Madras Agric. J., 105 (7-9): 346-351, September 2018

Uptake of Mercury by Marigold and Amaranthus on Spiked Soil

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Mercury is a global pollutant, highly toxic, is a non-essential element with no biochemical or nutritional function in the organisms and is ubiquitous in the environment. In order to elucidate the participation of plants in uptake of mercury an experiment was carried out to study the plant behavior on mercury contamination in the Department of Environmental sciences, Tamil Nadu Agricultural University, Coimbatore with amaranthus and marigold as test crops. Four different concentration of mercury were spiked to a soil along with control (without mercury). Relatively higher amount of Hg was found accumulating in the roots of Marigold (3.35 µg.g^{-1}) and Amaranthus (3.35 µg.g^{-1}) and the plants did not express any visual symptoms of toxicity. Regarding the partition of mercury in different plant parts, it ranked in the order of roots > shoots > leaves. Among the treatments 20 mg.kg^{-1} Hg recorded higher enzymatic activity in both the test crops. Marigold being a non-food chain crop can be recommended for Hg contaminated soils.

Key words: Mercury, Spiked, Amaranthus, Marigold

Mercury is a non-nutritive heavy metal that poses significant environment and health concerns. Mercury is the second toxic heavy metal which is present in the transitional d block of the periodic table and its pollution is a serious environmental problem throughout the world. Mercury is released by degassing of the earth's crust, from volcanoes, and from evaporation of oceans (Boeing, 1999). Mercury released from both natura land anthropogenic sources and widespread in nature. WHO has estimated that each year 10,000 tons of Mercury is released globally and it is used in agricultural, alkaline, batteries, chlorakali, and thermometer and in electrical apparatus. The main sources of contamination have been mining and smelting, burning of fossil fuels, industrial production of sodium hydroxide and chloride, wastes in fertilizers used on farmland. Three different oxidation states of namely, Hg° (metallic), Hg+ (mercurous) and Hg++ (mercuric) occurs in the environment. In case of organo metallic derivatives, mercury atom is tend to bind with carbon atoms. Mercury is a dense, silvery-white, shiny metal, which is liquid at room temperature and boils at 357 °C. And vapour pressure of the metal is 0.17 Pa (0.0013 mm Hg), and a saturated atmosphere at this temperature contains 14 mg/m. Even though it is ubiquitous in the environment, it is considered as non-essential and it has neither biochemical nor nutritional functions to the organisms (Karunasagar et al., 2006). Even though mercury (Hg) is a naturally occurring element, it is considered as a global pollutant that can impair both wildlife and human health. Exposure to mercury in the environment is inevitable. The various chemical reactions of mercury in soil depends on soil properties, soil organic matter (SOM), chloride concentration, and sulphide anions (Downey, 2012). Mostly in soil, it was found to be forming complex molecules either with humic acid or thio groups (Guo et al., 2013). Mercury exists in solid phase in soil by adsorbing with sulfides, clay particles and organic matter. In agricultural soils, divalent mercury is the predominant form. Many studies have focused on the distribution of mercury in the plant body in the natural environment. Most of the plants uptake Mercury to accumulate in roots and some accumulate moderate amount in the shoots, due to translocation or direct absorption in the shoots. High level of Hg+2 is strongly phytotoxic to plant cells. Toxic level of Hg+2 can induce visible injuries and physiological disorders in plants (Zhou et al., 2007). Plants exposed to elemental mercury vapor accumulated mercury in the shoots with no movement to roots (by day ten). Root-exposed plants showed accumulation of mercury in the roots with movement to shoots by day ten (Kumar, 2012). A pot study with potting mix where mercury was provided as HgCl2 solution the bioavailability and uptake of mercury was studied in clay loam soil. Therefore the present study was carried out to study the soil behavior and uptake of Hg in crops.

Material and Methods

Marigold and Amaranthus seeds of local varieties were obtained locally. The seeds were sown in plastic pots containing potting mix. The Marigold and Amaranthus plants were grown to about three weeks old before being exposed to mercury contamination. Plants were all grown in plastic pots containing potting mix. Mercury was supplied as $HgCl_2$ solutions. Marigold and Amaranthus plants were grown in pot containing 2.0 kg of soil. The mercury treatment was started at the same day for two plant species. Mercury treatments with four replicates in

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each group were supplied with 5, 10, 15, 20 mg\ Kg. All the treatment groups along with control were arranged in a completely randomized design. The mercury treatments were supplemented with water to avoid any water deficiencies. The plants were kept outdoors in an enclosed area except during extreme weather conditions. Plants were harvested after 60 days of exposure to mercury. Roots were washed with distilled water Both roots, shoots and leaves were dried at 60° C in an oven and then prepared for chemical analysis.

Preparation of plant samples

Plant samples of each part (leaves, stem, and roots) of the plants were oven dried and powdered. Dried 0.5 g of plant sample was taken in a conical flask after that 15 ml of triacid mixture was added and kept overnight for wet digestion. Then the flasks were kept in hot plates at 80-90˚C at which the samples were made to boil and digestion continued until a clear solution was obtained. After cooling, the solution was filtered through whatman No.42 filter paper, the samples transferred to 25 ml volumetric flask by adding Milli-Q water. The filtrate was analyzed for total mercury.

Treatments details

Crop - 1. Marigold (*Tagetes erecta L.*)

2. Amaranthus (*Amaranthus blitum L.*)

T_1 - Control

T_2 - 5 mg kg^{-1} Hg

T_3 - 10 mg kg^{-1} Hg

T_4 - 15 mg kg^{-1} Hg

T_5 - 20 mg kg^{-1} Hg

Root oxidation studies

Measurement of root oxidation activity was done using α - Naphthylamine. After taking out the plants from each pot, roots were washed thoroughly with tap water on a sieve and sponged to remove excess water before weighing. Approximately 1 g of root sample was taken for analysis from each plant and immersed in a flask containing 10 ml of 20 mg/l ȧ-naphthylamine and 10 ml of phosphate buffer (pH 7). The flask containing the roots was air plugged and kept at room temperature in total dark condition, since ȧ- naphthylamine is light sensitive. After 10 minutes 2 ml of solution was taken out and initial concentration (A1) of ȧ- naphthylamine solution was determined after adding 1 ml of sulphuric acid and 1 ml of 100 mg/l NaNo2 to the sample aliquot and absorbance was read at 530 nm using spectrophotometer. After an incubation period of 2 hour room temperature, the final concentration (A2) of α - naphthylamine was determined. The root activity was calculated as the amount of α - naphthylamine oxidized by the roots using the equation given by Chetan et al., (2011).

Root oxidation activity (mg g^{-1} dry weight $2h^{-1}$) = A1-A2-A0

Where,

A_1 is the initial value of ȧ-NA

A_2 is the final value of ȧ-NA

A_0 is the difference in the initial and final values of ȧ-NA sample without root that served as control.

Bioconcentration factor (BCF) and Translocation factor (TF)

BCF is the ratio of plant capability to accumulate heavy metal with respect to the heavy metal concentration. BCF>1 indicates the accumulation of heavy metal in the shoot. For TF, it is the ratio of plant ability to extract heavy metal from root to shoot, TF <1 means the accumulation of heavy metals in the root, and vice versa

BCF = [Metal concentration in plant shoot (mg/Kg)] / [Metal concentration in soil (mg/Kg)]

TF = [Metal concentration in plant shoot (mg/Kg)] / [Metal concentration in plant root (mg/Kg)] (Zhang et al., 2015).

Instrumentation

Apparatus

A Shimadzu (Model-1800) double beam UV-visible recording spectrophotometer used for measurements of absorbance.

Mercury stock solution

Mercury stock solution of 1000 mg/L was prepared by dissolving 135 mg of mercuric chloride in 100 ml of de-ionized water which was standardized with EDTA using xylenol orange as indicator. From this, working standards of 1 to 5 mg/L were prepared for a 100 ml with a few crystals of hydroxylamine hydrochloride in dilute sulphuric acid followed by boiling and diluting with de-ionized water to 100 ml.

Procedure

A series of standard solution ranging from1 mg/L to 5 mg/L in a 25 ml volumetric flask with 2ml of 0.0045 M of diphenylthiocarbazone reagent solution followed by the addition of 0.25 ml of 4.5 M sulphuric acid. After 1 min, 5 ml of 1, 4-dioxane was added and the mixture was diluted to the mark with de-ionized water. The absorbance was measured at 488 nm against a corresponding reagent blank. The mercury content in an unknown sample was determined using a concurrently prepared calibration graph. In case of plant samples, the digested sample was filtered and neutralized with dilute ammonia in presence of 1-2 ml of 0.01% tartarate solution which was then transferred into a 25 ml volumetric flask and diluted upto the mark. 1-2 ml of the aliquot of the final solution was pipette into a 25 ml volumetric flask and mercury content was determined as per the procedure. In case of soil samples, the digested content was filtered through Whatman No.42 filter paper into a 25 ml volumetric flask and neutralized with dilute ammonia. It was then diluted up to the mark. Aliquot of 1-2 ml were transferred and 2 ml of 4.5 M sulphuric acid

and followed by 1-2 ml of 0.01% tartarate solution. Mercury was then determined by the above procedure (Ahmed and Alam, 2003).

Results and Discussion

Initially two soil properties were evaluated namely, soil pH, soil organic matter content (SOM), in order to see the effect of the applied chemicals. Control plants were grown in the absence of the mercury metals mixture. The heavy metal – contaminated soils have lower pH values than the uncontaminated soil. Total Hg present in the pot culture experiment was 4.1 µg.g-1 in T2 and 18.32 µg.g-1 in T5 in Marigold and 4.00 µg.g-1 in T2 and 17.49 µg.g-1 in T5 in Amaranthus initially after the harvesting of both crops the values observed was 0.32 in T1 and 3.28 in T5 in Marigold and 1.32 in T1 and 3.98 in T5 in Amaranthus final it shows that there is uptake of Mercury by plants so it has been shown that the higher the metal application, the greater the reduction in soil pH (White et al., 1979). With a decrease in SOM there is an increase in the availability of heavy metals to plants due to fewer binding sites being present in the soil.

Table 1. Total uptake of Hg by root, shoot and leaves of marigold

Treatments mg kg^{-1}.Hg	Root (µg g^{-1})				Shoot(µg g^{-1})				Leaves(µg g^{-1})			
	S_1	S_2	S_3	Mean	S_1	S_2	S_3	Mean	S_1	S_2	S_3	Mean
control	0.02	0.05	0.02	0.03	0.03	0.05	0.03	0.04	0.01	0.04	0.06	0.04
5	0.24	0.78	1.86	0.96	0.22	0.40	0.74	0.45	0.04	0.20	0.27	0.17
10	1.12	2.06	2.74	1.97	0.32	0.98	1.70	1.00	0.18	0.31	0.49	0.33
15	2.05	3.15	4.75	3.32	1.01	2.11	1.79	1.64	0.29	0.63	1.08	0.67
20	2.04	3.20	4.80	3.35	1.19	2.01	2.60	1.93	0.54	0.97	1.90	1.14
Mean	1.09	1.85	2.83		0.55	1.11	1.37		0.21	0.43	0.76	
SEd	0.76	0.65	0.73		0.53	0.52	0.45		0.71	0.73	0.79	
CD (0.05)	1.46	1.48	1.50		0.93	0.98	0.83		1.46	1.42	1.50	

S_1- Vegetative stage, S_2-Reproductive stage, S_3- Harvesting stage *Mean of 3 replications

Effect on enzyme activity

Catalase

The enzyme activity has increased with the application of mercury in the soil in Marigold and Amaranthus. In (Fig.1) shows the catalase activity showed the range of 3.28 to 5.12 µ moles/ml in the Marigold crop and 3.98 to 5.07 µ moles/ml in Amaranthus. The maximum catalase activity in leaves was seen in the reproductive stage of both the crops values ranged from 3.90 to 4.35 µ moles/ml in Marigold and 4.32 to 5.42 µ moles/ml. This may

Table 2.Total uptake of Hg by root, shoot and leaves of amaranthus

Treatments	Root(µg g^{-1})				Shoot(µg g^{-1})				Leaves(µg g^{-1})			
mg kg^{-1}.Hg	S_1	S_2	S_3	Mean	S_1	S_2	S_3	Mean	S_1	S_2	S_3	Mean
control	0.01	0.01	0.02	0.01	0.05	0.07	0.08	0.07	0.07	0.09	0.09	0.08
5	0.29	0.73	1.90	0.97	0.21	0.38	0.79	0.46	0.02	0.23	0.29	0.18
10	1.19	2.01	2.59	1.93	0.4	0.88	1.62	0.97	0.13	0.36	0.50	0.33
15	2.10	3.04	4.80	3.31	0.19	0.18	1.65	0.67	0.19	0.70	1.28	0.72
20	2.04	3.18	4.50	3.24	1.28	2.09	2.40	1.92	0.64	0.90	1.80	1.11
Mean	1.13	1.79	2.76		0.42	0.72	1.31		0.21	0.45	0.79	
SEd	0.73	0.69	0.71		0.43	0.42	0.45		0.73	0.75	0.69	
CD (0.05)	1.56	1.48	1.53		0.93	0.88	0.90		1.56	1.49	1.53	

be due to application of mercury in different levels. In another study using wheat as test crop observed similar trends in enzyme activity due to induction of enzymes catalase was found in the leaves of wheat with increasing concentration of Hg (Makam et al., 2018). Similar trend showed in Hg-treated seedlings showed an increase in the catalase activity due to the higher concentration of mercury exposed between 250 and 500 µM of HgCl2 at 10 days. Taken together, our results suggest that mercury might have induced oxidation stress in Marigold and Amaranthus. Hg when accumulated in plant tissues produce alternatives in catalytic efficiency of enzymes, damage to cell membranes, and inhibition of root growth (Yadav,2010).

Peroxidase

The enzyme activity of Peroxidase (Fig. 1) shows significant increasing trends in all stages of Marigold and Amaranthus. The highest activity of 9.12 per g weight of sample in T_5 (20 mg.kg^{-1} Hg) of Marigold and 7.90 per g weight of samples in T_5 of Amaranthus were observed. In another studies using Mung bean as test crop have also observed an increasing trend in leaf.

Proline

There was a gradual increase in the proline activity in both Marigold and Amaranthus compared to control (Fig.1)This might be due to the activity of enzymes present in plant. The enzyme activity

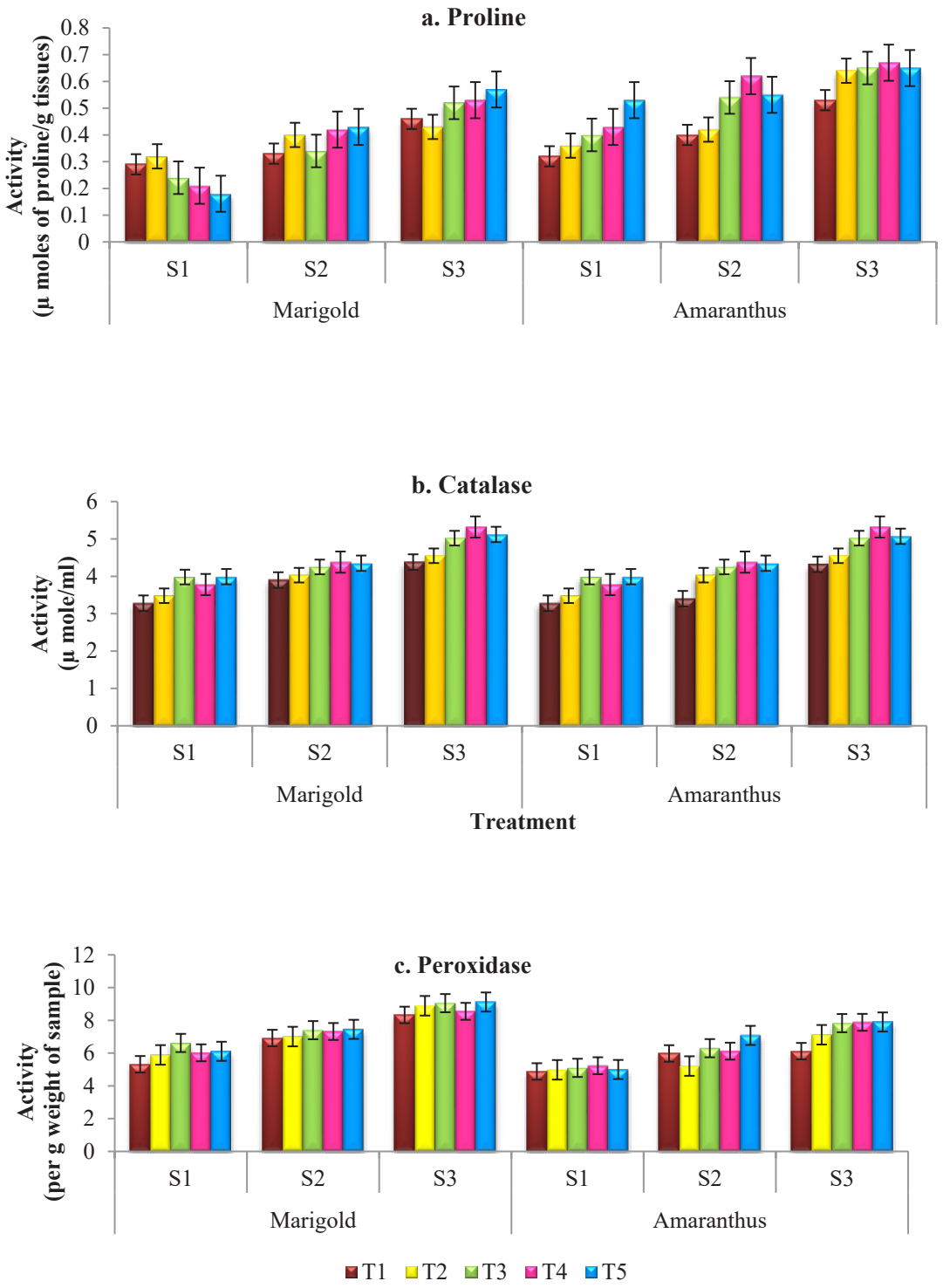
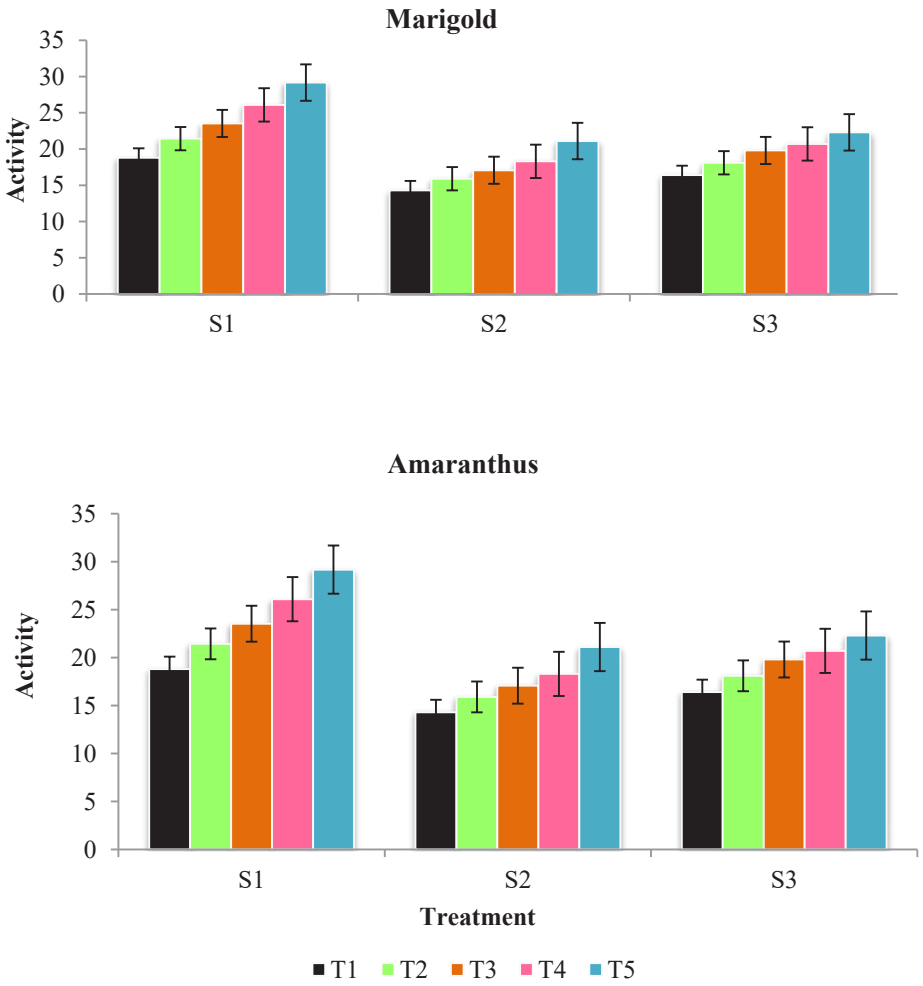


Fig.1. Effect of Hg on enzyme activities

increased with increasing concentration of Hg. In another study observed and they showed the similar trends in relation between the mercury and proline activity in rice has been resulted that Hg stress in rice plant increase the proline activity (Wang et al., 2009)

Total uptake of mercury by root, shoots and leaves of marigold and amaranthus

The influence of different doses of mercury in Marigold and Amaranthus showed that the maximum uptake of mercury was recorded in roots and less amount of Hg was taken by shoot and leaves of both crops (Table.1 and 2). In another study Hg accumulation by five plant sps, was observed and they showed similar trend of higher accumulation in the roots then in the aerial parts of plant (Du et al., 2017) and also it was reported that when cell wall is possessed with huge quantity of mercury



S_1 - Vegetative stage, S_2 – Reproductive stage, S_3 – Harvesting stage

T_1– control, T_2 – 5 mg/kg Hg, T_3 – 10 mg/kg Hg, T_4 – 15 mg/kg Hg, T_5 – 20 mg/kg Hg

Fig.2. Effect of Hg on Root oxidation

there is a mechanism which prevents toxic effects namely necrosis and chlorosis on various parts of the plant. In yet another study cucumber seedlings were exposed to HgCl2 during 10 and 15 days and mercury was readily absorbed by growing seedlings, and its content was greater in the roots than the shoot and leaf (Cargnelutti et al., 2006).

Table 3. Total accumulation and bioconcentration factor

Name of the plant	Treatments mg.kg^{-1}.Hg	Translocation factor	Bioconcentration factor
	control	0.10	0
	5	0.24	0.09
Marigold	10	0.36	0.01
	15	0.34	0.10
	20	0.40	0.40
	control	0.28	0
	5	0.18	0.36
Amaranthus	10	0.17	0.30
	15	0.18	0.48
	20	0.33	0.55
Mean		0.25	0.22
SEd		0.27	0.24
CD (0.05)		0.49	0.45

Root oxidation

There was a significant increase in the root oxidation for the different treatments (Fig. 2) It was ranged from 14.50 mg g^{-1} dry weight to 29.17 mg g^{-1} dry weight (Marigold) and 15.9 mg g^{-1} dry weight to 32.9 mg g^{-1} dry weight (Amaranthus) in the roots. Among the treatments, the T5 (20 mg.kg-1 Hg) showed higher root oxidation and it might be due to higher concentration of mercury in roots. This was also supported by Gopal Krishna et al., 2012 who stated that both leaves and root of plants treated with higher concentration of mercury subjected to comparatively greater root oxidation 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 crops.

In the present study, the enzyme activities (proline, peroxidase and catalase) and root oxidation increases with increase in concentration of Hg and this indicates stress condition of both plants. Accumulation of mercury content in various parts of the plant follows the order of roots > shoot > leaf in both Amaranthus and Marigold. In both crops, Amaranthus and Marigold, the values of BCF < 1 and TF < 1 indicates that the translocation of Hg in the upper parts and accumulation was found to be more in roots. Based on the results of plant uptake studies, although Hg accumulates more in the roots of both the plants, Marigold can be recommended as suitable crop in Hg contaminated soil being a non-food chain crop.

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Received : February 20, 2018; Revised : March 10, 2018; Accepted : March 25, 2018