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Original Research Article

Bioaccumulation of Mercury and Assessment of Suitable Biomarker in Vegetables Collected from East Calcutta Garbage Farming Area (*Dhapa*), India

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Abstract

The aims of the present study were to estimate mercury accumulation in the vegetables like *Ipomea aquatica* (Water Spinach), *Hygrophila spinosa* (locally known as *Kulĕkhära*), *Brassica oleracea capitata* (Cabbage), *Brassica oleracea botrytis* (Cauliflower), *Spinacea oleracea* (Spinach) and *Raphanus sativus* (Raddish) collected from East Calcutta Garbage farming area (88°20' - 88°35' E; 22°25' - 22°40' N) and also to find out the biochemical changes that has been taken place in those vegetables. In this multi-parametric approaches ascorbic acid, catalase, lipid peroxidation and non protein thiol content were measured. All oxidative stress parameters were positively correlated to mercury concentrations except non protein thiol content which exhibits negative correlation with the mercury accumulation in all collected samples. Since the results suggest that the non protein thiol content are sensitive parameters, could be useful as oxidative stress biomarkers in vegetables exposed to the mercury contaminated environment.

Keywords

Bioaccumulation
Biomarker
East Calcutta
Garbage farming area
Mercury

Introduction

Mercury is one of the major soil pollutants released by anthropogenic activities like mining, smelting, sewage sludge disposal and application of mercury containing fungicides. Since 1960's the mercury accumulation had received attention as a public health concern. Elevated concentrations of mercury have been found in vegetables grown in vicinity of chlor-alkali plants, sewage irrigated arable land and contaminated mining areas (Qian et al., 2009). Mercury tolerant plant species alter their biochemical path ways, involving different enzymes acting as metal chelators and detoxifiers (Cho et al., 1997). Bio-monitoring by tolerant plant has been widely used to assess the soil metal contamination. Presence of secondary metabolites, enzymes for detoxification and oxidative stress are common physiological biomarkers of metal contamination, which seem to be applicable in ecotoxicological diagnostic (Agarwal et al., 2007). Mercury is a transition metal able to induce oxidative stress in plants, which leads to lipid peroxidation, K^+ leakage, alteration of antioxidant enzyme activities and induction of thiol-containing compounds (Ali et al., 2000). The mercury induced oxidative damages in plants are linked with production of excess reactive oxygen species (Cho and Park, 2000) and the mercury induced genes are responsible to produce enzymes like superoxide dismutase, peroxidase and catalase (Sävenstrand and Strid, 2004). The molecular mechanisms of mercury induced oxidative damage and tolerance in plants have not been fully understood (Zhou et al., 2007).

Like other heavy metals mercury generally cause damage to plants, either directly or indirectly by triggering an increased level of production of reactive oxygen species (ROS).These ROS include superoxide radical, hydroxyl radical and hydrogen peroxide that are produced as byproducts during membrane linked electron transport activities as well as by a number of metabolic pathways (Baker et al, 2001). Plants have antioxidant systems to protect them against oxidative damage. In natural habits plants are surrounded by their natural enemies, the plant biochemical system actually produces some secondary metabolites and overcome the existing stress (Mazid et al., 2011). Some other *in vitro* study reported that *Paspalum vaginatum* absorbs mercury and accumulate in their tissues (Alrawiq and Mushrifah, 2013). Wang et al. (2014) also reported total mercury accumulation in the rice plants growing near coal mine areas and investigators also revealed that plant adsorbed mercury directly from the contaminated atmosphere. This may impose the biochemical change in the plant tissues (Fernández-Martíneza et al., 2015; Salem and Idris, 2015).

Kolkata, the capital of the State West Bengal, India is one of the major commercial and industrial cities of Eastern India. The city generates 3100 metric tons of solid waste per day, which are disposed in the *Dhapa* dumping ground (88°20' - 88°35' E; 22°25' - 22°40' N) for several decades. Part of this dumping ground (approximately 800 acre) irrigated by naturally treated effluent of Kolkata are utilized as garbage farmyard by the local people, producing at least 15 different types of vegetables round the year (Bhattacharyya and Santra, 2003). Bioaccumulation of mercury has been reported in the fish collected from sewage fed fisheries of this region (Bhattacharyya et al., 2010).

The aim of the present study was to investigate mercury accumulation level and to assess a few oxidative stress parameters of some vegetables collected from East Calcutta garbage farming area. The other objective was to establish appropriate relation in mercury concentration and oxidative stress parameters which help to identify a suitable biomarker of mercury pollution in the study area.

Materials and methods

Sampling and sample preparation

Thirty four plant samples of *Ipomea aquatica* (Water Spinach), *Hygrophila spinosa* (locally known as Kulekhara), *Brassica oleracea capitata* (cabbage), *Brassica oleracea botrytis* (cauliflower), *Spinacea oleracea* (spinach) and *Raphanus sativus* (radish) were collected from the East Calcutta garbage farming area (88°20' - 88°35' E; 22°25' - 22°40' N) round the year for determining total mercury level. Candipur village of Notrh 24 Parganas (22°49'15.43"N 88°47'20.17"E) was selected as control site, because no garbage farming and sewage water irrigation was practiced in this area (Fig. 1). About 1 gm of sample was taken for acid digestion by using Bethge's apparatus (Bhattacharyya et al., 2010).

Determination of total mercury

The mercury was determined by a cold-vapour atomic absorption spectrophotometer (Mercury Analyzer-5840, ECIL, detection limit 0.1μgm/l) at 253.6 nm using 20 % $SnCl_2$ and 10% HNO_3. Relative standard solution was run with the samples simultaneously to check the precision of the instrument. A quantitative recovery of standard used during analysis was 95% and the accuracy and precision data were found to be ± 5 μg m/l.

Assessment of stress parameters

Twenty percent (Weight/volume) of fresh plant extract were prepared by using oxalic acid, phosphate buffer, 20% trichloracetic acid and 6% trichloracetic acid for estimation of ascorbic acid, catalase, lipid peroxidation and non protein thiol content respectively. Ascorbic acid was estimated by using 2, 6 dichlorophenol indophenol (Sadasivam and Manickam, 1991); catalase was measured following the method of Chance and Machley (1955); lipid peroxidation was measured colorimetrically by using thioberbituric acid (Rellan-Alvarez et al., 2006) and 5, 5 bis dinitro thiobenzoic acid was used to measure non protein thiol content of the vegetable extract (Ellman et al., 1961).

Fig. 1: Map of the study area with control site.

Statistical analysis

Student t-test was performed and results were expressed as the mean ± S.D., and the value of $p<0.05$ was considered significant. Pearson correlation between mercury content and oxidative stress parameters was also calculated. The Wilcoxon Ranked-Sum test was used to determine the statistical significance of differences ($p>0.05$) between mercury concentration in vegetables at test and control sites.

Results

The mercury concentrations in collected vegetables grown in the East Calcutta garbage farming area are higher than that of the uncontaminated control areas (Table 1). The Lowest accumulation was observed in *Spinacea oleracea* followed by *Hygrophila spinosa*, *Raphanus sativus*, *Brassica oleracea botrytis*, *Ipomea aqutica* and *Brassica oleracea capitata.*

Four oxidative stress parameters namely ascorbic acid, catalase, lipid peroxidation and non protein thiol content has shown significant distinguishable profile of action with compare to the vegetables collected from control area. The ascorbic acid content of the test site decrease in all vegetables except *Brassica oleracea capitata* and *Raphanus sativus* (Fig. 2). In *Raphanus sativus* the ascorbic acid content remain almost unaltered whereas it was increased in *Brassica oleracea capitata* with respect to control site. Catalase was found to be higher in test vegetables like *Brassica oleracea capitata, Raphanus sativus* and *Ipomea aquatica* but lower in *Brassica oleracea botrytis* and *Spinacea oleracea* with respect to the control (Fig. 2). Some difference in test samples is also observed in lipid peroxidation study with compare to control site where test vegetables of *Ipomea aquatica, Brassica oleracea capitata, Brassica oleracea botrytis, Spinacea oleracea* and *Raphanus sativus* expressed lower lipid peroxidation activity than the control site (Fig. 2).The total non protein thiol content, however, has been found to be lower in all samples collected from the East Calcutta garbage farming area with compare to control area (Fig. 2).

Table 1. Status of mercury concentration in collected vegetables with Indian[1] and WHO[2] standard.

Sample	Mercury level (mg/kg)		Standard (mg/kg)
	Control	Test	
Ipomea aqutica	0.001	0.54	1.0[1]
Hygrophila spinosa	0.15	0.38	
Brassica oleracea capitata	0.25	0.634	0.23 – 0.47 / day[2]
Brassica oleracea botrytis	0. 12	0.52	
Spinacea oleracea	0.09	0.21	
Raphanus sativus	0.08	0.47	

[1] World Health Organization, 1990; [2]PFA Act, 1954.

Fig. 2: Ascorbic acids (mg/g), lipid peroxidation (% change/g), non-protein thiol content (micromole/g) and catalase (%) in vegetables collected from both test and control site.

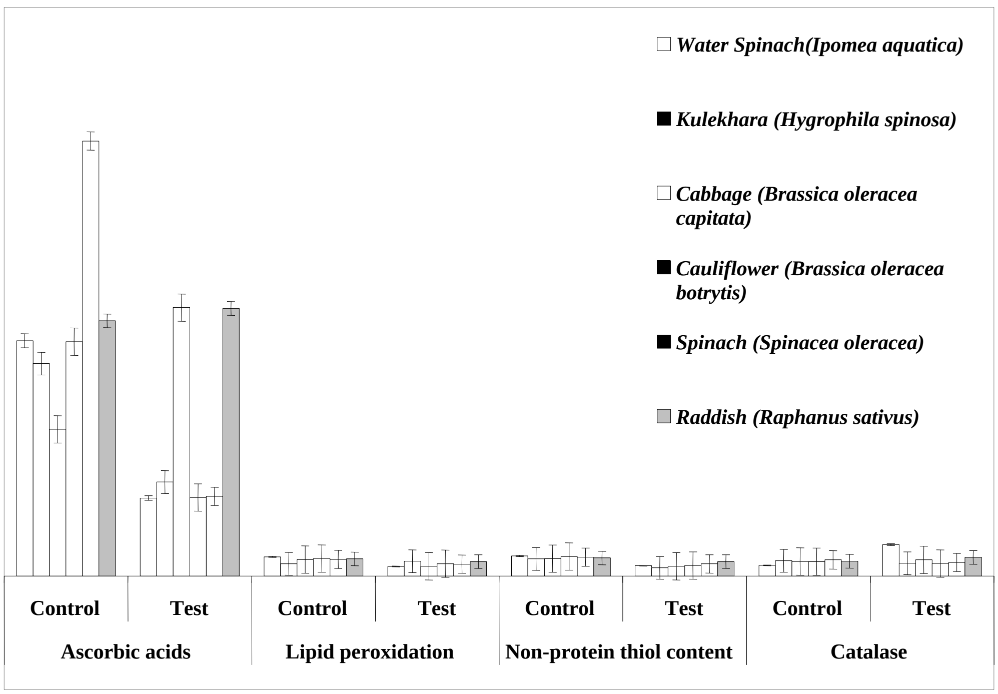
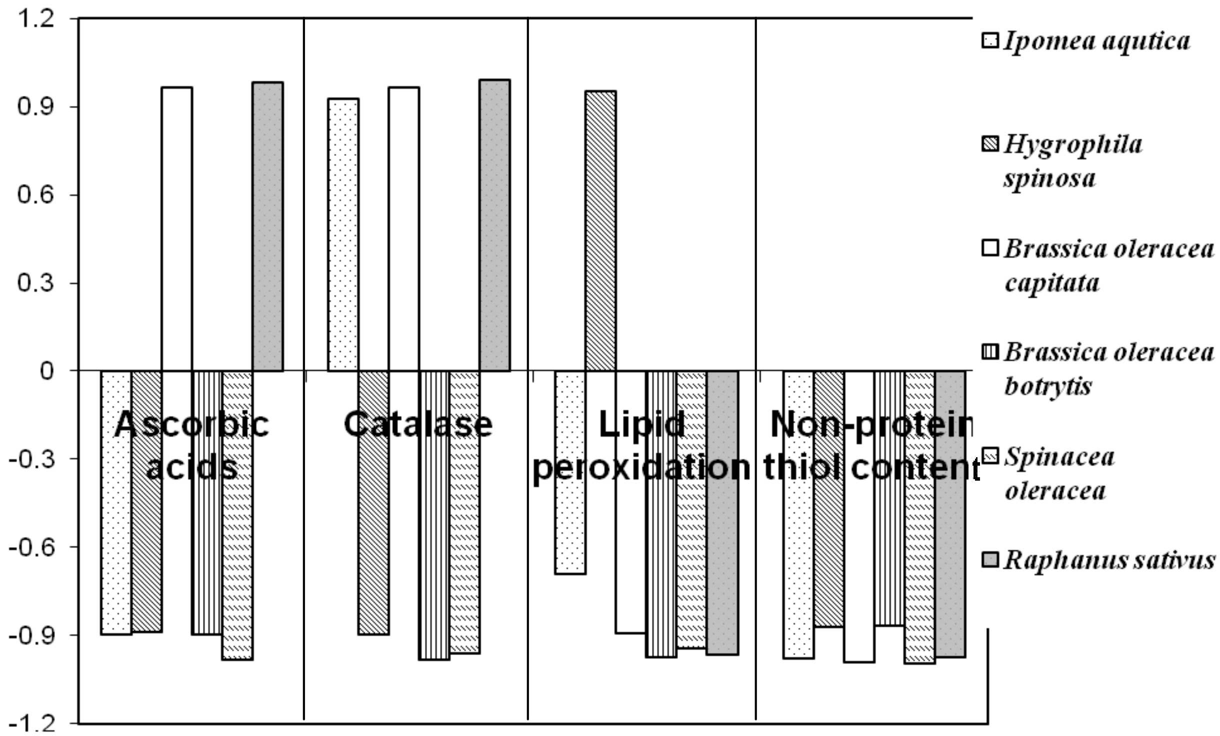


Table 2. Pearson Correlation values of four oxidative stress parameters with the total mercury content in plant tissues.

Plant species	Ascorbic acids	Catalase	Lipid peroxidation	Non-protein thiol content
Ipomea aqutica	-0.898	0.9255	-0.691	-0.9797
Hygrophila spinosa	-0.8896	-0.8963	0.9527	-0.87
Brassica oleracea capitata	0.9633	0.9642	-0.8922	-0.9924
Brassica oleracea botrytis	-0.8991	-0.9852	-0.973	-0.8689
Spinacea oleracea	-0.9824	-0.9604	-0.9447	-0.9972
Raphanus sativus	0.9815	0.9879	-0.9676	-0.9764

Fig. 3: Comparative study of Pearson Correlation between four oxidative stress parameter and mercury content in vegetables collected from East Calcutta garbage farming area.



The statistical result showed that only non protein thiol content was negatively correlated with mercury concentration among all collected vegetable samples where as the other stress metabolites like ascorbic acids, catalase and lipid peroxidation were not shown consistence correlation with the tested vegetable (Table 2 and Fig. 3).

Discussion

The test results revealed that total mercury accumulation was higher in study area in compare with control site. Bakare and his co-workers (2004) reported considerable amount of mercury in both fresh and boiled vegetables collected from a Nigerian farm situated beside the highway. An *in-vitro* study reported that mercury was accumulated in both root and stem of three plant species (Manikandan et al., 2015). The findings of the present study are also comparable with the works of Gothberg et al. (2002) Qian et al. (2009) and Meng et al. (2014) where the presence of mercury in vegetables was due to wastewater irrigation soil contamination and crop collected from contaminated mining areas. East Calcutta garbage farming area is an old dumping ground and data of mercury contamination was previously reported (Bhattacharyya et al., 2010). After analysis of Wilcoxon Ranked-Sum test, it could be inferred that vegetables from garbage farming areas produces significant difference with the agriculture practices in the rural areas (p>0.05). *Brassica oleracea capitata* has shown the highest mercury level in their tissue and all others of them are within the regulatory limit/permissible level for human consumption (1.00 µg mercury /gm of plant tissue) as recommended by the statutory bodies of United Kingdom, United States (Gammons et al., 2006), The Prevention of Food Adulteration Act in India (PFA, 1954) amended in 2002 and World Health Organization (1990). Consumption of those vegetables is supposed to be safe even for pregnant women also.

Another objective of the present study was to identify suitable mercury sensitive biochemical marker. For this purpose a multiparametric approach was followed and After student t-test it could be inferred that the level of all four oxidative stress parameters of test site has significant variation with the vegetable collected in the uncontaminated rural areas (p>0.05). The result of Pearson correlation between mercury bioaccumulation and oxidative stress has shown significant inference for the current paper (Fig. 3). Except ascorbic acid content of *Brassica oleracea capitata* and *Raphanus sativus*; catalase activity of *Ipomea aquatica* and *Raphanus sativus* and lipid peroxidation level of *Hygrophila spinosa*, all other collected vegetables were negatively correlated with oxidative stress parameters (Fig. 2). Almost similar result was observed by Rellan-Alvarez et al. (2006) where they observed change in ascorbic acids, lipid peroxidation and non protein thiol content in their mercury induced test plants. The current variation in catalase activity and lipid peroxidation data is also similar to the work of Cargnelutti et al. (2006). They concluded that bioaccumulation of mercury in plant cells trigger oxidative metabolic pathway. The other contemporary study revealed that mercury trigger antioxidant defense mechanism by producing stress metabolites in plant cells (Sobrino-Plata et al., 2009).

All this supporting literature and current correlation study help to prepare stress indices of the plant grown in mercury contaminated area. Mercury induced toxicity symptoms and plant's resistance to mercury was well studied (Moreno-Jimenez et al., 2009). In the present observation only non protein thiol content has shown negative correlation with the mercury bioaccumulation of the all test plants. This is probably that like other heavy metals mercury also reflects its defensive mechanism through the production of oxidative stress metabolites (Zhou et al., 2008; Gupta et al., 2015). Our finding indicates that since non protein thiol content has been found as a mercury induced stress metabolite can be used as biomarker for the waste dumping or municipal sewage irrigated areas. Very recent Oztetik (2015) also suggests that the thiol content or glutathione reductase of evergreen plant tissues can be considered as biomarker of urban environment. Instead of using cumbersome method of mercury determination from consumable vegetables the measurement of non protein thiol can be a tool for easy investigation of mercury contamination.

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