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Abolition of heavy metal mercury by low cost domestic organic wastes from the *Phaseolus vulgaris*

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Abstract

Contamination of agricultural land by mercury has become a threat to all living beings in some way or the other. Utilization of low cost domestic organic wastes like tea waste and potato peels for the neutralization of mercury toxicity from soil is the recent and promising detoxification method which is easy to apply and have no side effects. The present study discusses the capabilities of tea waste and potato peels to remove heavy metal mercury from *Phaseolus vulgaris* growing in green house in a randomized block design. The experimental plants were supplied with varying concentrations of mercury ie., 0, 10, 20, 30 ppm Hg whereas one set of treated plants was provided with 6gm of tea waste and other set with potato peels in same amount with all applied concentrations of mercury. The data revealed that adapting this free of cost organic waste strategy for detoxification of mercury was very significant. Rate of germination, rate of growth with respect to root and shoot length, leaf area, chlorophyll contents, free carbohydrates, proline and phenol contents were determined and found significantly improved under this dynamic technology. Results showed that tea waste has much potential for remediation of heavy metal mercury than potato peels. Present study not only demonstrated the adverse effects of mercury and its adsorption by organic domestic waste but it also help to solve the disposal issue of various domestic wastes.

Keywords: Adsorption, Mercury, Tea waste, Potato peels, *Phaseolus vulgaris*, stress indicators

Introduction

The presence of mercury in environment has become a major threat to all forms of life due to its bioaccumulation tendency and toxicity [1, 2, 33]. Mercury inhibits the growth and induced disorientation in roots and shoots at high concentrations [3, 4]. It causes inactivation of enzyme system, carcinogenic and mutagenic effects [5, 6]. In the environment it comes from both natural processes and industrial activities [7], where it persists for decades by means of continuous cycling between air, water, and land [8]. Adsorption is an engrossing alternative for an effective purification and separation technique used in industry especially in water and wastewater treatments [9]. Adsorption of Hg by solid materials is a suitable choice for efficient removal of metal [10]. At molecular level, adsorption is due to attractive interactions between a surface and the species being adsorbed. Low cost and risk-free adsorbent material can be use for the plant growth and reclamation of heavy metal from the contaminated soil [3]. The adsorption abilities of a number of low-cost adsorbents (e.g., clay, coal fly ash, sewage sludge, agriculture waste, tea waste, potato peels, rice husk, coconut husk, neem leaves, Oil palm shell) have been determined for the removal of heavy metals from water [11, 12, 13, 14, 15, 16, 17]. Tea and potato are widely used and inexpensive food of not only Indo-Pak but of all developed and under developed countries. These domestic wastes poses increasing disposal problems [18, 19]. Tea waste and potato peels were used as the low-cost adsorbents for removing heavy metal ions from aqueous solutions [20, 17, 32]. The current research was focused to observe the adsorbing potentials of the applied adsorbents for the abolition of heavy metal mercury from the contaminated agriculture land.

Material and methods

Preparation of Adsorbents: Tea waste was collected from teashops and residential areas of the town. It was washed with distilled water, shade dried for 24 hours, ground, sieved and stored in plastic bags at room temperature. Potato peels were collected from French fries shops and residence. They were washed with distilled water and shade dried at room temperature. The dried material was ground in an electric grinder and stored in sealed polythene bags.
Preparation of Solutions: Stock Solution (1000ppm) of Mercurous chloride (Hg_2Cl_2) was prepared and required concentrations (0ppm, 10ppm, 20ppm, 30ppm) were made from Stock Solution.

Preparation of Soil Bags: Three sets (each with 12 soil bags) were prepared provided with 1Kg garden soil/ bag. First set named experimental plants was provided with different concentrations of mercury ie., 0ppm, 10ppm, 20ppm, and 30ppm Hg respectively all with three replicates. Second set was tagged as treated 1, supplied with 6gm/kg tea waste powder along with different concentrations of applied mercury. Third set was labeled as treated 2 supplied with Potato peel powder instead of tea waste. Three soil bags from first set provided with 0ppm mercury and distilled water were considered as control.
Sterilization and Sowing of Seeds: Healthy seeds of *Phaseolus vulgaris* L. were surfaced sterilized, soaked and sown 4 seeds/soil bag.
Biophysical Parameters: Percentage germination, rate of growth with respect to root shoot length and leaf area were determined.
Biochemical Parameters: All the reagents used for the experimental studies were of analytical grade.
Chlorophyll contents: Chlorophyll a & b were estimated by Duxbury & Yentsh and Machalachan & Zalik formula. The supernatant was collected and its absorbance was recorded at 645 and 663nm respectively on schamdzu 160a uv/vis spectrophotometer.
Free Carbohydrates: Carbohydrate contents were determined by method described by [21]. 5ml of anthrone reagent was added to 0.5ml of protein free filtrate, and heated on a boiling water bath for 15 minutes and immediately cooled in ice water. Green color was obtained, optical density was recorded at 620nm against a reagent blank.
Proline: Proline contents were estimated by [22] method. Absorbance was recorded at 520 nm.
Estimation of phenols: Phenol contents were determined by [23] method. Absorbance was recorded at 660nm.
Data Analyzed: Data was analyzed by using ANOVA (analysis of variance), statistica ® version 5, ($P < 0.05$).

Results and discussions
Percentage Germination
Percentage germination of *Phaseolus vulgaris* was 100% at 0ppm and 10ppm Hg, whereas it was decreased to 91.66% ± 0.33 at 20ppm and 30ppm Hg. Decrease in rate of germination in the presence of mercury may attribute to the toxic effect of metal applied [24]. According to literature surveyed Abscisic acid induces seed dormancy, so a decline in rate of germination may attribute to the synthesis of absicisic acid which might be produced in stress due to mercury [25]. Inhibition in the rate of germination may also attribute to the shortage of water because water activates hydrolytic enzymes which break down stored food (proteins or oils). Hg blocked the path of water to dormant embryo. Germinating seeds also required oxygen for the metabolism. A sort of suffocation due to accumulation of mercury clumps on the surface of seeds might cause inhibition in germination. [26] reported that Hg_2Cl_2 was found to be the most toxic heavy metals with 94% inhibition in seed germination. By the application of tea waste and potato peel powder rate of germination was enhanced almost upto 100% at all applied levels of mercury.

Growth of Plant (Shoot, Root Length and Leaf Area)
Shoot Length: The results revealed that shoot length was significantly decreased in experimental plants at all applied levels of mercury (Table 1) [25, 27]. Reduction in shoot length may attribute to the disturbance in mitotic activity and this may be due to denaturation of protein and deoxyribonucleic acids by toxic metal mercury.
The shoot lengths were significantly improved by the application of tea waste upto 38.38 ± 3.11, 35.54 ± 1.01, 30.48 ± 2.32 and 27.57 ± 2.10 respectively at all given doses of mercury (Table 1). In the presence of potato peel powder along with different concentration of Hg applied, the shoot lengths were improved upto 33.84 ± 4.98, 29.94 ± 3.90, 29.21 ± 3.85and 27.57 ± 4.64. The results showed that tea waste act as good adsorbent over the potato peel.
Root Length: Pragmatic studies of experimental plants showed that there was a significant decrease in root length of *Phaseolus vulgaris* at all applied levels of mercury upto 13.48 ± 0.13, 12.16 ± 0.14, 11.72 ± 0.12, 11.03 ± 0.13 cms. Application of tea waste significantly enhanced the root length of experimental plants at all applied levels of mercury upto 19.86 ± 0.91, 19.72 ± 2.25, 19.11 ± 0.60 and 15.99 ± 1.01cms. Under the influence of potato peel at all applied levels of mercury root length of plants were improved significantly over control upto 16.71 ± 0.31, 15.83 ± 1.20, 15.53 ± 0.90 and 15.19 ± 0.44. The results showed that tea waste act as good adsorbent over the potato peel (Table 1).
Leaf Area: The results revealed that leaf area was significantly decreased in experimental plants from 25.55 ± 1.73, 20.97 ± 0.38 to 16.19 cm² ± 0.93over control which was found to be 36.6 ± 2.34 at all supplied levels of mercury. The Leaf area was improved by the application of tea waste upto 41.13 ± 2.11, 35.12 ± 0.96, 34.48 ± 1.81and 26.27 cm^2 ± 3.52 at all applied levels of mercury (Table 1**).** Whereas application of potato peel powder also enhanced leaf area significantly in comparison with experimental plants upto 37.13 ± 2.00, 34.64 ± 1.85, 29.02 ± 1.18, 18.93 ± 2.81 cm^2. The results showed that the performance of tea waste was good as compared to the potato peel powder. Reduction in the leaf area is a xerophytic character it might occur due to the less availability of water in plants.
Photosynthetic Pigments: Adverse effects were found on chlorophyll a and b with increasing concentration of Hg. This was happened due to the replacement of Mg from porphyrin ring of chlorophyll by mercury. Both chlorophyll a and b content decreased with increased metal concentration [28]. Reduction of chlorophyll content is common symptom of heavy metal toxicity which badly destructs the process of photosynthesis. The photosynthetic pigments, chlorophyll a, b and total chlorophyll in *Phaseolus vulgaris* L. exposed with the different concentrations of Hg (10ppm, 20ppm and 30ppm) showed a significant decrease over the control i.e. 0ppm of Hg. The results revealed that total chlorophyll content was significantly decreases in experimental plants with all supplied levels of mercury as 2.36 ± 0.02, 2.02 ± 0.21, 1.82 ± 0.01 and 1.45 ± 0.02 mg/gm fresh wt (Table 2). The total chlorophyll content was improved by the application of tea waste and potato peel adsorbents along with different concentration of Hg. The tea waste improved the total chlorophyll as 2.72 ± 0.02 at 0ppm, 2.56 ± 0.02 at 10ppm, 2.08 ± 0.02 at 20ppm and 1.92 ± 0.03 (mg/gm fresh wt) at 30ppm. Under the application of potato peel the total chlorophyll content was improved at all supplied levels of mercury as 2.46 ± 0.01, 2.28 ± 0.01, 2.09 ± 0.02 and 1.95 ± 0.02 (mg/gm fresh wt). The results showed that tea waste act as good adsorbent over the potato peel.
Carbohydrate Contents: Carbohydrates spare protein so that protein can concentrate on building, repairing, and maintaining body tissues instead of being used up as an energy source. Our results revealed that the carbohydrate

content in the shoot was significantly decreased to all applied concentrations of Hg in experimental plants as 579.1 ± 23.49, 507.43 ± 1.73, 375.29 ± 2.51 and 367.54 ± 1.19 µmol/gm fresh wt. The carbohydrate contents were significantly improved by the application of tea waste as compared to control. The application of tea waste improved the carbohydrate content as 679.27 ± 1.95, 533.79 ± 3.48, 485.44 ± 7.54 and 382.56 ± 21.59 µmol/gm fresh wt. at all applied levels of mercury. Under the application of potato peel the carbohydrate content was improved upto 731.33 ± 65.59 at 0ppm Hg, whereas it was reduced to 383.72 ± 4.94, 375.65 ± 1.65 and 328.2 ± 1.27 (µmol/gm fresh wt.) in all supplied concentrations of mercury respectively. The results showed that tea waste acted as a good adsorbent and improved carbohydrate contents at all applied levels of mercury [29], it may attributed to its antioxidant nature and affinity to adsorb heavy metal mercury on its surface. Whereas potato peels actively improved carbohydrate contents in the absence of mercury may be due to presence of nutritionally and pharmacologically important compounds [30], while failed to show any improvement in carbohydrate contents at all applied ranks of mercury this may attribute to the fermentation of cell wall carbohydrates into phenolic acids or alkaloids [31].

Proline Content: Proline is an osmoprotectent amino acid. It is a major constituent of cell wall structural proteins in plants. Proline is a stress substance, its production increases as the concentration of stress increases. The proline contents were directly proportional to the concentration of Hg. The contents of proline in experimental plants were increased as the concentration of Hg increased upto 4.85 ± 0.03, 4.85 ± 0.03, 7.28 ± 0.02, and 7.54 ± 0.02 (µ mol/ gm fresh wt.) at all applied levels of Hg [1]. Application of tea waste significantly reduced proline contents upto 4.73± 0.01, 4.35± 0.03, 5.14± 0.03 and 5.74± 0.02 µmol/ gm fresh wt. at all supplied levels of mercury.

Potato peels under varying concentrations of mercury failed to overcome the stress as 5.37 ± 0.01, 1.5 ± 0.05, 2.89 ± 0.02 and 2.95 ± 0.02 µmol/ gm fresh wt. at all supplied levels of mercury. Results revealed that proline contents of the plant sample were increased at 0ppm mercury this may attribute to the fermentation of polysaccharides of Potato peels to Phenolic compounds [31]. Proline contents of *Phaseolus vulgaris* were significantly decreased at all applied levels of mercury may attribute to the ineffectiveness of potato peels as adsorbent due to fermentation of polysaccharides to phenolic compounds. Due to this reason plants were badly affected by mercury.

Phenol Content: Phenol is a stress substance which increases with increasing concentrations of toxic substances. Data of current work revealed that Phenol contents of *Phaseolus vulgaris* increased upto 643.49 ± 2.43, 991.24 ± 2.12, 731.08 ± 2.05 and 776.03 ± 2.43 µ mol/ gm fresh wt. at all applied levels of mercury [1], whereas it was reduced significantly by the application of tea waste as 637.89 ± 4.12, 646.5 ± 3.18, 693.05 ± 1.16 and 690.51 ± 2.45 µ mol/ gm fresh wt. at all experimental values of applied Hg. Phenol contents at 10 ppm mercury with tea waste were very close to control, it means that 10ppm Hg may be the tolerable amount of mercury for *Phaseolus vulgaris.* According to the data obtained the phenol content in potato peel was 536.57 ± 2.22, 298.38 ± 0.63, 364.95 ±2.58 and 457.14 ± 2.82 µ mol/ gm fresh wt. at all applied levels of mercury. Phenol contents of *Phaseolus vulgaris* in the presence of potato peel were decreased even in the absence of mercury (0ppm Hg), whereas it further lowered down drastically at all applied levels of mercury. This condition may attribute to the fermentation of potato peels [31] and disturbance in molecular structure of organic and inorganic substances, due to this reason potato peels failed to adsorb mercury which badly damaged the plant and stress inhibitor phenol were not produced by the plant.

Conclusions

The current research work presented an efficient and multipurpose technique utilizing domestic organic waste for the adsorption of heavy metal mercury. Plant exhibited deleterious effects on almost all biophysical and biochemical parameters, especially on root length, shoot length, leaf area, chlorophyll contents, carbohydrates, proline and phenols at all applied levels of mercury. These drastic effects of toxic metal mercury were lessen by the application of tea waste and potato peels. Use of domestic organic waste is a new scientific tool, which is a non-polluting, low cost, chemical free and very effective for adsorption methods. It is concluded that tea waste is more efficient adsorbent as compare to potato peel. This can be collected not only from Hotels, restaurants and caterers but also from door to door collections. Collection of tea waste not only provides an efficient fertilizer but would also be helpful to municipal authorities. Use of these organic fertilizers would improve crop productivity. In future new free of cost adsorbents should be investigated for adsorption of different toxic metals.

Table 1: Effect of Hg and its adsorption by tea waste and potato peels on root, shoot length and leaf area of *Phaseolus vulgaris*

	Root Length (cms)			Shoot Length (cms)			Leaf Area (cm²)		
Hg [ppm]	Experimentals	Tea waste	Potato	Experimentals	Tea waste	Potato	Experimentals	Tea waste	Potato
0	13.48 ± 0.13	19.86 ± 0.91	16.71 ± 0.31	26.8 ± 0.89	38.38 ± 3.11	33.84 ± 4.98	36.6 ± 2.34	41.13 ± 2.11	37.13 ± 2.00
10	12.16 ± 0.14*	19.72 ± 2.25**	15.83 ± 1.20***	23.93 ± 0.89*	35.54 ± 1.01**	29.94 ± 3.90***	25.55 ± 1.73*	35.12 ± 0.96**	34.64 ± 1.85***
20	11.72 ± 0.12*	19.11 ± 0.60**	15.53 ± 0.90***	22.78 ± 1*	30.48 ± 2.32**	29.21 ± 3.85***	20.97 ± 0.38*	34.48 ± 1.81**	29.02 ± 1.18***
30	11.03 ± 0.13*	15.99 ± 1.01**	15.19 ± 0.44***	19.9 ± 1.18*	27.57 ± 2.10**	27.57 ± 4.64***	16.19 ± 0.93*	26.27 ± 3.52**	18.93 ± 2.81***

Results expressed as Mean ± SEM of triplicate, marked effects are significant at p< 0.0500 * indicated significant values of experimental plants, ** indicated significant values of tea waste and *** indicated significant values of Potato peel plants over their controls.

Table 2: Effect of Hg and its adsorption by tea waste and potato peels on Chlorophyll and Carbohyderate contents of *Phaseolus vulgaris*

	Total Chlorophyll ((mg/gm fresh wt.)			Carbohydrate (µmol/gm fresh wt.)		
Hg [ppm]	Experimental Plants	Tea waste	Potato	Experimental Plants	Tea waste	Potato
0	2.36 ± 0.02	2.72 ± 0.02	2.46 ± 0.01	579.1 ± 23.49	679.27 ± 1.95	731.33 ± 65.59
10	2.02 ± 0.21*	2.56 ± 0.02	2.28 ± 0.01	507.43 ± 1.73*	533.79 ± 3.48**	383.72 ± 4.94***
20	1.82 ± 0.01*	2.08 ± 0.02**	2.09 ± 0.02***	375.29 ± 2.51*	485.44 ± 7.54**	375.65 ± 1.65***
30	1.45 ± 0.02	1.92 ± 0.03**	1.95 ± 0.02	367.54 ± 1.19*	382.56 ± 21.59**	328.2 ± 1.27***

Results expressed as Mean ± SEM of triplicate, marked effects are significant at p< 0.0500 * indicated significant values of experimental plants, ** indicated significant values of tea waste and *** indicated significant values of Potato peel plants over their controls.

Table 3: Effect of Hg and its adsorption by tea waste and potato peels on Phenol and Proline of *Phaseolus vulgaris*

	Phenol (µ mol/ gm fresh wt.)			**Proline (µ mol/ gm fresh wt.)**		
Hg [ppm]	Experimental Plants	Tea waste	Potato	Experimental Plants	Tea waste	Potato
0	643.49 ± 2.43	637.89 ± 4.12	536.57 ± 2.22	4.85 ± 0.03	4.73 ± 0.01	5.37 ± 0.01
10	991.24 ± 2.12*	646.5 ± 3.18**	298.38 ± 0.63***	4.85 ± 0.03*	4.35 ± 0.03	1.5 ± 0.05
20	731.08 ± 2.05*	693.05 ± 1.16**	364.95 ±2.58***	7.28 ± 0.02	5.14 ± 0.03	2.89 ± 0.02
30	776.03 ± 2.43*	690.51 ± 2.45**	457.14 ± 2.82***	7.54 ± 0.02	5.74 ± 0.02	2.95 ± 0.02

Results expressed as Mean ± SEM of triplicate, marked effects are significant at $p< 0.0500$ * indicated significant values of experimental plants, ** indicated significant values of tea waste and *** indicated significant values of Potato peel plants over their controls.

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