original sol / protein) fresh weight	Peroxidase hg+Se(mg enz./ml original sol / protein) fresh weight	Catalase Hg(μ mol/min/gm fresh weight	Catalase (μ mol/min/gm fresh weight Hg+Se
C	69.66 ± 18.94	36.11 ± 0.07	9.79 ± 2.37	32.19 ± 1.07	1.58 ± 0.35	1.2 ± 0.51	45.91 ± 1.08	17.76 ± 3.18
0.001	47.51 ± 15.30	36.33 ± 0.04	19.62 ± 2.30**	37.68 ± 0.81**	0.48 ± 0.15**	2.56 ± 0.3	47.39 ± 1.19	20.76 ± 2.74
0.01	29.36 ± 3.51**	36.26 ± 0.08	6.85 ± 2.32	27.32 ± 1.52**	1.19 ± 0.3	1.48 ± 0.21	47.76 ± 1.01	26.1 ± 2.15
0.1	63.93 ± 11.69	36.22 ± 0.18	8.14 ± 2.31**	33.82 ± 0.97	0.64 ± 0.25**	2.36 ± 1.31	3.22 ± 0.4**	21.34 ± 3.45
1	No growth							

*=p<0.05 **=p<0.01

Table 3: When mercury concentration supplied to seed (four hours) and comparisons of this values to when mercury treated seed (four hours) excised leaf was treated with selenium different concentration i.e. co-exposer of selenium with mercury during seed (four hours) treatment.

treatment whereas in four hour activity shows constant result in all concentration (Table 3).

Hg inhibited endo nr activity in seed treated two hour at 0.1 mM concentration whereas in four hour 0.001 mM concentration significantly (Tables 2 and 3). When Se supplied with mercury, enhanced activity in 0.001 then 0.1mM concentration was observed during two hour seed treatment in comparison to this four hour seed treatment shows maximum activity in 0.001 mM than 0.1 and 0.01 mM concentration (Tables 2 and 3).

Antioxidant enzymes: Peroxidase activity when Hg treatment supplied to seed with two hour only in 1mM conc shows significantly increase in activity whereas in four hour 71 times more fold inhibition in 0.1mM followed by 0.001mM conc. or least inhibition in 0.01 mM conc. When Hg was co exposure supplied with selenium, peroxidase activity at 0.001 mM and 0.1mM shows more inhibition and 100 times more enhanced activity than control in both two hour and four hour (Tables 2 and 3).

During catalase activity, when only Hg treatment was given during seed treatment with two hour only in 1mM conc shows significantly decrease in activity whereas in four hour 93 times more fold inhibition in 0.01mM followed by 0.001mM conc. or least inhibition in 0.1 mM conc. When co exposure with Se mercury treatment supplied the activity enhanced and shows more detoxification. During two hour 0.1 mM shows more inhibition than 0.01mM, 0.001mM and 1 mM with compare to control. During four hour, 0.01 mM and 0.1mM shows more inhibition (Tables 2 and 3).

Discussion and Conclusion

Obtained from the present study showed reduced enzyme activity under Hg stress which might be associated with inhibited contribution of essential component required for the particular enzyme activity of plant. Similar research also done earlier for identify mercury toxicity on plants or ecosystem. The growth pattern of phaseolus seedlings in the presence of Hg was different from pea and spear mint [15], ryegrass [16,17], tomato [18], *Sesbania grandiflora* [19], *Mentha arvensis* [20].

The activities of anti-oxidative enzymes in the seedlings of *Phaseolus aureus* found that effects of Hg and cadmium (Cd) had little primary damaging effect on membranes [21]. Antioxidant effect of $HgCl_2$ was in selected plant *Clitoria ternatea* L. It increases H_2O_2 content and the antioxidant enzyme activities such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) were observed in $HgCl_2$ treated plants when compared with control [22].

Hg causes phyto toxicity and oxidative stress in wheat (*Triticum aestivum* L.) plants [23]. Plants treated with higher concentration of Hg were subjected to comparatively greater oxidative damage and demonstrated that the antioxidative components were not able to remove the stress due to higher concentration of Hg and thus might affect the productivity in plants. Hg also showed inhibition property towards physiological parameters such as chlorophyll, protein, nitrate, and endogenous pool. Higher concentrations of $HgCl_2$ were found to be more toxic [24].

The selenium content and species of both plant and animal foodstuffs depend on environmental conditions, in particular, the quantity and species of selenium to which the animal/plant is exposed. Selenomethionine is predominant in cereals, and selenium concentrations vary from 0.01 to 0.55 lg/g fresh weight [25], whereas in other plant foods the content is generally lower. Se existence supports the expression of the selenoprotein, which have important antioxidant and detoxification function [26]. These forms of selenium can combine with Hg^{2+} to form insoluble Hg-Se complex in rice root surrounding environment or root surface [27].

Co-exposure of Se with Hg enhanced NR enzyme activity and improved antioxidant mechanisms seedlings which could be attributed to their synergistic effect and the role of Se to regulate the plant growth and detoxifying mercury toxicity.

The interaction between mercury (Hg (II)) with selenium Se (VI) and Se (IV) in Tomato (*Lycopersicum esculentum*) experimented in Sand and soil culture resulted in Decreases Hg uptake [28]. Another study shows that Hg (II) and Se (VI) and Se (IV) in Radish plants (*Raphanus sativus*) performed in Pot culture decreases Hg uptake and possibly forms Hg-Se insoluble complex in soil [29]. Mercury Hg (II) Se (IV) in *Glycine max* (soybean) experimented with Soil and a 50:50 mix of soil and ProMix resulted forms a high molecular weight entity containing Se and Hg in plants [8].

Se may play an important role in limiting the bioaccessibility, absorption, and translocation/bioaccumulation of mercury in the aerial rice plant, which may be related to the formation of an Hg-Se insoluble complex in the rhizospheres and/or roots [30].

Se antagonistic study with other heavy metal also studied such as arsenic, cadmium etc. Arsenic interaction with Se in Hydroponic culture and found that decreases As uptake and lipid peroxidation; enhances levels of antioxidants, chlorophyll, MTs, thiols and GST; increases cellular viability, and rebuilds membranes. Enhances As concentration, Se induces more production of thiols and GSH to counterbalance the negative effects of increased As and inhibits lipid peroxidation in *Pteris vittata L* [31,32]. In Garlic (*Allium sativum*) found decreases Cd uptake and stimulates growth of root and seedlings. Another study in Rape (*Brassica napus L.,* Polish genotype cv. Bojan) and wheat (*Triticum aestivum*, Polish genotype cv. Kamila and a Finnish genotype cv. Manu) results shows Promotes growth, reduces lipid peroxidation, enhances membrane stability, and counterbalances the Cd-induced changes in nutrients [33,34] (Figures 1-3).

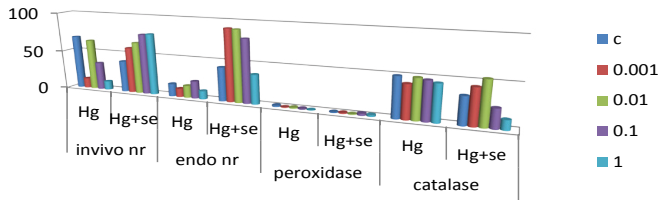

Figure 1: Showing co- exposure of selenium with mercury during sand treatment. On x axis concentration of metal treatment in mM and on y axis inhibition of different enzymes.

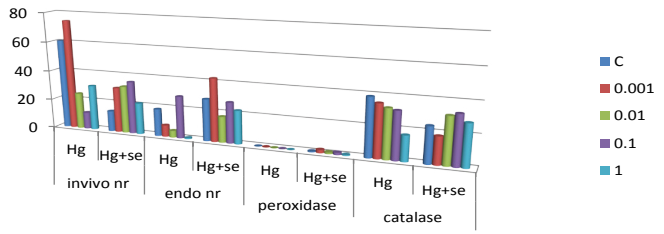

Figure 2: Showing co-exposure of selenium with mercury during two hour seed treatment. On x axis concentration of metal treatment in mM and on y axis inhibition of different enzymes.

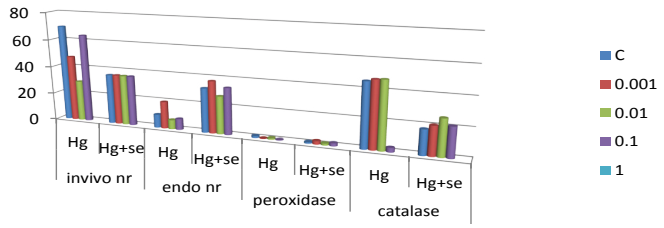

Figure 3: Showing co- exposure of selenium with mercury during four hour seed treatment. On x axis concentration of metal treatment in mM and on y axis inhibition of different enzymes.

The robust data presented in the above work shows that co-exposure of Se is more effective in the alleviation of Hg stress. Improved Hg tolerance in the presence of Se involves physiological, and biochemical interaction in a synergistic or additive way. Therefore, the present study gives a new strategy to minimize the Hg toxicity in crop plants, especially in rajmah *(phaseolus vulgaris)* using beneficial application of Se as protective mechanism against mercury toxicity.

Acknowledgment

Sincerely obliged to Dr. Archana Shrivastava and Dr. Jot Sharma for their encouragement and providing necessary administrative and research facility.

References

1. Bell FG, Bullock SET, Halbich TFJ, Lindsay P (2001) Environmental impacts associated with an abandoned mine in the Witbank Coalfield, South Africa. International Journal of Coal Geology 45: 195-216.
2. Schwartz C, Gerard E, Perronnet K, Morel JL (2001) Measurement of in situ phytoextraction of zinc by spontaneous metallophytes growing on a former smelter site. Science of the Total Environment 279: 215-221.
3. Passariello B, Giuliano V, Quaresima S, Barbaro M, Caroli S, et al. (2002) Evaluation of the environmental contamination at an abandoned mining site. Microchemical Journal 73: 245-250.
4. Sahni SK (2011) Hazardous metals and minerals pollution in India. A Position Paper, August 2011. Indian National Science Academy, New Delhi.
5. Ellis DR, Salt DE (2003) Plants, selenium and human health. Curr Opin Plant Biol 6: 273-279.
6. Sager M (2006) Selenium in agriculture, food, nutrition. Pure and Applied Chemistry 78: 111-133.
7. Whanger PD (1992) Selenium in the treatment of heavy metal poisoning and chemical carcinogenesis. J Trace Elem Electrolytes Health Dis 6: 209-221.
8. Yathavakilla SKV, Caruso JA (2007) A study of Se-Hg antagonism in Glycine max (soybean) roots by size exclusion and reversed phase HPLC-ICPMS. Analytical and Bioanalytical Chemistry 389: 715-723.
9. Srivastava HS (1974) In vivo activity of nitrate reductase in maize seedlings. Indian J Biochem Biophys 11: 230-232.
10. Aslam M (1981) Reevaluation of anaerobic nitrite production as an index for the measurement of metabolic pool of nitrate. Plant Physiol 68: 305-308.
11. Maehly AC (1954) The assay of catalase and peroxidase. In: Glick D (edr) Methods of biochemical analysis. Interscience publ, Newyork.
12. Zhou Q (2001) The measurement of malondialdehyde in plants. In: Zhou Q (edr) Methods in Plant Physiology. China Agricultural Press, Beijing.
13. Zhang ZL (1990) The measurement of chlorophyll in plants. In: Zhang ZL (edr) Methods in Plant Physiology. China Higher Education Press, Beijing.
14. Cui Y, Wang Q (2006) Physiological responses of maize to elemental sulphur and cadmium stress. Plant soil environ 11: 523-529.
15. Beauford W, Barber J, Barringer AR (1977) Uptake and distribution of Hg within higher plants. Physiol Plant 39: 261-265.
16. Al-Attar AF, Martin MH, Nickless G (1988) Uptake and toxicity of cadmium, mercury and thalium. Chemosphere 17: 1219-1225.
17. Godbold DL, Huettermann A (1988) Effect of zinc, cadmium and Hg on root elongation Picea (Karst) seedlings and the significance of these metals to forest die-back. Environ Pollut 53: 375-381.
18. Cho U, Park J (2000) Mercury-induced oxidative stress in tomato seedlings. Plant Sci 156: 1-9.
19. Malar S, Sahi SV, Favas PJC, Venkatachalam P (2014) Assessment of mercury heavy metal toxicity-induced physiochemical and molecular changes in *Sesbania grandiflora* L. Int J Environ Sci Technol 12: 3273-3282.
20. Manikandan R, Sahi SV, Venkatachalam P (2015) Impact Assessment of Mercury Accumulation and Biochemical and Molecular Response of *Mentha arvensis*: A Potential Hyperaccumulator Plant. Hindawi Publishing Corporation? Scientific World Journal 2015: 1-10.
21. Shaw BP (1995) Effects of mercury and cadmium on the activities of antioxidative enzymes in the seedlings of *Phaseolus aureus.* Biologia Plantarum 37: 587-596.
22. Priya M, Balakrishnan V, Kiruthika Lakshmi A, Aruna R, Ravindran KC (2014) Mercury induced oxidative stress of antioxidants in *Clitoria ternatea.* L International Letters of Natural Sciences 23: 1-8.
23. Gopal KS, Seema U, Bibhuti BS (2012) Mercury induced phytotoxicity and oxidative stress in wheat (*Triticum aestivum* L.) plants. Physiol Mol Biol Plants 18: 21-31.
24. Sharma J, Shrivastava S (2014) Physiological and morphological responses of Phaseolus vulgaris caused by mercury stress under lab conditions. Recent Advances in Biology and Medicine 1: 7-12.
25. Fairweather-Tait SJ, Collings R, Hurst R (2010) Selenium bioavailability: current knowledge and future research requirements. Am J Clin Nutr 91: 1484S-1491S.
26. Sun HW, Ha J, Liang SX, Kang WJ (2010) Protective role of selenium on garlic growth under cadmium stress. Communications in Soil Science and Plant Analysis 41: 1195-1204.
27. Feng R, Wei C, Tu S, Sun X (2009) Interactive effects of selenium and arsenic on their uptake by *Pteris vittata* L. under hydroponic conditions. Environ Exp Bot 65: 363-368.
28. Shanker K, Mishra S, Srivastava S, Srivastava R, Daas S, et al. (1996) Effect of selenite and selenate on plant uptake and translocation of mercury by tomato (*Lycopersicum esculentum*). Plant and Soil 183: 233-238.
29. Shanker K, Mishra S, Srivastava S, Srivastava R, Dass S, et al. (1996) Study of mercury-selenium (Hg-Se) interactions and their impact on Hg uptake by the radish (*Raphanus sativus*) plant. Food Chem Toxicol 34: 883-886.
30. Zhang H, Feng X, Zhu J, Sapkota A, Meng B, et al. (2012) Selenium in soil inhibits mercury uptake and translocation in rice (*Oryza sativa* L.). Environ Sci Technol 46: 10040-10046.

31. Malik JA, Goel S, Kaur N, Sharma S, Singh I, et al. (2012) Selenium antagonises the toxic effects of arsenic on mungbean (*Phaseolus aureus* Roxb.) plants by restricting its uptake and enhancing the antioxidative and detoxification mechanisms. Environmental and Experimental Botany 77: 242-248.

32. Srivastava M, Ma LQ, Rathinasabapathi B, Srivastava P (2009) Effects of selenium on arsenic uptake in arsenic hyperaccumulator *Pteris vittata* L. Bioresour Technol 100: 1115-1121.

33. Zembala M, Filek M, Walas S, Mrowiec H, Korna´s A, et al. (2010) Effect of selenium on macro and microelement distribution and physiological parameters of rape and wheat seedlings exposed to cadmium stress. Plant and Soil 329: 457-468.

34. Xinbin Z, Wenhua W, Shuhui Y, Yongxiang Z (2013) Interactive Effects of Selenium and Mercury on Their Uptake by Rice Seedlings. Research Journal of Applied Sciences, Engineering and Technology 5: 4733-4739.