British Biotechnology Journal

British Biotechnology Journal
5(3): 148-155, 2015, Article no.BBJ.2015.014
ISSN: 2231–2927

SCIENCEDOMAIN *international*
www.sciencedomain.org

Identification of a Biochemical Indicator in Plants for Heavy Metal Contamination

Sonali Hazra Das[1], Mahasweta Paul[1], Shankha Ghosh Dastidar[1], Tulika Das[1] and Srabanti Basu[1*]

[1]Department of Biotechnology, Heritage Institute of Technology, Kolkata, India.

Authors' contributions

The work has been carried out in collaborations between all authors. Authors SHD, MP, SGD and TD managed literature survey, performed the experiments and did the statistical analysis. Authors MP and SGD wrote the first draft of the manuscript. Author SB designed the experiments, supervised the study and prepared the final manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BBJ/2015/14303
Editor(s):
(1) Juan Pedro Navarro – Aviñó , Technical University of Valencia, Spain.
(2) Kuo-Kau Lee, Department of Aquaculture, National Taiwan Ocean University, Taiwan.
Reviewers:
(1) Anonymous, Ireland.
(2) Echem, Ogbonda G, Science Laboratory Technology, Rivers State Poly Technic, Bori, Nigeria.
(3) F.B.G.Tanee, Plant Science and Biotechnology Department, University of Port Harcourt, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=803&id=11&aid=7565

Original Research Article

Received 26th September 2014
Accepted 4th December 2014
Published 31st December 2014

ABSTRACT

Aims: To identify a biochemical parameter in a plant(s) that can serve as an indicator of heavy metal pollution.
Study Design: Design of the study includes collection of plant samples from a contaminated site and a rural site and to check their biochemical parameters related to oxidative stress. Based on the experimental findings, attempts were made to identify a parameter that can serve as an indicator of heavy metal contamination.
Place and Duration of Study: The study was conducted in Department of Biotechnology, Heritage Institute of Technology, India, during the period from August 2013-June 2014.
Methodology: Plant samples were collected from East Calcutta Wetlands (metal contaminated site) and Binogram, a village 90 Km away from Kolkata (control site). The samples were checked for the following parameters: non-protein thiol, ascorbic acid, superoxide dismutase (SOD), lipid peroxidation. This was followed by a statistical analysis.
Results: Non-protein thiol content was found to be less in the *Ipomoea, Hygrophila* and cabbage

---

**Corresponding author: Email: srabanti_b@yahoo.co.uk;*

samples collected from ECW. The values were 65-75% of the village samples. Cauliflower leaves did not show any alteration in the parameter. Ascorbic acid was altered significantly in cabbage and *Hygrophila*. Activity of SOD decreased in all samples. Lipid peroxidation increased in all samples except *Ipomoea*. Of the biochemical parameters tested, maximum alteration was found with non-protein thiol, in which glutathione (GSH) is the major constituent. Of the vegetables studied, cabbage showed an alteration in all biochemical parameters.

Conclusion: GSH has the potential for being used as an indicator of heavy metal pollution. Cabbage can be used as a model vegetable for testing the biochemical parameters. The method will be helpful in identification of a contaminated site before estimation of heavy metals.

Keywords: Oxidative stress; non-protein thiol; East Calcutta Wetlands; heavy metals; plants.

1. INTRODUCTION

Agricultural soils in many parts of the world are slightly-to-moderately contaminated by heavy metals. This could be due to the long term use of phosphatic fertilizers, sewage sludge applications, dust from smelters, industrial waste and bad watering practices in agricultural lands [1-3]. With industrial development, the production and emission of heavy metals have increased. Some metals like arsenic, cadmium, chromium, lead etc., when present at high concentrations, have strong toxic effects and pose an environmental threat. Presence of heavy metals in different food causes serious health hazards, depending on their relative levels.

The primary response of plants toward stress is the generation of Reactive Oxygen Species (ROS) upon exposure to high levels of heavy metals. Various metals either generate ROS directly through Haber-Weiss reactions or create oxidative stress through indirect mechanisms [4,5]. The indirect mechanisms include their interaction with the antioxidant system, disrupting the electron transport chain or disturbing the metabolism of essential elements [6-8]. To countermine the toxicity of ROS, living organisms possess defence systems called anti-oxidative or anti-oxidant defence systems, comprising both enzymatic and non-enzymatic constituents. The non-enzymatic anti-oxidants are generally small molecules that include the tripeptide glutathione, cysteine, hydroxy-quinone, ascorbate, the lipophilic anti-oxidants α-tocopherol, carotenoid pigments and a variety of other compounds [9]. The enzymatic anti-oxidant components include the enzymes capable of removing, neutralizing or scavenging ROS and the intermediates, such as catalase, glutathione peroxidase, ascorbate peroxidase, superoxide dismutase, glutathione reductase, monodehydroascorbate reductase and dehydroascorbate reductase. The non-enzymatic and enzymatic components work in close co-ordination for the effective removal of ROS. Thus, a balance is maintained between the formation and destruction of ROS. A shift in the balance between pro-oxidative and anti-oxidative systems will lead to Oxidative Stress or accumulation of the toxic ROS.

East Calcutta Wetlands (ECW), located approximately between latitudes 22º25’ to 22°40’ N to longitudes 88°20’ to 88º35’ E covering 12,500 hectares area, acts as the sewage treatment and resource recovery system of Kolkata. The resource recovery takes place mainly by three processes – sewage-fed fisheries, agriculture on the garbage substrate and paddy cultivation using pond effluent. The Kolkata Municipality generates 600 million litres of wastewater daily. The wastewater flows through underground sewers to the pumping stations at the eastern fringes of the city and is then pumped into open channels. The wastewater is received by the sewage-fed fisheries and local farms. The wetland has been incorporated in the list maintained by the Ramsar Bureau established under the Ramsar Convention and is recognised as a ‘Wetland of International Importance’. The site receives mixed waste containing domestic wastewater as well as industrial effluents. Reports are available on presence of heavy metals in the soil of this area [10,11].

The objective of the present study is to check the biochemical stress parameters of plants grown in the East Calcutta Wetlands and to identify a biochemical parameter that can serve as an indicator of heavy-metal contamination.

2. MATERIALS AND METHODS

2.1 Collection of Samples

Plant and soil samples were collected from the East Calcutta Wetlands (experimental site or the site contaminated with heavy metals), and Binogram, a village of Hooghly district, 90 Km away from Kolkata (control site). Location map of the sample collection site is given in Fig. 1. Plant samples included *Hygrophila auriculata, Ipomoea aquatica, Brassica oleracea* (Cauliflower: Botrytis cultivar group), *Brassica oleracea* (Cabbage: White cabbage: *B. oleracea* L. *var. capitata* L. *f. alba DC.*). The plant samples were collected in the season of their cultivation and the biochemical tests were carried out within five days of collection. Nine samples of each plant were tested for each biochemical parameter.

2.2 Estimation of Heavy Metals in Soil Samples

Soil samples were dried keeping it at 70ºC and weighed periodically till constant weight is achieved. The dried samples were crushed and digested with concentrated hydrochloric acid and 70% perchloric acid. Arsenic, chromium, cadmium and lead were estimated in the digests by atomic absorption spectrophotometry.

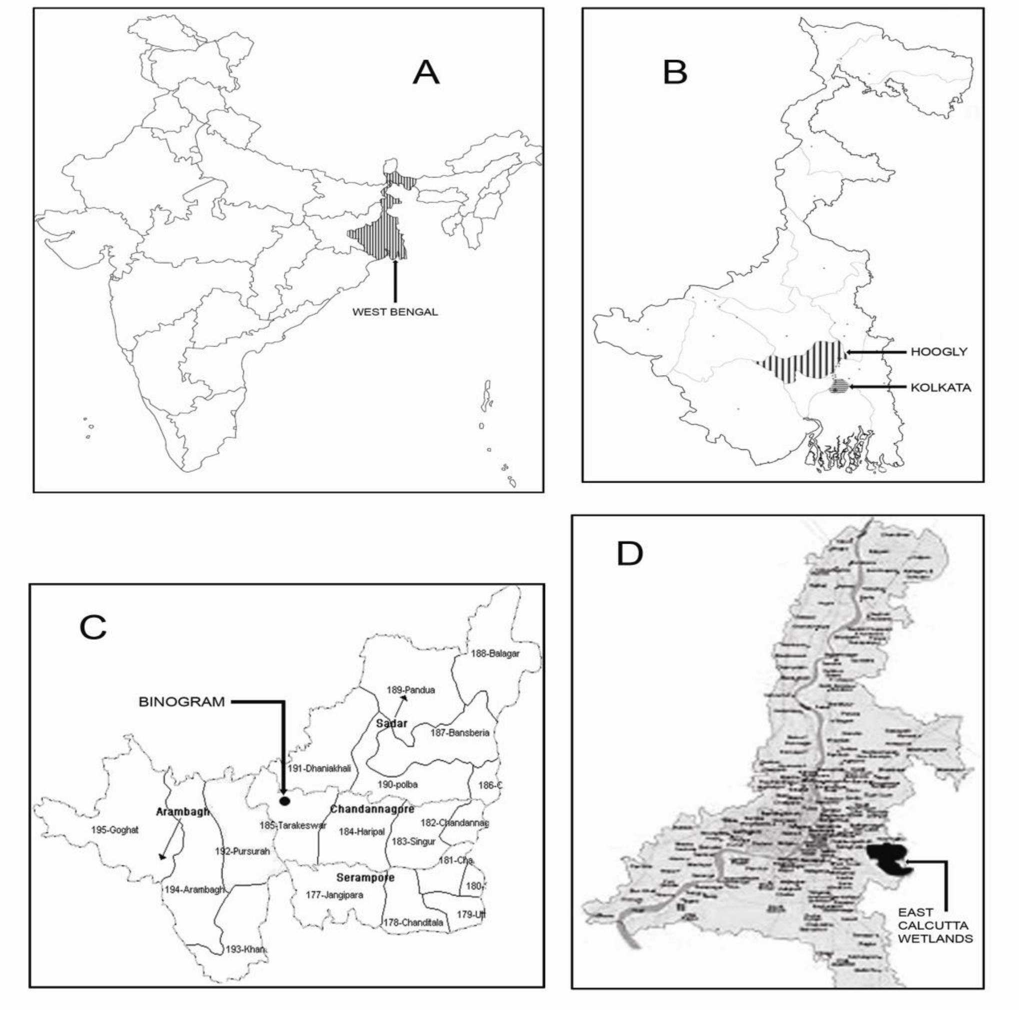

Fig. 1. Location map of the sample collection site (A): location of West Bengal in India, (B): location of Hooghly district and Kolkata in West Bengal, (C) location of Binogram in Hooghly, (D): location of East Calcutta Wetlands in Kolkata

2.3 Estimation of Non-protein Thiol

Plant extract (20% w/v) was prepared in 6% (w/v) TCA and centrifuged at 5,000 rpm for 10 minutes to separate the proteins. Non-protein thiol was estimated in the supernatant spectrophotometrically (Shimadzu UV1700) using 5,5'di-thio-bis [nitro-benzoic acid] (DTNB) reagent [12]. Absorbance was measured at 440 nm (Shimadzu UV1700).

2.4 Estimation of Ascorbic Acid

Aqueous extracts of plant samples (30% w/v) were centrifuged at 5000 rpm for 5 minutes and the supernatant was collected. Ascorbic acid was estimated by volumetric method following the procedure of Madhava-Rao [13] using 2, 6 dichlorophenol indophenol as an indicator.

2.5 Assay of Superoxide Dismutase (SOD)

Aqueous extracts of plant samples (20% w/v) were centrifuged at 5,000 rpm for 5 minutes and the supernatant was collected. SOD was estimated spectrophotometrically following the prevention of auto-oxidation of pyrogallol [14]. A reduction of 50% auto oxidation of pyrogallol is considered 1 unit of SOD.

2.6 Estimation of Lipid Peroxidation

Plant extract (20% w/v) was made in 0.5M saline-phosphate buffer, pH 7.4. Lipid peroxidation was estimated using thiobarbituric acid (TBA). Determination of TBA-reactive substances (TBARS), mostly consisting of malonaldehyde, was performed spectrophotometrically with the supernatant [15].

Statistical analysis of all the experimental data was done using Student's t test (unpaired).

3. RESULTS AND DISCUSSION

3.1 Estimation of Heavy Metals in Soil Samples

Soil samples collected from the East Calcutta Wetlands are highly contaminated with heavy metals as found from the study. Soil samples of the control site contained much lower amounts of chromium, cadmium and arsenic than the experimental site (Table 1). However, a considerable amount of lead is present in the soil sample of the control site which might have come from exhausts of automobiles passing through the village roads. However, the amount is lower than that found in the soil sample of the experimental site.

3.2 Estimation of Non-protein Thiol

The non-protein thiol content was decreased in *Ipomoea, Hygrophila* and cabbage samples collected from the ECW (Fig. 2). The decrease was comparable in cabbage and *Hygrophila* (74% and 75% respectively of the control-site), whereas decrease in *Ipomoea* was a little less (65% of the control-site). Cauliflower was an exception where no change in thiol content was found. The finding is supported by Schutzendubel [16] who reported decrease in glutathione (GSH), the major non-protein thiol present in living systems, due to cadmium stress.

GSH is a key component in metal scavenging mechanisms due to its high affinity towards heavy metals, especially arsenic, lead, cadmium and mercury. Apart from binding the heavy metals directly, GSH serves as the precursor of phytochelatin, a protein synthesized under the condition of heavy-metal stress [17]. Induction of phytochelatin synthesis has been best reported in cases of cadmium toxicity. However, it can be induced in the presence of other heavy metals like mercury, copper, zinc, lead and nickel [18]. The depletion of non-protein thiols in the plant samples collected from ECW may have resulted from higher synthesis of phytochelatin in those plants due to the presence of heavy metals in soil.

3.3 Estimation of Ascorbic Acid

Ascorbic acid has been decreased significantly in cabbage and *Hygrophila* collected from the ECW and remained unchanged in cauliflower and *Ipomoea* (Fig. 3). The decrease was comparable in both the samples (10% and 12% of the control sites respectively). Ascorbic acid is a component of anti-oxidant defence mechanism and plays an important role in protection of plants against several environmental stresses including heavy metal stress [19].

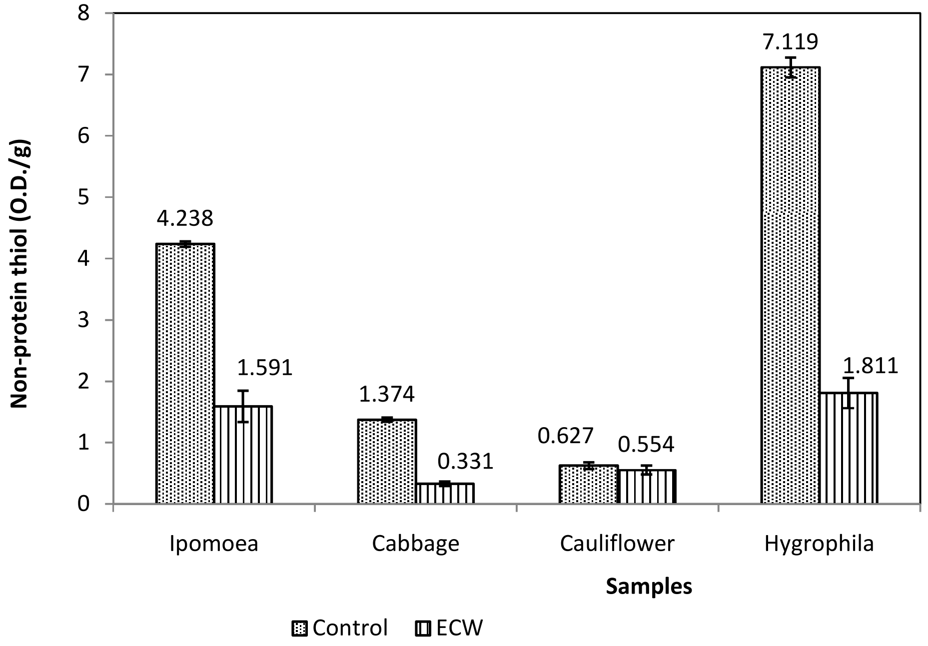

Fig. 2. Non-protein thiol-contents in plant samples collected from East Calcutta Wetlands and the control sites

Significant changes: Ipomoea (P<0.0001) cabbage (P<0.0001), hygrophila (P<0.001)

Table 1. Heavy metal content of the soil samples

Metal	Binogram (control site) (mg/kg)	East Calcutta Wetlands (experimental site) (mg/kg)
Chromium	6.935	31.23
Cadmium	1.395	2.16
Lead	30.9	60.3
Arsenic	<0.2	0.79

Previous studies have shown that ascorbic acid content in the roots and shoots of two cultivars of pigeon pea (*Cajanus cajan*) decreased when zinc and nickel was supplied from outside. Moreover, ascorbic acid content showed a significant negative correlation with increasing concentrations of the two metal ions [13]. Soto et al. [20] have reported lower ascorbic acid level in *M. muscorum* in presence of organic compounds. Reduced ascorbic acid content may be due to direct oxidation of ascorbic acid caused by lead (II). It is also possible that ascorbic acid has been consumed by the antioxidant defence enzyme ascorbate peroxidase. The possibility of consumption of ascorbic acid by ascorbate peroxidase can be substantiated by the observation of Okamoto et al. [21]. The group has reported high activity of ascorbate peroxidase content in *Gonyaulax polyedra* due to heavy metal stress induced by lead (II), mercury (II), cadmium (II) and copper (II).

3.4 Assay of SOD

The activity of SOD has been found to be decreased significantly in all three samples tested for it (Fig. 4). In an earlier study, John et al. [22] have reported higher SOD activity due to exposure to cadmium at both low and high concentrations. However, Zhang et al. [23], who studied heavy metal stress on oxidative enzymes in leaves of two mangrove plant seedlings, namely *Kandelia candel* and *Bruguiera gymnorrhiza*, reported that SOD activity fluctuated in plants under different stressed conditions. From the above information from other studies and the observation of the present study, it can be stated that SOD may not be a good parameter to be used as biochemical indicator of heavy metal stress in plants.

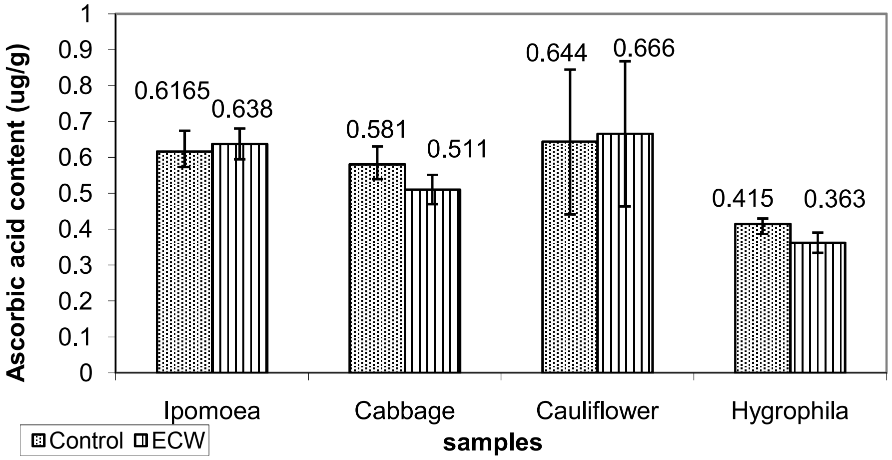

Fig. 3. Ascorbic acid contents in plant samples collected from East Calcutta Wetlands and the control sites

Significant changes: cabbage (P<0.005), hygrophila (P<0.05)

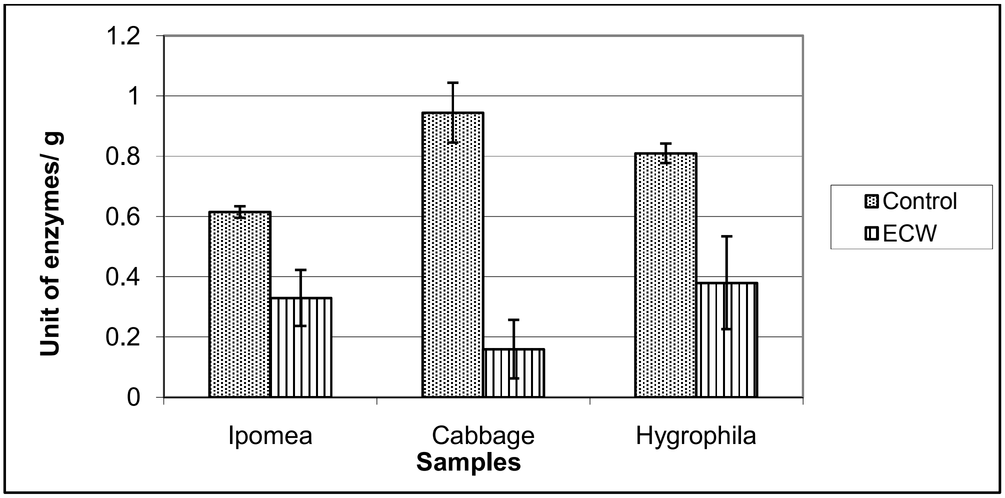

Fig. 4. Activity of superoxide dismutase (SOD) in plant samples collected from East Calcutta Wetlands and the control sites

Significant changes: Ipomoea (p<0.01), cabbage (p<0.005), hygrophila (P<0.005)

3.5 Estimation of Lipid Peroxidation

Lipid peroxidation was significantly increased in cabbage, cauliflower and *Hygrophila* and remained unaltered in *Ipomoea* (Fig. 5). There is a possibility of enhanced lipid peroxidation in tissues as heavy metals are known to induce oxidative stress in plants. The reason may be that heavy metals, particularly the transition metals, can induce production of hydroxyl radicals which could lead to lipid peroxidation [16]. High lipid peroxidation was observed by Zhang et al. [23] in the leaves of *Bruguiera gymnorrhiza* under heavy metal stress. Excess cobalt, nickel and cadmium produced increased amount of TBARS in spinach [24]. John et al. [22] have reported high amount of malondialdehyde, a product of lipid peroxidation, in *Lemna polyrrhiza* due to cadmium stress. The increase in lipid peroxidation may be a direct effect of the metal toxicity caused by increased production of ROS or an indirect effect caused by depletion of GSH and/or components of anti-oxidant defence mechanism.

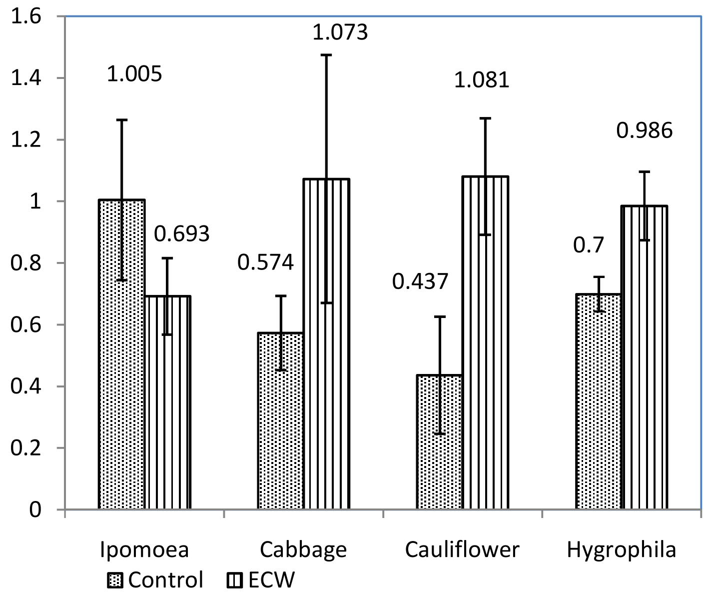

Fig. 5. Lipid peroxidation in plant samples collected from East Calcutta Wetlands and the control sites

Significant changes: cabbage (P<0.01), cauliflower (P<0.0002), hygrophila (P<0.002)

4. CONCLUSION

Of the biochemical parameters tested, maximum alteration was found with the parameter non-protein thiol, in which GSH is the major constituent. Therefore it can be concluded that GSH content of plant samples has the potential for being used as an indicator of heavy metal pollution of a contaminated site. Of the vegetable studied, cabbage showed an alteration in all biochemical parameters. Hence cabbage perhaps can be used as a model plant species in which the number of parameters showing variability is higher in a contaminated site among the species studied; thereby indicating a maximum level of sensitivity in regard to those parameters. However, more field studies are required to validate the present observation. Moreover, GSH content and other biochemical parameters are required to be tested under laboratory conditions. The process will be helpful for identification of a contaminated site before going for estimation and identification of heavy metals.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Bell FG, Bullock SET, Halbich TFJ, Lindsay P. Environmental impacts associated with an abandoned mine in the Witbank Coalfield, South Africa. Int J Coal Geol. 2001;45:195–216.
2. Schwartz C, Gerard E, Perronnet K, Morel JL. Measurement of in-situ phytoextraction of zinc by spontaneous metallophytes growing on a former smelter site. Sci Total Environ. 2001;279:215–21.
3. Passariello B, Giuliano V, Quaresima S, Barbaro M, Caroli S, Forte G Garelli G Iavicoli I. Evaluation of the environmental contamination at an abandoned mining site. Microchem J. 2001;73:245–50.
4. Wojtaszek P. Oxidative burst: An early plant response to pathogen infection. Biochem J. 1997;322:681–92.
5. Mithofer A, Schulze B, Boland W. Biotic and heavy metal stress response in plants: Evidence for common signals. FEBS Lett. 2004;566:1–5.
6. Srivastava S, Tripathi RD, Dwivedi UN. Synthesis of phytochelatins and modulation of antioxidants in response to cadmium stress in Cuscuta reflexa—an angiospermic parasite. J Plant Physiol. 2004;161:665–74.

7. Qadir S, Qureshi MI, Javed S, Abdin MZ. Genotypic variation in phytoremediation potential of Brassica juncea cultivars exposed to Cd stress. Plant Sci. 2004;167:1171–81.
8. Dong J, Wu FB, Zhang GP. Influence of cadmium on antioxidant capacity and four microelement concentrations in tomato seedlings (*Lycopersicon esculentum*). Chemosphere. 2006;64:1659–66.
9. Larson RA. The antioxidants of higher plants. Phytochemistry. 1988;27:969-78.
10. Raychaudhuri S, Mishra M, Nandy P, Thakur A R. Waste management: A case study of ongoing traditional practices in East Calcutta Westland. Am J Agric Biol Sci. 2008;3(1):315-20.
11. Kundu N, Pal M, Saha S. East Kolkata Wetlands: A resource recovery system through productive activities. Proceedings of Taal 2007: The 12th World Lake Conference. 2008;868-81.
12. Owens CWI, Belcher RVA. Colorimetric micro-method for the determination of glutathione. Biochem J. 1965;94:705–11.
13. Madhava Rao, Sresty TVS. Antioxidative Parameters in the seedlings of Pigeonpea (*Cajanus cajan* (L.) Millspaugh) in response to Zn and Ni stresses. Plant Sci. 2000;1572:113-28
14. Marklund S, Marklund G. Involvement of superoxide anion radical in the auto-oxidation of pyrogallol and a convenient assay for superoxide. Eur J Biochem. 1972;47:3170-5.
15. Janero D. Malonaldehyde and thiobarbituric acid reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free Rad Biol Med. 1990;9:515-40.
16. Schutzendubel A, Polle A. Plant responses to abiotic stresses: Heavy metal-induced oxidative stress and protection by mycorrhization. J Exp Bot. 2002;53:1351-65.
17. Jozefezak M, Remans T, Vangronsveld J, Cuypers A. Glutathione is a key player in metal-induced oxidative stress defences. Int J Mol Sc. 2012;13:3145-75.
18. Yadav SK. Heavy metals toxicity in plants: An overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. South African J Bot. 2010;76:167-79.
19. Khan TA, Mazid M, Mohammad F. A review of ascorbic acid potentialities against oxidative stress induced in plants. J Agrobiol. 2011;28(2):97-111.
20. Soto P, Gaetel G, Hidalgo ME. Assessment of catalase activity, lipid peroxidation, chlorophyll-a, and growth rate in the fresh water green algae *Pseudokirchneriella subcapitala* exposed to copper and zinc. Lat Am Aquat Res. 2011;39(2):280-5.
21. Okamoto M, Kuwahara A, Seo M, Kushiro T, Asami T, Hirai N et al. CYP707A1 and CYP707A2, Which encode abscisic acid 8-hydroxylases, are iIndispensable for proper control of seed dormancy and germination in arabidopsis. Plant Physiol. 2006;141:97-107.
22. John R, Ahmad P, Gadgil K, Sharma S. Antioxidative response of *Lemma polyrrhiza* L. to cadmium stress. J Env Biol. 2007;28(3):583-9.
23. Zhang FQ, Wang YS, Lou ZP, Dong JD. Effect of heavy metal stress on antioxidative enzymes and lipid peroxidation in leaves and roots of two mangrove plant seedlings (Kandeliacandel and Bruguiera gymnorrhiza), Chemosphere. 2007;67:44-50.
24. Pandey N, Pathak G, Pandey D, Pandey R. Heavy metals Co, Ni, Zn and Cd produce oxidative damage and evoke differential antioxidant responses in spinach. Braz J Plant Physiol. 2009;21:103-111. DOI.org/10.1590/S1677-04202009000rg/10.1590/S1677-04202009000.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=803&id=11&aid=7565