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Contamination of Heavy Metals in Aquatic Vegetables Collected from Cultivation Sites in Sri Lanka

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Abstract: *Heavy metal contamination in vegetables is a major concern indetermining food safety, quality and understanding adverse health impacts. In this study,we measured the contamination of heavy metals (Zn, Cu, Ni, Pb, Cd, Mn, Cr, Hg and As in three aquatic vegetables namely water spinach, Lasia roots and lotus roots which were collected from different cultivation sites. The metal concentrations were measured using ICP OES and the results showed that all vegetables exceeded the permissible levels in the concentrations of Pb, and Cr. Both Water spinach andLasia roots showed Cd ranging from 1.01 – 2.77mg/kg and0.84 -9.23 mg/kg respectively with two samples each exceeding permissible levels. No Cd was detected in Lotus roots. A significant difference between the three vegetables were observed in their Ni and Mn concentrations. The metal ion concentrations did not vary with the sites of collection for water spinach and Lasia root. However, the Mn and Cr concentrations in the Lotus roots were different depending on the site of collection. In an attempt to understand the pattern of metal contamination depending on the cultivation sites our results highlights the importance of investigating the soil and water in the area of study to identify the source of contamination.*

Keywords: *Heavy Metals, Lasia Spinosa, Ipomoea aquatic, Nelumbo nucifera*

I. Introduction

Vegetables are an important component of daily meals since they supply vitamins, minerals, and fibers required for a healthy living. Vegetables in the form of leaves, roots, and fruits are used in the regular diet not only for nutritional values but also for their medicinal values. The FAO/WHO recommends a dietary intake of 400 g a day for vegetables and also many developed countries have campaigned for promoting consumption of vegetables, especially through the International Fruits and Vegetables Alliance [1]. Hence, cultivation of green vegetables for commercial purposes in the suburban areas bordering the urban areas have been rapidly increasing. In general, most of the green leafy vegetable are grown along the bank of rivers, besides paddy fields, banks of irrigating ponds, natural wetlands and also few plots are available in home gardens closer to water outlets of the house or closer to the water wells. Wastewater from industrial waste, from paddy field and other vegetable farms have the possibility of being contaminated with fertilizers and pesticides in excessive amount [2].

Rapid and unorganized urbanization, massive industrialization, use of various chemicals in agriculture and most importantly, human activities are factors that poses a threatening to human life by increasing pollution in air, water, soil and the environment [3]. These activities have caused an increase in the heavy metal concentration in the urban environment in most developing countries [4]. Heavy metals are non-biodegradable and persistent environmental contaminants. These heavy metals deposited on the surfaces of soil and plant are absorbed into the plant tissues and are accumulated in different parts of the plants [5]. Due to the increased pollution of the water bodies with industrial effluents, wash out from cultivation field etc. the contamination of the human food chain has become inevitable. With the increasing demand for green vegetables, the excessive application of nitrogen, other inorganic fertilizers, organic manures, and pesticides have increased leading to accumulating high levels of pesticide residues, nitrate, and other anions as well as heavy metals both in the soil and the plant [6].

Polluted water, soil, and air in the cultivated areas have been identified as major roots of contaminating plants with heavy metals. One of the main routes that heavy metals enter the human food chain leading to harmful effects on health is through plants. Hence, many countries have studied the uptake of heavy metals by plants grown in polluted soils and water bodies extensively [7, 8, 9, 10, 11]. Wastewater and industrial effluent contaminating agricultural soils with heavy metals is of serious concern due to its implications on human health. Vegetables can absorb metals from soil and from the air of polluted environments and deposit on different parts of the vegetables [12]. Over 20 metals such as Arsenic, Antimony, Beryllium, Cobalt, Chromium, Lead, Manganese, Mercury, Molybdenum, Nickel, and Tin are reported to cause toxic effects [13, 14]. Their toxic effects to human have been known for centuries and their carcinogenic activities have also been reviewed by Frust (1977) [15]. Heavy metals such as Cu, Zn, Mn, and Fe are essentiallyrequiredin plant and human nutrition, yet exceeding concentration or their accumulation would lead to many heath issues. International regulations such WHO, FDA, EU etc. have defined the maximum permissible levels of toxic metals in food items with the increased awareness of the risks and health effects that metals pose through the food chain contamination [16, 17, 18]. Therefore, the level of heavy metal contamination of the food is one of the most important factors in determining the food quality.

Previous Studies performed in Sri Lanka showed the vegetables grown on contaminated soil and green leafy vegetables collected from local markets are contaminated with heavy metals [19, 20]. Aquatic plants such as *Ipomoea aquatica* and *Lasia Spinosa* common to wetlands in the tropical and subtropical regions of the world are commonly found in many ponds, streams, rivers banks and wetlands in Sri Lanka. *Ipomoea aquatica* (English name-Water Spinach, Sinhala name-Kankun) is a popular green leafy vegetable and both leaves and roots of *Lasia Spinosa*(English name-Lasia, Sinhala name--Kohila) are used as a vegetable. *Nelumbo nucifera* (English name-Lotus, Sinhala name-Nelum) roots are also a popular vegetable among most Asian countries including Sri Lanka and is a seasonal vegetable. The Lotus grow in the irrigation ponds and lakes naturally, especially in the North Central Province and the Southern Province of Sri Lanka.The roots are harvested seasonally (after the rainy season) and sold to the local markets for consumption. Water Spinach, Lasia and Lotus are well-investigated plants and reported to be a source of vitamins, fibers and with many medicinal values[21, 22, 23].

In most developing countries,it is a common practice to grow vegetables on banks of rivers passing through urban areas, banks of irrigation ponds and other water outlets which are often polluted by heavy metals [24, 25, 26]. In this study, we collected the plant material from the cultivation sites grown for commercial purposes. Kasbewa is a suburban area close to Colombo (the commercial capital of Sri Lanka) in the Western Province of Sri Lanka. The Water Spinach and Lasia roots collected from Kasbewa area and Lotus roots collected from irrigation pond in southern and north central province of Sri Lankawereanalyzedto determin theconcentrations of heavy metal namely Cadmium (Cd), Chromium (Cr), Copper (Cu), Lead (Pb), Manganese (Mn), Mercury(Hg), Nickel (Ni), Zinc (Zn), and Arsenic (As).

II. Materials and Methods

2.1 Plant Collection

The cultivation sites for sample collection were selected randomly and all samples were collected randomly from the site.

2.1.1 Water spinach

Water spinach samples were collected from five different commercial cultivation sites at Kasbawa. Four samples from wetlands (WL) and one sample from a home garden (HG) (where only organic fertilizer was used) were collected for analysis

2.1.2 Lasia

All Lasia samples were also collected from the Kasbawa area. One sample was collected from a wetland commercial cultivation site(WL), one sample from a cultivation site close to a stream (S)one sample from a cultivation plot grown beside a paddy field (PF) and one sample from a site where home waste water is collected (HG) and one sample from a site close to a domestic well (DW).

2.1.3 Lotus root

The lotus root samples were collected from irrigation lakes: one from Debarawewa (DB) in the southern province, and two from the north central province Galewela (GW) and Dambulla (DM)

2.2 Sample preparation

The collected samples were labeled according to the location of collection and were washed thoroughly with distilled water and air dried for one week . Each sample was cut into small pieces and was dried at $70\,^{o}C$ until a constant weight was obtained. The dried samples were ground using a blender and sieved using a 2 mm sieve. The samples were stored in a clean bottle at room temperature until further use.

2.3 Samples digestion

A sample of 2.00 g of each of the dried ground plant material was weighed into 100 ml beakers and a 10 ml of conc. Nitric acid (conc. HNO_3) was added. The sample was heated at $95\,^{o}C$ on a hot plate for 15 minutes and was cooled for about 5 minutes. Another 5 ml of conc. HNO_3 was added to the sample and was heated at $95\,^{o}C$ for an additional 30 minutes without letting it boil until the volume of the sample was reduced to 5 ml. The resulting sample was cooled and 2 ml of distilled water and 3 ml of 30% hydrogen peroxide was added. The sample was heated once again gently to initiate the peroxide reaction. When the effervescence became vigorous, the heating was stopped and 30% hydrogen peroxide was added with gentle heating until the effervescence was low. A 5 ml of conc. HCl and 10ml of distilled water were added to the sample and was heated for an additional 15 minute without boiling. The sample was cooled and filtered through a Whatman No. 42 filter paper and diluted to 5 ml with distilled water. The digestion sample (the filtrate) obtained was diluted to

25.00 ml using distilled water in a volumetric flask. A blank sample was prepared in the same manner as stated above except instead of the 2.00 g of the sample 2.00 g of distilled water was added [27]

2.4Determination of Heavy metals

The heavy metals such as Zn, Cu, Ni, Pb, Cr, Cd, Hg and As were determined using ICP-OES instrument (ICP-OES 720ES axial Varian). All metal concentrations were estimated using calibration curves for each metal and the concentrations were expressed in mg/L except in Hg where the concentration was determined in μg/L.

III. Results and Discussion

The contamination of heavy metals in plants is of great concern due to its potential impact on human and animal health. Table 1 presents the heavy metal concentrations (mean, ranges and recommended permissible levels) in water spinach, Lasia roots and lotus roots collected and analyzedfrom different cultivation sites grown for commercial purposes. Results revealed variable metal levels in among the three vegetable samples under investigation. The analysis performed on water spinach, Lasia root, and Lotus root showed that all samples exceeded the permissible levels in the Pb and Cr content. Arsenic (As) was not observed in any of the samples at the detection limits of our experiment. Aquatic plants tested for the ability to accumulate heavy metals havealso shown the abilityto remove metals from contaminated water bodies[28]. Aquatic plants can accumulate heavy metals at concentration 100,000 times greater than in the associated water [29, 30]. Therefore, the aquatic plant does carry a greater potential of being contaminated with heavy metals than other plants. Hence, understanding the levels of contamination of the edible aquatic plants are essential.

3.1 Water Spinach

The maximum level of Zn observed in water spinach was 18.81 mg/kg and the minimum was 16.80 mg/kg. The Cu content was between 1.49 -6.92 mg/kg where the Pb content was ranging between 1.01 – 1.36 mg/kg exceeding the permissible level of 0.3 mg/kg. Ni content of the water spinach sample ranged from 1.00 – 1.64 mg/kg and the Mn contents ranged from 16.32 -28.68 mg/kg. The Hg content ranged from 4.15 -12.15 μg/kg and the Cr content ranged from 1.50 -2.05 mg/kg exceeding the permissible level of 0.1. Cd was observed only in one sample collected from wetlands with a concentration of 0.44 mg/kg which also exceeded the permissible level of 0.2 mg/kg (Table 1, Figure 1) [17,18]. Comparison of the average metal content in water spinach showed that Mn > Zn > Cu > Cr > Pb > Cd > Hg. However, previous studies performed in India have shown that the uptake of heavy metals by *Ipomoea aquatica* is significantly high and has reported accumulating Cd, Cu, and Pb in its roots stems and leaves [31, 32, 33]. *Ipomoea aquatica*is also reported to accumulate arsenic and is widely used for wastewater treatment [34].

3.2 Lasia roots

The Zn content of Lasia roots showed the highest range compared to that of water spinach and lotus roots which ranged from 122.85 -732.7 mg/kg and the Cu content was 2.63-9.11 mg/kg. The Pb content exceeded the permissible value of 0.3 mg/kg in all samples ranging from 1.66- 2.77 mg/kg [17,18]. The Ni content and the Mn content ranged from 0.73 -3.78 mg/kg and 25.81 -221.43 mg/kg respectively. The Mn content was highest in Lasiarootsamples with a maximum of 221.43 mg/kg. The Hg content ranged from 9.3 - 9.9 μg/kg and the Cr and the Cd content ranged from 0.84 -2.45 and 0.18 - 0.41 mg/kg respectively (Table 1, Figure 1). Three samples showed Cd over the permissible limit where one sample at the border of the limit and the sample collected from the home water-well area did not show any Cd at our detection levels. Comparison of the average heavy metal content in Lasia showed that Zn > Mn> Cu > Pb > Cr > Ni > Cd > Hg..

3.3 Lotus roots

The Zn content of the Lotus roots was within the range of 13.39 -17.00 mg/kg and the Cu content was ranging from 6.54 - 9.95 mg/kg. The Pb content in the samples was ranging from 1.22- 1.97 mg/kg where the Ni content ranged from 1.52 – 2.43 mg/kg. The Mn content in the samples ranged from 34.09 - 73.08 mg/kg. The Hg content in the sample was highest in the lotus roots as compared to water spinach and Lasia root, ranging from 12.17 -17.72 μg/kg. The Cr content of the sample ranged from 2.05 -9.23 mg/kg exceeding the recommended permissible levels and showed the highest level of 9.23 mg/kg (Table 1, Figure 1) [17,18]. However, no Cd or As was detected in any of the lotus root samples. Comparison of the average metal content in Lotus root showed that Mn > Zn >Cu > Cr > Ni > Pb >Hg. The Cr and the Mn content collected from Dambulla was high compared to the other two sites with a value of 9.23 mg/kg (P=0. 000083) and 73.08 mg/kg(P= 0.0053) respectively.

In this study, significant differences were found in concentrations of Zn (P = 0.00057, one-way ANOVA), Pb (P= 0.0024, one-way ANOVA) and Mn (P= 0.027, one-way ANOVA) between the test vegetables but not within the Cu, Ni, Cd, Hg and Cr concentrations. However, no significant difference was observed depending on the site of the collection in Kasbaewa for Water spinach and Lasia.

Kanake et al., 2104 also reported accumulation of heavy metals such as Ni, Cd, Cr and Pb above permissible levels in green leafy vegetables (*Ipomoea aquatic, Alternanthera sessilis, Amaranthus viridis* , *Basella alba* and *Lasiaspinosa*) collated from the markets in Piliyandala Sri Lanka [20] and Premarathna et al., 2011 provided evidence of leafy vegetables grown on soils that are extensively cultivated in Sri Lanka to carry trace levels of heavy metals [11]. The uptake of heavy metals by plants is influenced by plant species, growth, soil type, metal species and also by various factors such as soil pH, physical and chemical properties of soil, clay content etc. [12, 35]. Apart from the above-mentioned factors, human activities have also influenced in polluting both soil and water and eventually has influenced the contamination of the plants that grow on them [36].

Table 1: Concentration of Zn,Cu, Pb, Ni, Mn, Hg, Cr, Cd and As in Water Spinach (*Ipomoea aquatic*), Lasia roots (*Lasia Spinosa*) and Lotus roots (*Nelumbo nucifera*)

	Zn	Cu	Pb	Ni	Mn	Hg	Cr	Cd	As
	mg/L	mg/L	mg/L	mg/L	mg/L	µg/L	mg/L	mg/L	mg/L
Water spinach									
S 1 (WL)	18.69	6.49	**1.09**	1.64	17.61	ND	**2.05**	**0.44**	ND
S 2 (WL)	18.08	4.61	**1.17**	1.22	28.68	ND	**2.02**	ND	ND
S 3 (WL)	17.78	1.49	**1.36**	1.19	22.93	4.15	**1.82**	ND	ND
S 4 (WL)	16.80	4.15	**1.01**	1.00	24.33	12.15	**1.50**	ND	ND
S 5 (HG)	18.81	6.92	**1.04**	1.10	16.32	ND	**1.55**	ND	ND
Average of all Water spinach samples	18.03	4.73	1.13	1.23	21.97	8.15	1.79	0.44	
Max. level	18.81	6.92	**1.36**	1.64	28.68	12.15	**2.05**	0.44	-
Min. level	16.80	1.49	**1.01**	1.00	16.32	4.15	**1.50**	-	-
Lasia									
S 1 (WL)	520.15	3.09	**2.77**	1.03	221.43	9.9	**0.84**	**0.41**	ND
S 2 (S)	517.4	3.21	**2.69**	1.54	59.83	ND	**2.26**	0.18	ND
S 3 (PF)	578.6	2.63	**1.66**	3.78	152.58	8.7	**2.45**	**0.25**	ND
S 4 (HG)	732.7	3.23	**2.48**	0.73	176.77	ND	**2.33**	**0.44**	ND
S 5 (DW)	122.85	9.11	**1.81**	1.57	25.81	ND	**1.04**	ND	ND
Average of all Lasia samples	494.34	4.25	**2.28**	1.73	127.28	9.3	**1.78**	0.32	
Max. level	732.7	9.11	**2.77**	3.78	221.43	9.9	**2.45**	0.41	-
Min. level	122.85	2.63	**1.66**	0.73	25.81	9.3	**0.84**	0.18	-
Lotus root									
S 1 DB	17.00	6.54	**1.22**	1.59	34.09	ND	**2.52**	ND	ND
S 2GW	13.39	7.44	**1.39**	1.52	39.14	12.17	**2.05**	ND	ND
S 3 DM	14.43	9.95	**1.97**	2.43	73.08	17.72	**9.23**	ND	ND
Average of all Lotus root samples	14.94	7.98	1.53	1.85	48.77	14.95	4.60	-	-
Max. level	17.00	9.95	**1.97**	2.43	73.08	17.72	**9.23**	-	-
Min. level	13.39	6.54	**1.22**	1.52	34.09	12.17	**2.05**		
Permissible levels mg/kg *	No ML	40	0.3	4#	-	0.03	0.1	0.2	0.1

Bold numbers indicate the values above the permissible limits.
ND = Not detected- Levels were below the detection limit.
***Source:** FAO/WHO (1999/ 2001) [17,18]

Edible material contaminated with an excessive quantity of heavy metals are linked with etiology of various disorders, particularly with cardiovascular, nervous, kidney as well as bone disorders [37, 38]. It is reported that food contaminated with heavy metals can severely reduce some vital nutrients in the body leading to incapacities related with malnutrition, reduced immunological defenses, growth retardation, gastrointestinal cancer etc. [39, 40]. Nriagu and Pacyna, (1988) reported that continuous exposure of Cd in edibles and water can result in accumulation of Cd in kidneys causing kidney diseases [41]. Higher contact with Cd could also result in lung disorders like bronchiolitis, emphysema, and alveolitis [42]. Though the increase in the concentration of Ni, Zn, and Mn has not been considered as a major concern since these metals are identified as elements required for biochemical reactions in the body, is reported to be deadly if it surpasses the allowed amount in edibles. Lead is also categorized as a principally poisonous metal and is reported to cause many negative impacts on human health due to its toxicity [43].

Pesticides and fertilizers are identified as the main sources of heavy metal pollution of the soil and water in agricultural areas[2]. Studies performed in the dry zone of Sri Lanka showed that both agricultural and non-agricultural soils are not contaminated to an environmental risk level for Cd, Cu, Pb, Ni and Zn [19]. However, studies have shown that continuous irrigation of agricultural land with waste water and sewage could cause heavy metal accumulation both in the soil and vegetables [44].The FAO/WHO is promoting consumption of vegetables as a major dietary component in many developing countries as an easy and cost-effectivesource of many vitamins and for other health benifits[1,45]. However, consuming vegetables that are contaminated in large quantities could pose a health risk to the consumers. Studies performed by. Shakya and Khwaounjoo, 2103, showed that vegetables make a remarkable contribution to the daily intake of Cd, Pb, and Zn [2].

Our present study showed that Cd and Pb concentrations in all three vegetables were above the permissible limits set by FAO/WHO for human consumption [17,18]. The Cd concentrations were also above the recommended limits in one water spinach sample and in two Lasia root samples. However, consumption of vegetables with elevated levels of heavy metals for long period may lead to high level of accumulation in the body causing related health disorders. It is therefore suggested that regular monitoring of heavy metals in vegetables is essential in order to prevent excessive build-up of these metals in the human food chain. It is also important to further investigate the soil and the water in the cultivation area and search for the root and entry points of heavy metals to the environment. Furthermore, it must be discouraged to cultivate on contaminated soil and also discourage the use of pollutedwater.

IV. Conclusion

The results of this study show that the vegetables under this investigation namely Water spinach, Lasia root and Lotus root are contaminated with heavy metals such as Zn, Cu, Pb, Ni, Mn, Hg, Cr and Cd in detectable quantities. The contamination levels of Cr and Pb concentrations exceeded the permissible levels in all vegetable samples tested and Cd concentrations were exceeded at three locations. Arsenic was not present in any of the samples tested at the detection limits of our experiments. Since consumption of heavy metal contaminated vegetables could lead to many heath issues, regular monitoring for heavy metal contamination must be promoted to ensure the safety of the food for the consumers.

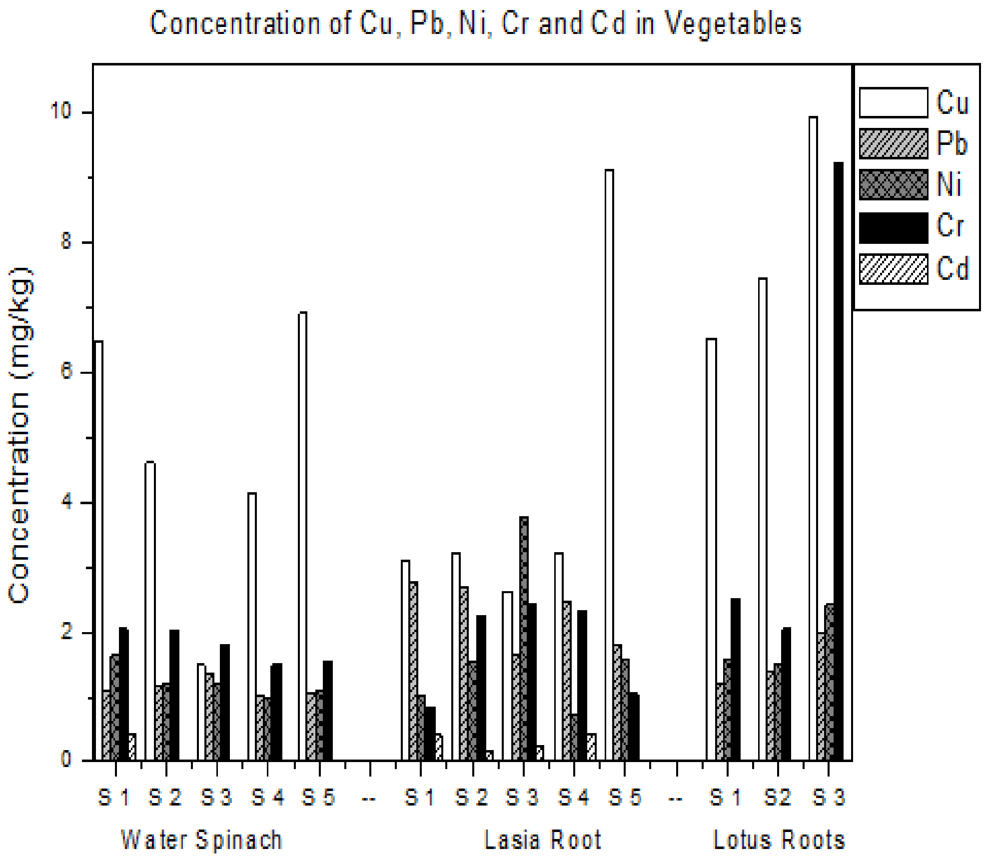

Figure 1. Concentration of Cu, Pb, Ni, Cr and Cd (mg/kg) in Water spinach, Lasiaroots and Lotus roots

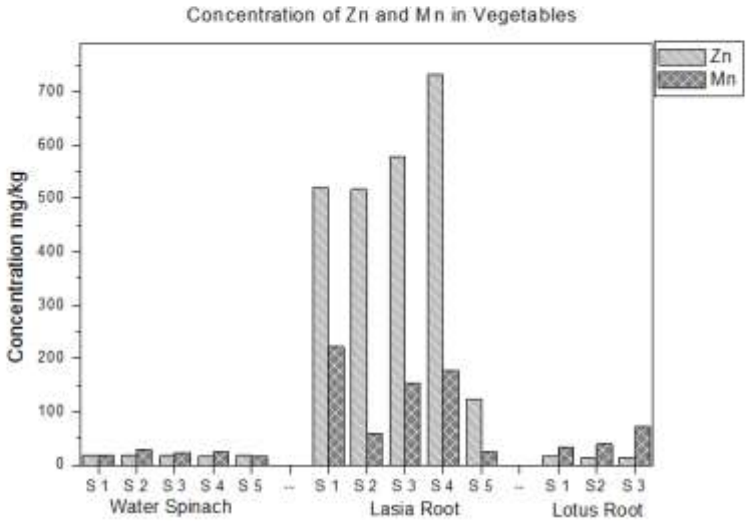

Figure 2. Concentration of Zn and Mn (mg/kg) in Water spinach, Lasia roots and Lotus roots

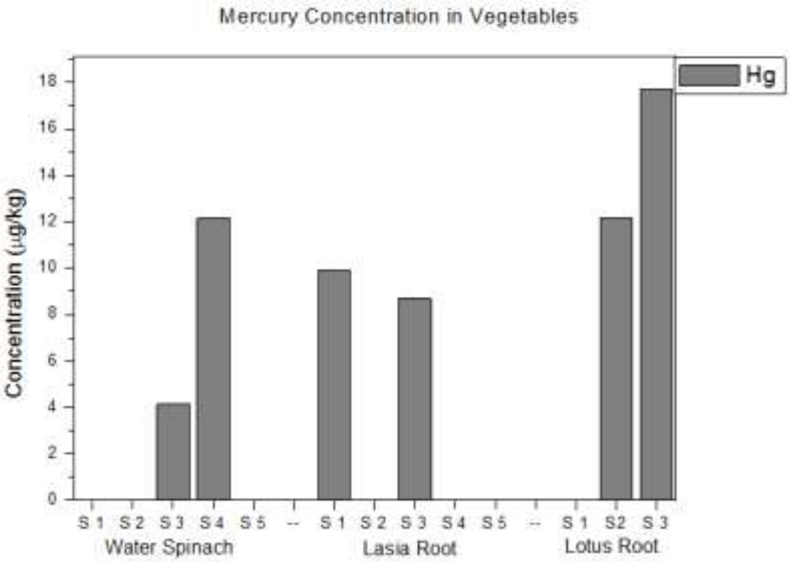

Figure 3. Concentration of Hg (μg/kg) in Water spinach, Lasia roots and Lotus roots

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