International Journal of Theoretical & Applied Sciences, 9(2): 45-50(2017)

ISSN No. (Print): 0975-1718
ISSN No. (Online): 2249-3247

Assessment of Mercury Toxicity on Seed Germination, Growth and Antioxidant Enzyme Expression of *Sorghum vulgare* var. SG- 1000 Seedlings

Deepti Mukhraiya and J.L. Bhat
Department of Life Sciences,
School of Sciences, ITM University, Gwalior, (Madhya Pradesh), India

(Corresponding author: Deepti Mukhraiya, dptmkhr800@gmail.com, jawaharbhat@gmail.com)
(Received 22 July, 2017 accepted 20 August, 2017)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: The present study was undertaken to assess the effect of mercury on seed germination, growth and expression of antioxidant enzyme defense system in *Sorghum vulgare* var. SG-1000 seedlings. Seed germination and seedling growth of *Sorghum vulgare* was reduced by exposure of mercury chloride of different concentrations, however, no effect was observed by 5 mgL^{-1} mercury treatment. Root length, shoot length, fresh weight and dry weight of seedlings were reduced. The water retention capacity of seedlings was reduced. EDTA partially offsets the adverse effects on germination and growth of seedlings due to mercury treatment. Protein content of seedlings was reduced by mercury exposure. SOD activity was also reduced by mercury exposure, however SOD activity increased by 5 mgL^{-1} mercury treatment. Proline content of seedlings increased with increase in mercury concentration. The proline content further increased when the seedlings were exposed to mercury and EDTA simultaneously.

Key words: Mercury, seedling, proline, water retention capacity, growth.

I. INTRODUCTION

Heavy metal pollution is serious environmental problem all over world affecting adversely soil fertility, limiting crop yield and lowering crop quality [1,2,3] and is health hazard to animals and human population [4]. Heavy industrialization and modern agriculture practices and other anthropogenic activities have caused heavy metal contamination [5]. Heavy metals occur naturally in earth's crust, however their release in excess due to anthropogenic activities have contaminated arable land worldwide. Heavy metal contaminants in soil inhibits seed germination and adversely effects growth, mineral nutrition, water relations and metabolism in plants [6,7,8].

Mercury is highly toxic heavy metal that reduces soil fertility and inhibits plant growth. Mercury occurs naturally throughout the world and is also released into environment through human activities. Anthropogenic emission sources of mercury are coal and oil combustion, solid waste incineration, metallurgical processes of metals, urban and industrial waste discharge [9]. In the past pesticides and fungicides containing mercury were used in agriculture which resulted in mercury contamination in arable lands. Mercury contamination of soil also occurs due to addition of heavy metal contaminated sludge and manures [10]. Mercury has strong persistence in soil, therefore mercury contamination in soils lasts for pretty long time [11].

High concentration of heavy metals causes stress in plants, which results in oxidative damage to plant cells triggering the formation of increased level of reactive oxygen species (ROS) such as superoxide radical (O_2^-), singlet oxygen ($^1O_2$), hydroxyl radical (-OH) and hydrogen peroxide (H_2O_2). ROS causes damage to membrane lipids, proteins, nucleic acids, chlorophyll and enzymes [12]. To neutralize oxidative damage plant cells have a complex antioxidant system capable of scavenging ROS by redox homeostasis. Defense against ROS occurs through enzymatic and non enzymatic antioxidants [13] and they play a key role in antioxidant buffers [14], enzymatic antioxidants include superoxide dismutases (SOD, EC1.15.1.1), Catalase (CAT 1.11.1.6), ascorbate peroxidase (APX 1.11.1.11), glutathione peroxidase (GPX1.11.1.9), and peroxiredoxin (Prxs 1.11.1.5). These enzymes are present in almost all sub cellular compartments. Non-enzymatic oxidants include tocopherol, flavonoids, phenols and carotenoids [15].

Proteins are important constituents of plant cells and are effected by heavy metals. Heavy metal toxicity causes changes in soluble protein content in plants which reflects defense mechanism in response to oxidative stress [8].

Protein has protective effect on plants against metal toxicity stress, salinity stress, drought stress etc. Proline content accumulation occurs with increase in concentration of heavy metals. Proline protects plants against damage by ROS. Proline plays role in osmoregulation [16], inhibition of lipid peroxidation [17], protection of enzymes [18], scavenging of free radicals [19] and singlet oxygen quenching [20].

Synthetic chelates have been widely used to enhance phytoextraction of heavy metals from contaminated soils [21] and commonly used chelate is ethyldiamine tetraacetic acid (EDTA). Chelates induce plants to take up more heavy metal than they normally accumulate [22]. Most species differ in tolerance, uptake, translocation and accumulation of mercury in different parts of plants. Several researchers have studied effect of mercury on growth, physiology and metabolism of several plant species including *Triticum aestivum* [23], *Medicago sativa* [24], *Sesbania drummondi* [25], *Pteris vittata* and *Nephrolepsis exaltata* [26], *Oryza sativa* [27], *Brassica oleraceae*, *Brassica campestris*, *Brassica rapa* and *Spinacia oleraceae* [28], *Jatropha cureas* [29] , *Lycopersicon esculentum*[30], *Mentha* arvensis [31], *Patanus occidentalis, Pinus echinata* and *Pinus taeda* [32], *Vigna radiata* [33], *Sesbania grandiflora* [34], *Albizzia lebbeck* [35]. The present study was undertaken to assess the effect of mercury on seed germination, growth and expression of antioxidant enzyme defense system in *Sorghum vulgare* var. SG-1000 seedlings.

II. MATERIALS AND METHODS

A. Seed germination and growth of seedlings

Seed germination was conducted on filter papers in glass petridishes. Twenty seeds were placed in each petridish. Filter papers were moistened with aqueous mercury chloride solution while controls were moistened with deionized water. Petridishes were incubated at 30°C ± 1°C. Experiment was run for 7 days. Counting of germinated seeds and measurement of growth parameters of seedlings such as root length, shoot length and biomass was done on 7th day. Radicale and plumule emergence was taken as criterion of seed germination. Results are based on five replicates.

Determination of root and shoot length: Seedlings were removed from filter paper with the help of foreceps on 7th day of treatment. The length of root and shoot of each seedling was measured with the help of scale. Growth inhibitory rate (GIR) of roots and shoots was calculated using the formula:

$$GIR\ (\%) = \frac{(X-Y)}{Y} \times 100$$

Where X was average length in the control i.e. without mercury metal treatment and Y was average length in tested mercury metal concentration [36].

Determination of biomass: Fresh weight (FW) of seedlings was determined on 7th day of treatment. For determination of dry weight (DW) plant samples were kept in petridishes and placed in hot air oven at 70°C for 48 hours, and then weighed.

RWC was calculated as per formula [26].

$$RWC\ (\%) = [(FW-DW/FW)] \times 100$$

Extraction of protein and enzymes: About 200 mg of fresh seedlings were homogenized in pre chilled pestle and mortar under ice-cold condition with 5.0 ml of extraction buffer (100mM k- Phosphate buffer, pH7.8), 0.1 mM EDTA, 0.1% (v/v)Triton X-100 and 2% w/v) polyvinyl pyrrolidone (PVP). The homogenate was centrifuged at 10,000 rpm for 15 minutes at 4ºC. The supernatant was used for protein estimation and enzyme assays.

Total soluble protein: The protein content was estimated according to the Lowry's method [38] using bovine serum albumin (BSA) as standard.

SOD assay: The measurement of activity of superoxide dismutase was done according to method suggested by [39]. The SOD activity was estimated by measuring its ability to inhibit the photochemical reduction of nitrobluetetrazolium (NBT). The activity was measured at 560 nm by UV- visible Spectrophotometer (Perkin-Elmer).

Proline estimation: The measurement of proline was done according to method suggested by [40]. Activity was measured at 420 nm by UV spectrophotometer (Perkin- Elmer).

III. RESULTS AND DISCUSSION

Seed germination and seedling growth was reduced by exposure to mercury chloride of different concentrations, however no effect was observed by 5 mg L^{-1} treatment of mercury chloride (Table 1). Seed germination is the most important phase in the life cycle of a plant and is highly responsive to heavy metal toxicity [37]. Higher doses effected both germination of seeds and seedling growth. The germination was 50.5 % and 10.4 % by mercury treatment of 25 mgL^{-1} and 100 mgL^{-1} respectively. Root length and shoot length was reduced by 76.4% and 37.90 % by 25 mgL^{-1} treatment. Seedling biomass, fresh weight and dry weight were also reduced. The fresh weight and dry weight of seedling was 5.5 mg and 0.66 mg respectively at 25 mgL^{-1} mercury treatment.

Table 1: Effect of heavy metal mercury on seed germination and seedling growth of *Sorghum vulgare* var. SG-1000.

Treatment Hg (conc. mg L^{-1})	Germination (%)	Root length (mm)	Shoot length (mm)	Biomass of seedling		Growth inhibition (%)		Relative water content of seedling (%)
				Fresh weight (mg)	Dry weight (mg)	Root length	Shoot length	
Control	90.2 ± 3.1[a]	56.4±5.0[a]	52.5±4.5[a]	53.6±40[a]	5.36±0.7[a]	0	0	90.0[a]
5	90.1±3.4[a]	56.2± 3.8[a]	52.4± 3.8[a]	53.6±4.1[a]	5.36±4.1[a]	0	0	90.0[a]
10	75.3 ± 2.8[b]	34.5± 4.9[b]	41.2± 4.5[b]	37.5±3.7[b]	4.32± 3.3[b]	38.5	21.50	88.4[b]
25	50.5 ± 2.7[c]	11.33 ±0.5[c]	32.6±2.0[c]	5.5 ±0.4[c]	0.66±0.04[c]	76.41	37.90	88.0[b]
50	30.8 ± 1.6[d]	10.7 ±0.3[d]	22.9±1.5[d]	4.1±0.3[d]	0.54±0.03[d]	81.02	56.38	87.1[c]
75	20.6 ± 1.2[e]	07.2 ±0.5[e]	10.4±1.0[e]	3.3±0.2[e]	0.47±0.02[e]	87.23	80.19	86.2[d]
100	10.4 ± 1.1[f]	05.3± 0.2[f]	7.2 ± 0.2[f]	2.2±0.1[f]	0.33±0.01[f]	90.60	86.30	85.4[e]

Values represent mean ± standard error, (n=3). Means with different letters within a column are significant at $P \leq 0.05$ level.

Table 2: Effect of heavy metal mercury and EDTA (50 mg L^{-1}) on seed germination and seedling growth of *Sorghum vulgare* var. SG-1000.

Hg (mgL^{-1})	EDTA (mgL^{-1})	Germination (%)	Root length (mm)	Shoot length (mm)	Biomass of seedlings		Growth inhibitory rate GIR (%)		Relative water content (RWC) of seedling (%)
					Fresh weight (mg)	Dry weight (mg)	Root length	Shoot Length	
Control		90.2 ±3.1[a]	56.4±5.0[a]	52.5±4.5[a]	53.61±40[a]	5.36±0.7[a]	0	0	90.0[a]
5	50	90.0 ± 3.5[a]	56.3± 4.9[a]	52.3± 3.8[a]	53.6± 3.1[a]	5.35± 0.6[a]	0	0	90.0[a]
10	50	82.0 ± 3.7[b]	41.3± 5.1[b]	47.2± 2.6[b]	42.5± 2.9[b]	4.78± 3.0[b]	26.64	10.9	88.7[b]
25	50	70.3 ±3.6[c]	20.4±2.0[c]	39.6±1.1[c]	6.50±0.3[c]	0.72±0.04[c]	63.82	24.57	88.9[b]
50	50	60.7 ±2.9[d]	19.4±1.8[d]	27.6±1.0[d]	5.12±0.2[d]	0.62±0.03[d]	65.60	47.42	88.0[b]
75	50	50.9 ±2.8[e]	15.3±1.4[e]	15.2±0.9[e]	4.34±0.1[e]	0.56±0.02[e]	72.87	71.04	87.1[c]
100	50	40.2 ±1.8[f]	12.5±1.0[f]	13.4±0.7[f]	3.21±0.1[f]	0.44±0.01[f]	77.82	74.47	86.5[d]

Values represent mean ± standard error, (n=3). Means with different letters within a column are significant at $P \leq 0.05$ level.

Water retention of seedlings was 85.4% by 100 mgL^{-1} mercury treatment in comparison to control where water retention was 90%. The combined treatment of mercury and EDTA (50 mgL^{-1}) (Table 2), resulted in moderation of effect of mercury in all mercury concentrations with reference to germination, root and shoot length, biomass and water retention by seedlings. The germination was 40.2% and inhibition of root and shoot length was 77.82% and 74.47% respectively by 100 mgL^{-1} mercury + EDTA treatment, while germination was 10.4% and inhibition of root and shoot length was 90.65% and 86.30% respectively by 100 mgL^{-1} mercury treatment alone. Several studies have reported similar findings on growth reduction on Hg^{+} exposure [41, 42]. Several authors have reported that water deficiency in seedlings due to Hg^{+} toxicity. [29, 34]. In this study gradual decrease in RWC of seedlings occurred with increase in Hg^{+} concentration. About 4-5% reduction in water retention capacity was observed (Table 1, 2).

Biochemical changes in seedlings occurred upon exposure to different concentration of mercury. Protein, proline and SOD content of seedlings did not change on exposure of seedling to Hg^{+} concentration of 5 mgL^{-1}. Changes occurred when concentration of Hg^{+} was above 5 mgL^{-1}.

Protein content started decreasing at 10 mgL^{-1} Hg^{+} exposure, protein content was reduced by 57% at this concentration and by 100 mg L^{-1} Hg^{+} treatment, protein content was reduced by 80% in comparison to control (Fig. 1). EDTA had slightly moderating effect and at 10 mgL^{-1} Hg^{+} + EDTA (50 mgL^{-1}) the reduction was 46% in comparison to control, while reduction was 57% without EDTA. Synthetic chelates such as EDTA have been widely used to enhance phytoremediation of heavy metals [21].

On heavy metal exposure, the protein pool has shown increase in many plants [41] and many have reported decrease in protein content. Decrease in protein content is the result of several effects, oxidative stress, protein utilization for detoxification and increased ribonuclease activity.

The SOD activity increased by 5 mgL^{-1} mercury treatment. SOD activity of seedlings decreased on exposure to mercury concentrations above 5 mgL^{-1} (Fig. 2). This may be due to enzyme damage from the excess production of free radicals. In most of research studies several authors have reported increase in SOD content on exposure to heavy metals [34, 41]. The SOD is considered as a first line of defense against oxidative damage.

Proline content of seedlings increased on exposure to mercury chloride exposure (Fig. 3). At 50 mg L^{-1} mercury concentration the proline content increased by 50%. The proline content further increased when the *Sorghum* seedlings were exposed to mercury and EDTA simultaneously. Many authors have reported increase in proline content in response to stress to heavy metals [43, 44]. Under stress condition many plant species accumulate proline as an adaptive response to adverse stress condition to contain stress injury and also proline plays a role in protein synthesis. Proline is an excellent osmolyte, plays three major roles during stress as a metal chelator, an oxidative defense molecule and a signaling molecule [44].

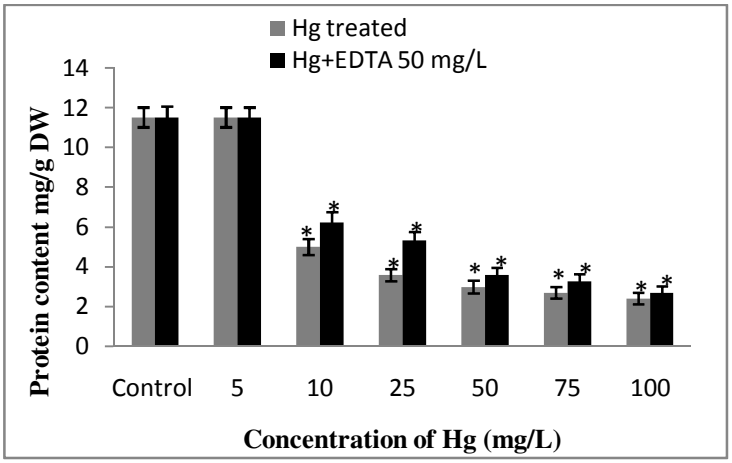

Fig. 1. Effect of mercury (Hg) metal stress and mercury + EDTA on protein content in *Sorghum vulgare* var SG-1000 seedlings. The values represent (mean ± standard error, n=3). *Significant at P ≤0.05 level.

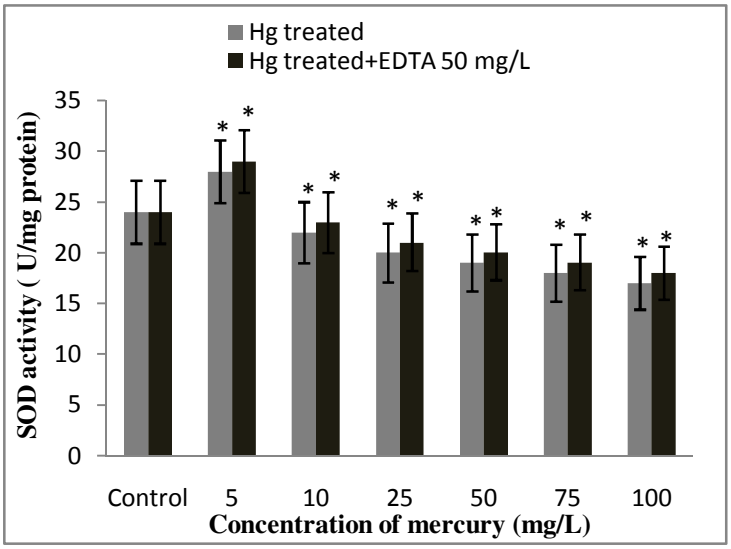

Fig. 2. Effect of mercury (Hg) metal stress and mercury + EDTA on SOD activity in *Sorghum vulgare* var SG- 1000 seedlings. The values represent (mean ± standard error, n=3). * Significant at P ≤0.05 level.

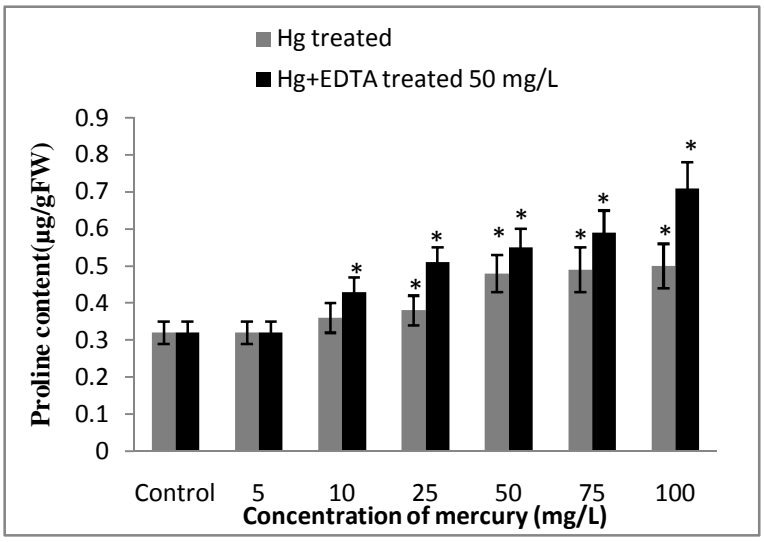

Fig. 3. Effect of mercury (Hg) metal stress and mercury +EDTA on proline content in *Sorghum vulgare* var SG-1000 seedlings. The values represent (mean ± standard error, n=3). *Significant at P ≤0.05 level.

ACKNOWLEDGEMENT

We are thankful to Hon'ble vice chancellor, Prof. Kamal Kant Dwivedi and management, ITM University, Gwalior (Madhya Pradesh) for all the necessary facilities for the present study.

REFERENCE

[1]. Singh, S., Parihar, P., Singh, R., Singh, V. P. and Prasad, S. M. (2015). Heavy metal tolerance in plants. Role of transcriptomics, Proteomics, metabolomics and ionomics. *Front. Plant Sci.,* **6**: 1143.

[2]. Sherene T (2010). Mobility and transport of heavy metals in polluted soil environment. *Biological Forum-An Int J*., **2**: 112–121

[3]. Hasanabadi, T., Shahram, L., Mohammad, R., Hosein, G. and Adel, M. (2015). Effect of clinoptilolite and heavy metal application on some physiological characteristics of annual Alfalfa in contaminated soil. *Biological Forum- An Int. J*., **7**(2): 361-366

[4]. Conceicao, F.T., Navarro, G.R.B., and Silva, A.M. (2013). Anthropogenic influence on Cd, Cr, Cu, Ni, Pb and Zn concentrations in soil and sediments in a watershed with sugarcane crops at Sao Paulo state Brazil. *Int. J. Environ Res.,* **7**(3): 551-560.

[5]. Kavamura, V.N., and Esposito, E. (2010). Biotechnological strategies applied to the decontamination of soils polluted with heavy metal. *Biotechnol. Adv,*. **28**: 61-69.

[6]. Goldbold, D.L., and Kettner, C. (1991). Lead influence root growth and mineral nutrition of *Picea abies* seedlings. *J. Plant Physiol.,* **139**: 95-99.

[7]. Moustakas, M., Lanaras, T., Symeonidis, L., and Karataglis, S (1994). Growth and some photosynthetic characteristics of field grown *Avena sativa* under copper and lead stress. *Photosynthetica*, **30**: 389-396.

[8]. Rastgoo, L. and Alemzadeh, A. (2011). Biochemical responses of Gouan (*Aeluropus littoralis*) to heavy metal stress. *Australian Journal of Crop Science,* **5**(4): 375-383.

[9]. Pirrone, N., Costa, P., Pacyna, J.M., and Ferrara, R. (2001). Mercury emissions to the atmosphere from natural and anthropogenic sources in the mediterranen region. *Atmospheric Environment*. **35**: 2997-3006.

[10]. Azevedo, R. and Rodriguez, E. (2012). Phytotoxicity of mercury in plants: *A review Journal of Botany.* 2012. doi: 10.1155/2012/848614.

[11]. Wang, O., Kim, D., Dionysiou, D. D., Sorial, G.A. and Timberlake, D. (2004). Sources and remediation for mercury contamination in aquatic ecosystem- a literature review. *Environmental pollution,* **131**(2): 323-336.

[12]. Gallego, S., Benavides, M. and Tomaro, M. (2002). Involvement of an antioxidant defense system in the adaptive response to heavy metal ions in *Helianthus annus* cells*. Plant Growth Regul.,* **36**: 267-273.

[13]. Scandalios, J.G. (2005). Oxidative stress: Molecular perception and transduction of signals triggering antioxidant gene defenses. *Braz. J. Med. Biol. Res.,* **38**: 995-1014

[14]. Foyer, C.H., & Noctor, G. (2005). Redox homeostasis and antioxidant signaling. A metabolic interface between stress perception and physiological responses. *Plant cell,* **17**: 1866-1875.

[15]. Gratao, P.L., Polle, A., Lea, P.J., and Azevedo, R.A., (2005). Making the life of heavy metal stressed plants a little easier. *Functional plant Biol.* **32**: 481-494.

[16]. Ahmed, I. and Hellebust, J. A. (1988). The relationship between inorganic nitrogen metabolism and proline accumulation in osmoregulatory responses of two *euryhaline* microalgae. *Plant Physiol.,* **88**: 348-354.

[17]. Mehta, S.K. and Gaur, J.P. (1999). Heavy metal induced proline accumulation and its role in ameliorating metal toxicity in *Chlorella vulgaris*. *New Phytol.* **143**: 253-259.

[18]. Nikolopoulos, D. and Manetas. (1991). Compatible solutes and in vitro stability of *Salsola soda* enzyme in proline incompatibility. *Phytochemistry,* **30**: 411- 413.
[19]. Smirnoff N. and Cumbes, Q. J. (1989). Hydroxyl radical scavenging activity of compatible solutes. *Photochemistry,* **28**: 1057-1060.
[20]. Alia, Mohanty, P. and Matysik, J. (2001). Effect of proline on the production of singlet oxygen. *Amino acid,* **21**: 195-200.
[21]. Greman, H., Veliknonja-Bolta, S., Vodnik, D., Kos, B. and Lestan, D. (2001). EDTA enhanced heavy metal Phytoextraction: metal accumulation, leaching and toxicity. *Plant and soil,* **235**: 105-114.
[22]. Liphadz, S. M., Kirkham, M.B., Douglas- Mankin, K.R., and Paulsen, G.M. (2003). EDTA assisted heavy metal uptake by poplar and sunflower grown at a long term. *Plant and Soil*, **257**(1): 171-182.
[23]. Jamal, S.N., Iqbal, M.Z., and Athar, M., (2006). Evaluation of two wheat varieties for phytotoxic effect of mercury on seed germination and seedling growth. *Agriculture Conspectus Scientificus,* **71**(2): 41-44.
[24]. Zhou, Z.S., Huang, S.Q., Guo, K, Mehta., S.K., Zhang, P.C., and Yang, Z. (2007). Metabolic adaptation to mercury induced oxidative stress in roots of *Medicago sativa* L. *J. Inorg. Biochem.,* **101**: 1-9.
[25]. Venketachalam, P., Srivastava, A.K., and Raghothama, K.G. and Sahi, S. V. (2009). Gene induced in response to mercury ion exposure in heavy metal hyperaccumulator *Sesbania drummondii. Environ. Sci. Technol.* **43**: 843-850.
[26]. Chen, J., Shiyab, S., Han, F.X., Monts, D.L., Waggoner, A. W. and Su, Z. X. (2009). Bioaccumulation and physiological effects of mercury in *Pteris Vittata* and *Nephrolepis exaltata*. *Ecotoxicology,* **18**: 110-121.
[27]. Meng, B., Feng, X., Qiu, G., Cai, Y., and Wang, D. (2010). Distribution patterns of inorganic mercury and methyl mercury in tissues of rice (*Oryza sativa* L.) plants and possible bioaccumulation pathways. *J. Agric. Food. Chem.,* **58**: 4951-4958.
[28]. Ling, T., Fangke, Y., and Jun, R. (2010). Effect of mercury to seed germination, coleoptile growth and root elongation of four vegetables. *Research Journal of Phytochemistry,* **4**: 225-233.
[29]. Gao, S., Yang, C., Tang, L., Xu, Y., Wang, S. and Chen, S.F. (2010). Growth and antioxidant responses in *Jatropha curcas* seedling exposed to mercury toxicity. *J. Hazard Mater.,* **182**: 591-597.
[30]. Shekar, C., Sammaiah, D., Shasthree, T., and Reddy, K.J., (2011). Effect of mercury on tomato growth and yield attributes. *International Journal of Pharma and Biosciences,* **2**(2): 358-364.
[31]. Manikandan, R. and Venkatachalam, P. (2011). Risk assessment of mercury ion heavy metal exposure on physiological and biochemical changes and DNA damage using RAPD analysis in *Mentha arvensis* seedlings. *Plant Cell Biotech. Mol. Biol.,* **12**: 41-50.
[32]. Jean-Philippe, S.R., Labbe, N., Damay, J., Franklin, J.A., & Hughes, K. (2012). Effect of mercuric compounds on pine and sycamore germination and early survival. *American Journal of plant Sciences,* **3**: 150-158.
[33]. Jagatheshwari, D., Ranganathan, P. (2012). Influence of mercuric chloride on seed germination, seedling growth and biochemical analysis of *Gram* (*Vigna radiate* L. Wilczek. Var. Vamban-3). *International Journal of Pharmaceutical and biological Archives,* **3**(2): 291-295.
[34]. Malar, S., Sahi, S.V., Favas, P.J.C., & Venkatachalam, P. (2015). Assessment of mercury heavy metal toxicity-induced physicochemical and molecular changes in *Sesbania grandiflora* L. *Int. J. Environ. Sci. Technol.,* **12**: 3273-3282.
[35]. Iqbal, M.Z., Shafiq, M., and Athar, M. (2014). Phytotoxic effects of mercury on seed germination and seedling growth of *Albizia lebbbek* (L) Benth. *Advances in Environmental Research,* **3**(3): 207-216.
[36]. Korpe, D.A. and Aras, S., (2011). Evaluation of copper-induced stress on eggplant (*Solanum melongena* L.) seedlings at the molecular and population levels by use of various biomarkers. *Mutat. Res.***, 719**: 29–34.
[37]. Kuriakose, S.V. and Prasad, M. N. V. (2008). Cadmium stress affects seed germination and seedling growth in *Sorghum bicolor* (L.) Moench by changing the activities of hydrolyzing enzymes. *Plant Growth Regul.,* **54**: 143-156. DOI 10.1007/s10725-007-9237-4.
[38]. Lowry, O.H., Rosebrough, N.J., Farr, A.L., & Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.,* **193**: 265-275.
[39]. Beauchamp, C. and Fridovich, I. (1971) Superoxide dismutase: improved assays and assay an applicable to acrylamide gels. *Anal. Biochem.,* **44**: 276-287.
[40]. Bates, L., Waldren, R.P. and Teare, I.D., (1973). Rapid determination of free proline for water-stress studies. *Plant and Soil,* **39**: 205-207.
[41]. Manikandan R., Sahi, S.V. and Perumal, V. (2015). Impact assessment of mercury accumulation and biochemical and molecular response of *Mentha arvensis*: A potential hyper accumulator plant. *The Scientific World Journal,* DOI: 10.1155/2015/715217.
[42]. Mukherjee M. and Dalal T., (2014). Early seedling growth and accumulation of proline and phenol in *Trigonella foenum-graecum* under heavy metal stress. *International Journal of Science and Research,* **3**(8): 1271-1273.
[43]. Anayat M., Agnihotri R. K., Vamil R., Kumar S. and Sharma R. (2014). Role of Cd and Hg on biochemical contents of fennel and its reduction by exogenous treatment of nitrogen. *International Journal of Scientific and Research Publications*, **4** (3): 1-6.
[44]. Hayat S., Hayat Q., Alyemeni M. N., Wani A. S., Pichtel J. and Ahmad A. Role of proline under changing environments - A review. *Plant Signaling & Behavior*, **7**(11): 1–11.