Jurnal
Teknologi

Full Paper

PELARGONIUM RADULA AS A PLANT BIOINDICATOR IN MONITORING MERCURY IN DRINKING WATER

Noraishah A Majid, Nur Syazila Ramli, Ing Chia Phang*

Department of Biotechnology, Kulliyyah of Science, International Islamic University Malaysia, Jalan Sultan Haji Ahmad Shah, Bandar Indera Mahkota, 25200, Kuantan, Malaysia

Article history
Received
28 June 2015
Received in revised form
10 September 2015
Accepted
21 October 2015

*Corresponding author
ingchia@iium.edu.my

Abstract

Pollution by organic and inorganic toxic substances has raised public and environmental concern globally since last decade. However, due to rapid growth of world population along with excessive industrial development, this situation worsens. One of the non-essential heavy metals, mercury (Hg), is a widespread toxic pollutant commonly found in drinking water sources. Therefore, there is a need to monitor the level of Hg in the drinking water. Biomonitoring, which use biological response to assess environmental changes, is one of the approaches that are getting more attention recently. Plant bioindicator offers huge advantages over conventional water quality analysis. Other than cheap, easy to apply and give rapid results; most importantly, people in the rural area can apply this method to monitor water quality without the need of modern equipment and technical expertise. In this study, young leaves from terrestrial plants were treated with water containing Hg solutions. Plant that showed visible morphological changes was selected as potential bioindicator and further analyzed. Over 60 plants were screened including herbs, shrubs and flowering plants. At the 24th h of observation, 15 plants showed morphological changes with several obvious symptoms, including the presence of dark spot, chlorosis, browning of leaves and wrinkle. Among these plants, *Plectranthus amboinicus*, *Lantana camara* and *Pelargonium radula* treated with Hg solutions exhibited morphological changes at the 6th h of treatment, compared to the control. In the present study, *Pelargonium radula* was chosen as the Hg bioindicator as it gave the fastest and visible morphological changes, which is within 4 h of treatment. This new finding was promising, as it demonstrated that plants could be used for Hg biomonitoring for the safety of drinking water.

Keywords: Plant bioindicator, biomonitoring, Hg, drinking water, Pelargonium radula

1.0 INTRODUCTION

Water is essential to all life forms and geological process as it plays a critical role in all our daily activities such as irrigation, industrial, thermoelectric power, aquaculture and domestic uses; the demand for water increased sharply every year. In Malaysia alone, total demand for water is expected to reach 14 billion m^3 in 2020, and almost 99% of it comes from rivers and stream in the country [1]. Other than external uses of water, the most important purpose of water is as drinking water. However, water supply management in Malaysia is not centralized as it is managed according to state-by-state basis [2]. Water quality problem arose as a result from anthropogenic activities. Thus, maintaining the quality of water is crucial to ensure its safe consumption which literally should be free from any detrimental substances, such as microorganisms, heavy metals and any inorganic compound [3].

Of all causes, metals contaminants had widely reported to be one of major reasons that lead to water pollution [4, 5]. Heavy metals are commonly introduced into the environment through mining practices as well as manufacturing processes. Hg, in particular, can spread from contaminated to uncontaminated sites through expulsion from refineries and factories, runoff from landfills and croplands; and as dust from coal-fired power plants [6]. This situation became worsen as rain transports this metal from atmosphere and soil to the water bodies; particularly ground water and rivers.

Like other metals contaminants, Hg is harmful to human beings due to their long biological half-life and the tendency to accumulate in the human body. Hg is a non-essential element for the growth of plants and animals, and it does not involve in cell metabolism process [7]. Human generally exposed to Hg via consumption of contaminated fish in the form of methylmercury or by breathing the vaporous Hg [7, 8]. The most well documented cases of methylmercury poisoning are from Minamata Bay, Japan in 1956, which killed hundreds of people and causing thousands of them suffered with permanent nervous system damage [8]. In milder cases, mercury poisoning will reduce the motor skills in adults; and stunt the development of fetuses and newborn babies [6]. Therefore, there is a need to monitor the level of Hg in the drinking water.

In order to ensure the quality of drinking water, several approaches were introduced; including physical, chemical and biological monitoring techniques. In recent years, detecting environment abnormalities with the helps from biological materials including fishes, mollusks and plants has received considerable attention [9]. The usage of this method, or in other word; bioindicators are environmental friendly, cost effective and provide simpler alternative than conventional techniques for determining the Hg. level in drinking water [10]. In this paper, plant bioindicator was chosen to monitor the drinking water quality as it has the capability to accumulate various pollutants, respond rapidly to environmental oddities and readily available [11]. The efficiency of using plants as bioindicator has been proven by several studies. According to [12], *Helianthus annus* has been successfully used in the detection of lead in the environment. *Rosa indica* and *Dianthus caryophyllus* are good bioindicator in detecting air pollution [13]. In addition, *Phaseolus vulgaris* is able to perceive the presence of cadmium in the environment [14]. The effectiveness of plant bioindicator relies on the plants species and metal bioavaibility.

The best bioindicator typically shows rapid response to drinking water containing metal contaminants that is beyond the tolerable limit upon exposure [10]. Sensitive plants produce instant observable visual symptoms of toxicity, including changes in shape (e.g. wilting, curling of leaves and desiccation), color (e.g. appearance of black spot, chlorosis, and browning of leaves) and other possible changes shown on leaves [15]. These changes arise because metal contaminants may disturb the biochemical, physiological and metabolic process in plants [16]. These alterations may disturb the cellular mechanisms, and eventually lead to cell death [17]. Thus far, Hg plant bioindicators are not available for biomonitoring. Thus, this study aims to screen for potential plant species that can be used as mercury bioindicator for safety assessment of drinking water.

2.0 EXPERIMENTAL

Preparation of Hg solution

Hg stock solution of 10 ppm was prepared from the standard solution of 1000 ppm (Merck, Darmstadt, Germany). Drinking water containing heavy metal was prepared according to the acceptable limit as set in the National Drinking Water Guideline (Table 1). In order to observe the severity of leaves as the concentration increase, concentrations that double and half the acceptable limit were also been prepared. Ultrapure water without addition of Hg was used as controls.

Table 1 Concentrations of Hg used in this study

Acceptable limit according the National Drinking Water Guideline [18]	Hg concentrations (ppb)
Half of the acceptable limit	0.5
According to the acceptable limit	1.0
Double of the acceptable limit	2.0

Sampling of leaves

Young leaves from different types of plants (shrub, flowers, vegetables, herbs and trees) was collected from various places including Taman Pertanian Kuantan, Pusat Fertigasi Kuantan, Vegetable Farm Gambang, MARDI Serdang and MARDI Cameron Highland. The young leaves were translucent, in light green color, smaller in size, and were located at the shoot tips, compared to the mature leaves. The leaves were plucked and submerged into tubes containing different concentrations of Hg solution. Each tube contains at least five leaves; depending to the availability of leaves. All treatments were done in triplicate.

Observation of morphological changes on leaves

Observable morphological changes of leaves including wrinkle, chlorosis, presence of dark sport, change of color were observed at different time interval: 0, 6, 12, 18 and 24 h, right after the leaves were submerged into the Hg solution. Hg-treated leaves were compared with the control. All visible changes shown on leaves and time when the changes occurred were recorded.

Identification of potential Hg bioindicator

Plants that showed morphological changes in 6 h of Hg treatment were further screened and time when the changes occurred was recorded at every 1 h interval. Plant that show morphological changes at the shortest time was selected as potential Hg bioindicator.

3.0 RESULTS AND DISCUSSION

Leaves subjected to Hg solution suffered clear symptoms of phytotoxicity. The morphological changes became more severe as the Hg concentration became higher [19]. In this study, 63 plant species were screened. Fifteen plant species exhibited visible morphological changes within 24 h of Hg treatment (Table 2). Different plant species have different sensitivity level towards metal stresses. The degree of plant sensitivity to metal toxicity depends on the external and internal factors, including the bioavaibility of metals, plant genotypes, exposure limit and period, as well as physiological and morphological condition of plants [20, 21]. Various morphological changes were observed from these plant species; including color changes from green to yellowish-brown or dark brown, dark spots appearance, petiole and veins darkening, chlorosis and wilting.

Table 2 List of plants that showed morphological changes within 24 h of Hg treatment

Plants	Time when morphological changes was observed (h)			
	6	12	18	24
Antigonon leptopus		✓	✓	✓
Bougainvillea glabra				✓
Cosmos caudatus			✓	✓
Cucumis sativus		✓	✓	✓
Hibiscus rosa sinensis		✓	✓	✓
Ipomoea aquatic		✓	✓	✓
Ixora coccinea				✓
Lantana camara	✓	✓	✓	✓
Pelargonium radula	✓	✓	✓	✓
Piper betle		✓	✓	✓
Piper sarmentosum		✓	✓	✓
Plectranthus amboinicus	✓	✓	✓	✓
Spondias dulcis			✓	✓
Stevia rebaudiana		✓	✓	✓
Syzgium myrtifolium		✓	✓	✓

An ideal bioindicator is able to respond rapidly to metal toxicity and exhibit obvious morphological changes that can clearly been seen by naked eyes [10]. Thus, further screening was undergone for three plant species that showed changes within 6 h observation. *Pelargonium radula* gave the fastest morphological changes in the presence of 0.5, 1.0 and 2.0 ppb Hg, which at the 4^{th} h, followed by *Plectranthus amboinicus* and *Lantana camara* at the 5^{th} and 6^{th} h, respectively. Thus, *P. radula* was selected as the Hg plant bioindicator in the present study. As seen in Figure 1, *P. radula* treated with Hg experienced colour changes from green to yellowish-brown, chlorosis and wilted, in comparison to the control. The difference of the morphological changes in Hg-treated leaves was obvious and can be distinguished clearly, compared with the untreated leaves. As the concentration of Hg increases, the severity of Hg toxicity increases. These visible symptoms could also be seen in cabbage treated cadmium as they exhibited chlorosis, appearance of black spots and reddish-purple coloration along the leaf margin [22]. In *Spinacea oleracea* L., dark spots along the veins appeared upon exposed to nickel and cadmium [23].

Toxicity exhibited by metal elements commonly involves neurotoxicity, hepatotoxicity and nephrotoxicity [24]. Specific toxicity conditions in different plant species occurred due to variations in metal solubility, absorbability, transport and chemical reactivity towards metal [25]. The degree of metal tolerance mechanisms in plants differs from one to another. According to [26], two tolerance mechanisms are involved: internal tolerance mechanism and exclusion mechanism that protect plants from metal toxicity. The internal tolerance mechanism mainly involved the immobilization, compartmentalization and detoxification of metals in the symplasm with the aid from metal binding compounds. Meanwhile, exclusion mechanisms focus on the prevention of metals from entering or staying in the symplasm and interacting with other sensitive organelles in plants [27].

P. radula is a great garden plant (Figure 2). It is normally used as one of cooking ingredients, pest control and fragrance by the Asian communities. It has unique leaves shape and smell. Locally, *P. radula* is known as 'Jeramin' or 'Pokok Halau Nyamuk'[28]. It is popularly used as mosquitoes repellent as it contain terpene alcohols, which is believed to poses insect repellent activity [29]. *P. radula* is also widely used for interior landscape of building and indoor garden. *P. radula* belongs to the family of Geraniaceae and genus of *Pelargonium*. However, the potential of *P. radula* as a bioindicator has not been discovered. *P. radula* originates from Malaysia and thus is readily and widely available for environmental biomonitoring purposes.

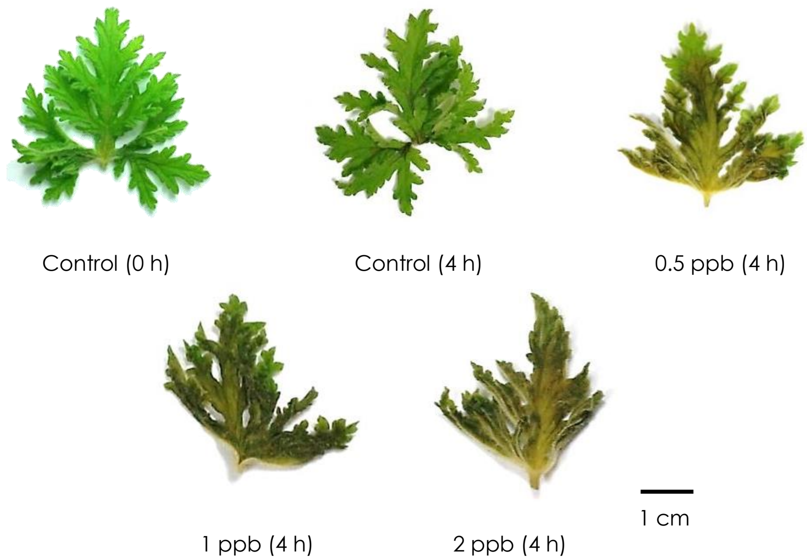

Figure 1 *P. radula* treated with different concentration of Hg solutions

Figure 2 *P. radula*

Metal toxicity is well-known to be responsible for many visual morphological changes in plants. Time taken for different plant to exhibit changes may due to the physiology of plants. Generally, leaves have cuticle layers, which protect them from environmental abnormalities. However, the cuticles layers of different leave differ in thickness. Thick cuticle layer is covered by wax layer and chlorenchymatous rind cells. These rind tissues are interconnected with parenchymatous tissues, which are assumed to be associated with plant defensive strategy [30]. In this study, younger leaves were chosen as they lack cuticle compounds. As a result, younger leaves have lower tolerance mechanisms to the presence of heavy metals, giving metals a higher chance to dominate the leaves and affect their system. Compared to mature leaves, younger leaves also contain lower amount of phenolic compound, which is responsible for adaptation and defense mechanisms towards metal stress [31].

In general, excess amount of Hg causes greater toxicity symptoms in leaves. This is due to disturbance of the metabolism processes including chlorophyll and protein content reduction, respiration inhibition, organelles' ultrastructure modification and enzymes activities alteration [32]. Heavy metals greatly affect the photosynthetic processes in leaves by disrupting the sensitive components of photosynthetic structures. Chlorophyll is the most important chloroplast components for photosynthesis, and is well known as a main light-absorbing pigment that gives plant its color [33]. Chlorophyll reduction in leaves mainly connected to its biosynthesis. The replacement of Ca^{+} and Na^{+} by metal ions on the thylakoid membrane tends to destroy its structure, causing it to lose its function. This would alter the enzymatic system of the chloroplasts and block its synthesis [34]. The sub-microstructure of chloroplasts will also be destroyed, lowered the capacity of protons capture in PS I and PS II. [35, 36]. As demonstrated in *Hydrocharis dubia* L. and *B. schreberi*, thylakoid membrane of chloroplasts was degraded upon exposure to Hg and cadmium. The alteration and destruction of chlorophyll structure and system in leaves is reflected by the color changes of leaves [37].

Additionally, the decrease of chlorophyll synthesis rate in leaves is also due to the deficiency of some essential elements such as iron and magnesium [37]. The chlorophyll pigment in the leaves may also be destructed by the action of constitutive peroxidases, lipoxygenase and fatty acid-dependent with chlorophyll-bleaching activities in the presence of phenolic compound, linolenic acid and H_2O_2 [38]. Grana cascade, mitochondria and thylakoid membrane of chloroplasts were shown to be altered and degraded under metal stress. Changes in mitochondria structures were a result of the penetration of K^+ and H_2O from lumen to the outside, and disruption of ATP activities.

In general, heavy metals induce oxidative damage in plants; directly or indirectly through the formation of superoxide radical (O^{2-}), hydrogen peroxide (H_2O_2) and singlet oxygen (O_2), which are collectively known as reactive oxygen species (ROS). ROS rapidly attack the molecules and structures in cells [21]. Over accumulation of ROS in leaves trigger the oxidative process including protein oxidation, membrane lipid peroxidation, enzyme inhibition, as well as DNA and RNA damage. These alterations then lead to the inability of leaves to synthesize protein and accelerate protein denaturation [39]. Furthermore, competition and replacement of essential ions with metal ions to the sulphydryl (SH) groups of the active sites of proteins cause disruption in protein functions, structures and metabolic processes in cells [40]. This also directly affects photosynthetic system in plants [32]. Ultimately, these alterations lead to permanent metabolic dysfunction in cells and cell death [41].

4.0 CONCLUSION

Among the 63 plants screened in this study, *P. radula* was chosen as the best bioindicator to detect the presence of Hg in drinking water. *P. radula* treated with Hg exhibited morphological changes including changes of color from green to yellowish-brown, chlorosis and became wilted within 4 h of Hg treatment. Hg toxicity became more severe as the concentration of Hg increased, and duration of exposure to Hg increased.

Acknowledgement

The authors would like to acknowledge the Ministry of Higher Education Malaysia (MOHE) for the Fundamental Research Grant Scheme (FRGS13-034-0275) as a financial support throughout this study. Grateful thanks also keen to the Taman Pertanian Kuantan, Pusat Fertigasi Kuantan, Vegetable Farm Gambang, MARDI Serdang and MARDI Cameron Highland board for the sample collections consent during this study.

References

[1] Azrina, A., Khoo, H. E., Idris, M. A., Ismail, A. and Razman, M. R. 2011. *Evaluation of Selected Metal Elements in Commercial Drinking Water and Tap Water in Peninsular Malaysia. Jurnal Sains Kesihatan Malaysia*. 9(1): 5-11.

[2] Chan, N. W. 2009. Issues and Challenges in Water Governance in Malaysia. *Iranian Journal of Environmental Health, Science and Engineering*. 6(3): 143-152.

[3] Khoo, H. E., Azrina, A., Idris, M. A., Ismail, A. and Razman, M. R. 2011. Evaluation of Selected Metal Elements in Commercial Drinking Water and Tap Water in Peninsular Malaysia. *Jurnal Sains Kesihatan*. 9(1): 5-11.

[4] Kita, G. and Skoblewski, P. 2010. Biosorption of Heavy Metals – Modern and Cheap Method of Polluted Wastewater Treatment. *Food Chemistry and Biotechnology*. 74: 99-105.

[5] Shahmansouri, M. R., Pourmoghaddas, H. and Shams, G. 2003. Leakage of Trace Metals by Internal Corrosion into Drinking Water Distribution System. *Diffuse Pollution Conference Dublin 2003*. 96-99.

[6] Khan, A. G., Kuek, C., Chaudhry, T. M., Khoo, C. S. and Hayes, W. J. 2000. Role of Plants, Mycorrhizae and Phytochelators in Heavy Metal Contaminated Land Remediation. *Chemosphere*. 197-207.

[7] Duruibe, J. O., Ogwuegbu, M. O. C. and Egwurugwu, J. N. 2007. Heavy Metal Pollution and Human Biotoxic Effects. *International Journal of Physical Sciences*. 2(5): 112-118.

[8] Emara, A. M and Zayet, H. H. 2011. Major Chemical Disasters in Egypt and Abroad Part 1: Abroad. *Egyptian Journal of Occupational Medicine*. 35(2): 237-255.

[9] Zurayk, R., Sukkariyah, B. and Baalbaki, R. 2001. Common Hydrophytes as Bioindicators of Nickel, Chromium and Cadmium Pollution. *Water, Air, and Soil Pollution*. 127: 373-388.

[10] Carignan, V. and Villard, M-A. 2002. Selecting Indicator Species to Monitor Ecological Integrity: A Review. *Environmental Monitoring and Assessment.* 78: 45-61.

[11] Lasat, M. M. 2000. Phytoextraction of Metals from Contaminated Soil: A Review of Plant/Soil/Metal Interaction and Assessment of Pertinent Agronomic Issues. *Journal of Hazardous Substance Research*. 2: 1-25.

[12] Krystofova, O., Shestivska, V., Galiova, M., Novotny, K., Kaiser, J., Zehnalek, J., Babula, P., Opatrilova, R., Adam, V. and Kizek, R. 2009. Sunflower Plants as Bioindicators of Environmental Pollution with Lead (II) Ions. *Sensors*. 9: 5040-5058.

[13] Saxena, P. and Ghosh, C. 2013. Ornamental Plants as Sink and Bioindicators. *Environmental Technology.* 34(23): 3071-3079.

[14] Benabid, H., Ghorab, M. F. and Djebaili, A. 2008. Cadmium as an Environmental Pollutant Use of Plant as Bio-Indicator of Pollution (*In vivo* Experimentation) Influence of Cadmium on Chlorophyll Content of Canadian Wonder Beans (*Phaseolus vulgaris*). *Research Journal of Applied Sciences.* 3: 66-69.

[15] Dobroviczká, T., Piršelová, B. and Matušíková, I. 2012. The Effect of Cadmium Epidermis of Leaves of Two Soybean Varieties. *International scientific conference of PhD students and young scientists and pedagogues*. Retrieved from http://conferences.ukf.sk/index.php/phdconf.

[16] Agrawal, V. and Sharma, K. 2006. Phytotoxic Effects of Cu, Zn, Cd and Pb on In Vitro Regeneration and Concomitant Protein Changes in *Holarrhena antidysenterica*. *Biologia Plantarum*. 50(2): 307-310.

[17] Sharma, P. and Dubey, R.S. 2005. Lead Toxicity in Plants. *Brazilian Journal of Plant Physiology*. 17(1): 35-52.

[18] Ministry of Health. 2004. National Standard for Drinking Water Quality. Engineering Service Division Ministry of Health, Malaysia.

[19] Rellán-Álvarez, R., Ortega-Villasante, C., Álvarez-Fernández, A., Del Campo, F.F. and Hernández,

L.E. 2006. Stress Responses of *Zea mays* to Cadmium and Mercury. *Plant and Soil.* 279: 41-50.

[20] An, Y. J. 2004. Soil Ecotoxicology Assessment Using Cadmium Sensitive Plants. *Environmental Pollution*. 127: 21-26.

[21] Shu, X., Yin, L., Zhang, Q. and Wang, W. 2012. Effect of Pb Toxicity on Leaf Growth, Antioxidant Enzyme Activities, and Photosynthesis in Cuttings and Seedlings of *Jatropha curcas* L. *Environmental Science Pollution Research*. 19: 893-902.

[22] Pandey, N. and Sharma, C.P. 2002. Effect of Heavy Metals Co^{2+}, Ni^{2+} and Cd^{2+} on Growth and Metabolism of Cabbage. *Plant Science*. 163: 753-758.

[23] Pandey, N., Pathak, G. C., Pandey, D. K. and Pandey, R. 2009. Heavy Metals, Co, Ni, Cu, Zn and Cd, Produce Oxidative Damage And Evoke Differential Antioxidant Responses In Spinach. *Brazilian Journal of Plant Physiology*. 21(2): 103-111.

[24] Benavides, M. P., Gallego, S. M. and Tomaro, M. L. 2005. Cadmium Toxicity in Plants. *Braz. J. Plant Physiol*. 17(1): 21-34.

[25] Viehweger, K. 2014. How Plants Cope with Heavy Metals. *Botanical Studies*. 55: 35.

[26] Yang, Y. Y., Jung, J. Y., Song, W. Y., Suh, H. S. and Lee, Y. 2000. Identification of Rice Varieties with High Tolerance or Sensitivity to Lead and Characterization of the Mechanism of Tolerance. *Plant Physiology*. 124: 1019-1026.

[27] Hue, N. V., Craddock, G. R. and Adams, F. 1986. Effect of Organic Acids on Aluminium Toxicity in Subsoils. *Soil Science Society of America Journal.* 50: 28-34.

[28] Zuraida, A. R.,Mohd Shukri, M. A., Erny Sabrina, M. N. and Ayu Nazreena, O. 2015. Improvement of Regeneration of Pelargonium radula via Somatic Embryogenesis. *British Biotechnology Journal*. 5(4): 166-173.

[29] James, K. S. 2003. *Pelargonium* species. In: Balchin, M. L. Geraniums or Pelargonium: *The Genera Geranium and Pelargonium*. New Fetter Lane, London: Taylor and Francis. 11-15.

[30] Coopoosamy, R. M. and Naidoo K. K. (2011). Anatomical Features of the Medicinal Importance of *Aloe excelsa* (berger). *African Journal of Biotechnology*. 10(39): 7622-7632.

[31] Raven, P. H. and Johnson, G. B. 2002. *Biology*. 6th Edition. Boston, Massachusetts: McGraw-Hill. 807-836.

[32] Yruela, I. 2005. Copper in Plants. *Brazilian Journal of Plant Physiology.* 17(1): 145-156.

[33] Percival, G. C., Keary, I. P. and Noviss, K. 2008. The Potential of a Chlirophyll Content SPAD Meter to Quantity Nutrient Stress in Foliar Tissue of Sycamore (*Acer pseudoplatanus*), English Oak (*Quercus robus*), and European Beech (*Fagus sylvatica*). *Arboriculture and Urban Forestry*. 34(2) 89-100.

[34] Brinza, L., Nygård, C. A., Dring, M. J., Gavrilescu, M. and Benning, L. G. 2009. Cadmium Tolerance and Adsorption by the Marine Brown Alga *Fucus vesiculosus* from the Irish Sea and the Bothnian Sea*. Bioresource Technology*. 100: 1727-1733.

[35] Hao, H. Q., Shi, G. X., Du, K. H. and Xu, Q. S. 2001. Toxic Effect of Hg^{2+} on Physiology and Ultrastructure of *Hydrocharis dubia. Journal of Lake Sciences*. 13(2): 163-168.

[36] Chen, Y. 1999. Bioremediation of Pollution in Soil. *Progress of Environmental Science*. 2: 7-11.

[37] Cheng, S. 2003. Effects of Heavy Metals on Plants and Resistance Mechanisms. *Environmental Science and Pollution Research*. 10(4): 256-264.

[38] Matile, P., Hortensteiner, S. and Thomas, H. 1999. Chlorophyll Degradation. *Annual Review of Plant Physiology and Plant Molecular Biology.* 50: 67-95.

[39] Khadijeh, B., Bahman, K. and Ali, M. 2011. Effects of Cadmium on Growth, Protein Content and Peroxidase Activity in Pea Plants. *Pakistan Journal of Botany.* 43(3): 1467-1470.

[40] Azevedo, R. and Rodriguez, E. 2012. Phytotoxicity of Mercury in Plants: A Review. *Journal of Botany*. 1-6.

[41] Farid, M., Shakoor, M. B., Ehsan, S., Ali, S., Zubair, M. and Hanif, M. A. 2013. Morphological, Physiological and Biochemical Responses on Different Plant Species to Cd Stress. *International Journal of Chemical and Biochemical Sciences.* 3(2013): 53-60.