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Heavy Metal Contamination in Green Leafy Vegetables Collected from Selected Market Sites in Lucknow Area of India

Punam Sadana
Department of Chemistry, M.S. College, Saharanpur (Uttar Pradesh), India
Email - punamsadana@gmail.com

Abstract: Nickel (Ni), cadmium (Cd), chromium (Cr), Lead (Pb), and Copper (Cu) concentration in 5 kinds of leafy green vegetables, namely spinach" (*Ipomoea aquatica*), " water spinach (*Spinacia oleracea*), "chaulai" *(Amaranthus viridis),*"Malbar spinach" *(Basella alba)* and "white goosefoot" *(Chenopodium album)* were analysed by utilizing spectrophotometer. Substantial variances in elements contents were found among leafy green vegetables studied. On a dry weight basis, the average amounts of heavy metals identified in leafy green vegetables were 0.71-15.89, 0.07-0.97, 0.18-5.05, and 0.18-1.59, 7.05-18.44 mg/kg for Ni, Cd, Cr, Pb, and Cu. Furthermore, the average concentrations of metals in leafy green vegetables were determined to be Cu>Ni>Cr>Pb>Cd in order of abundance. The presence of heavy metals in composite green leafy vegetables obtained from the 4 market sites, however, did not differ significantly ($p < 0.05$). The levels of Ni, Cd, Cr, and Pb were also found to be higher than the FAO/maximum WHO's acceptable limits for human consumption.

Key Words: *Heavy Metal, dietary fiber, metallic materials, Green Leafy Vegetables.*

1. INTRODUCTION :

GLVs (green leafy vegetables) are an important part of the Indian diet. Minerals, Vitamins, dietary fiber, and anti-oxidants are abundant in such vegetables. Recently, there has been a rise in GLV consumption, notably among the metropolitan population. GLV, on the other hand, contains both vital and lethal elements. Heavy metals can be absorbed through polluted soil as well as irrigation water (Abdulazeeza and Azizb 2014). GLV can also absorb metals deposited on plant surfaces that have been exposed to polluted environments. Even though some heavy metals (Cr, Mn, Ni, Zn, Cu, and Fe) are required for a variety of biological functions in the human body, excessive levels can have a variety of negative health consequences. Pb, Cd, Hg, and As, on the other hand, are non-essential, toxic elements linked to a variety of chronic diseases in humans. Contamination by heavy metals of fruits and vegetables gathered from manufacturing locations in many countries, including India, has been documented in a number of studies (Sobukola et al. 2010). Despite the fact that local experts have acknowledged the seriousness of heavy metal contamination of GLV on consumer health, there is no evidence that any research has been done to examine the degree of heavy metal pollution of GLV sold in Indian market places, unlike in other nations. Due to air precipitation, heavy metal levels in green vegetables from selected markets in Lucknow have increased (Sharma, Agrawal, and Marshall 2009). The prevalence of heavy metals pollution in leafy vegetables sold in marketplaces in Libreville, Gabon, has recently been documented (Hu et al. 2013), which is also attributable to air deposition. (Su et al. 2014) looked into the levels of heavy metals in four leafy vegetables sold in Abraka, Delta State, Nigeria, whereas (Parvin, Sultana, and Zahid 2014) looked into the quantities of heavy metals in fruits & leafy vegetables sold in Lagos, Nigeria. The majority of GLV marketing locations in Lucknow are near roadsides, where the environment is significantly contaminated by heavy metal-laden emissions. Increased traffic activity in and around the capital have resulted in significant accumulations of heavy metals in roadside surroundings as a result of rapid industrialisation and urbanisation (Olufunmilayo, Oludare, and Oluwatoyin 2014).

Furthermore, a variety of small-scale enterprises, such as textile, battery, galvanising, metal products, & cable coating industries; brick kilns; diesel engines; re-suspended road dust, and so on, have all linked to the metal ions contamination of the environment. As a result, hazardous metals can be accumulated on the surfaces of leafy greens during production, transportation, and marketing. Heavy metals may be present in these GLV, as farmers wash them in dirty water before transporting them to markets (Ekpo, Ukpong, and Udoumoh 2014). As a result, it is expected that the most often eaten green leafy vegetables sold at roadside open markets in Lucknow (UP) India are polluted with heavy metals. Furthermore, heavy metals have the potential to accumulate in food chains and create hazardous consequences in humans due to their persistent nature and cumulative tendency. As a result, these food items must be analyzed to confirm that the quantities of these trace metals are below legal limits set by local and international regulations. This is especially significant in the case of GLV, since there are few data on the heavy metal concentration of such widely used agricultural materials. As a result, the current study was established with the goal of measuring Cd, Ni, Pb, Cr, and Cu concentrations in five varieties of major green leafy vegetables gathered at random from four urban and suburban market sites in and around Lucknow area, India.

2. Material and Methods :

Sample Collection

GLV were purchased from four market sites *viz*. Patrakarpurum (market 1), kathauta chauraha (market 2), vithauli crossing (market 3) and Jankipurum (market 4) located in and around Lucknow, India. Four markets were chosen at random, with the ones located along roadside open areas with high traffic loads receiving priority. Market Sites 1 and 2 were chosen from the densely populated patrakarpurum and Kathuta chauraha areas, while Market Sites 3 and 4 were chosen from the suburban Vithauli crossing and Jankipurum areas. The GLV purchased were“water spinach" (*Ipomoea aquatica*), "spinach (*Spinacia oleracea*), "chaulai" (*Amaranthus viridis*),"Malbar spinach" (*Basella alba*) and “white goosefoot” (*Chenopodium album).* GLV samples were collected in clean polyethylene bags and brought to the laboratory for analysis.

Sample Preparation and Analysis

To remove soil, dirt, and other air-borne contaminants, GLV samples obtained from market sites were thoroughly cleaned with running tap water, as is done during the typical cooking procedure. Small chunks of the edible components were cut.

Drying

Test sections were dried in a drying oven at 105°C until they reached a constant weight, then cooled to room temperature and homogenized with a clean pestle and mortar. Following the dry ashing process outlined in AOAC 999.11, the ground samples were maintained at room temperature in hermetically sealed polyethylene bags until required for analysis by Atomic Absorption Spectrometry (AAS).

Instruments

Metal levels were measured using a background-corrected Thermo Scientific ICE 3000 Series Atomic Absorption Spectrometer (AAS). Each sample's result is based on the mean of three replicate readings. For each heavy metal, standard curve of absorbance versus quantities were constructed, and the quantity of each metal was estimated by interpolation from the calibration plot.

Assurance of Quality

To assure the credibility of the testing results, suitable safety measures & quality assurance processes were followed. Analytical & trace-metal grade chemicals & reagents were employed throughout the experiment. During the study, distilled water & properly cleaned glasses and utensils were used. To avoid cross-contamination, materials were handled carefully, and reagent blank tests were performed to correct instrument values.

Statistical Analysis

The average levels of heavy metals in GLV were calculated using the Excel spreadsheet programmed. Using Minitab Version 17, a one-way analysis of variance (ANOVA) was done to evaluate whether there was a significant difference ($p<0.05$) between groups.

3. Result and Discussion :

Figure 1: *Vegetable green leaves sample collected from market. (a) Spinach (b) water spinach (c) chaulai (d) Malbar spinach (e) white goosefoot*

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Tables shows the levels of Ni, Cd, Cr, Pb, and Cu in five GLV taken from four randomly chosen roadside open markets in and around Lucknow. The detected Ni, Cd, Cr, Pb, and Cu levels in the GLV were compared to the WHO prescribed levels to assure the safety and well-being of the consumer (Hu et al. 2013). In our findings the average heavy metal concentrations discovered in GLV obtained from the four major sites varied from 0.70-14.89 mg/kg for Ni, 0.08-0.98 mg/kg for Cd, 0.17-4.05 mg/kg for Cr, 0.17-1.49 mg/kg for Pb, and 6.05-17.44 mg/kg for Cu, on a dry matter basis. The mean amounts of heavy metals in leafy green vegetables were determined to be Cd <Pb <Cr <Ni <Cu in order of their abundance. Despite the fact that Cu, Ni, and Cr are considered important elements for a variety of biological activity in the human body, high quantities of these metallic materials can have a negative impact on consumer wellness. Copper is required for body pigmentation, the maintenance of the CNS, and the prevention of anemia, in addition to being related with the functions of Fe and Zn in the body (Su et al. 2014). Most plants do not have enough copper to grow normally, thus it must be supplemented with chemical or organic fertilizers on a regular basis (Parvin et al. 2014). Furthermore, copper poisoning in the body can cause lipid peroxidation, iron shortage, and membrane damage. Chromium is also a vital trace metal for maintaining blood glucose levels, which helps to prevent diabetes by allowing insulin to work more effectively.

It assists in the metabolism of lipids in the body and raises HDL cholesterol levels while lowering LDL cholesterol levels. Humans can experience erratic heartbeat, stomach trouble, itching, and flushing after consuming large dosages of chromium. Chromium can also cause ulcers, liver problems, & kidney problems. (Olufunmilayo et al. 2014).

Table 1: Heavy Metal Contamination in Green Leafy Vegetables Collected from Patrakarpurum Market

Commodity	Ni	Cd	Cr	Pb	Cu
Spinach	2.09	**0.90**	**6.04**	**0.32**	1.37
Water Spinach	**7.29**	**0.82**	**2.80**	**0.95**	12.67
Chaulai	2.08	**0.64**	2.00	**1.05**	12.09
Malbar spinach	**16.28**	0.20	0.55	0.28	8.84
White goosefoot	**16.99**	**0.98**	1.36	**1.38**	18.44

Table 2: Heavy Metal Contamination in Green Leafy Vegetables Collected from Kathauta Chauraha Market

Commodity	Ni	Cd	Cr	Pb	Cu
Spinach	**6.76**	**0.37**	0.94	0.22	16.03
Water Spinach	3.34	**0.51**	1.34	**0.54**	12.87
Chaulai	0.94	**0.22**	1.86	**1.09**	11.37
Malbar spinach	0.98	0.19	0.28	**0.45**	10.57
White goosefoot	**11.32**	**0.43**	1.12	**1.07**	16.12

Cd & Pb, on the other hand, are non-essential hazardous metals that, even at extremely low quantities, have carcinogenic effects and teratogenic defects in humans (Ekpo et al. 2014). According to reference (Domergue and Vedy 1992), when Pb is taken from the soil by plants, it accumulates primarily in the root area due to its inability to pass through the endodermis of roots. Leaves, on the other hand, may absorb a lot of Pb from the air.

Table 3: Heavy Metal Contamination in Green Leafy Vegetables Collected from Vithauli Crossing Market

Commodity	Ni	Cd	Cr	Pb	Cu
Spinach	0.79	0.07	**2.72**	0.18	16.25
Water Spinach	1.83	0.18	**3.37**	**0.64**	7.05
Chaulai	3.56	**0.34**	**2.32**	**0.54**	10.63
Malbar spinach	1.53	0.09	0.18	**0.39**	13.65
White goosefoot	3.81	**0.78**	0.28	**1.07**	12.87

As a result, GLV have the potential to acquire Pb from both the soil and the atmosphere via their leaves. Lead has been identified as a serious cumulative body toxin that enters the body via food, air, and water and is not removed by washing veggies. Pb levels in certain green vegetables may be enhanced as a result of pollutants in irrigation water, soil, or industrial and vehicular emissions, as lead is used as an anti-knocking agent in gasoline (Prudent et al. 2014).

Table 4: Heavy Metal Contamination in Green Leafy Vegetables Collected from Jankipurum Market

Commodity	Ni	Cd	Cr	Pb	Cu
Spinach	1.72	0.08	0.33	**1.32**	12.64
Water Spinach	2.35	**0.43**	1.04	**0.44**	10.31
Chaulai	0.71	0.11	**3.07**	**0.96**	9.21
Malbar spinach	2.21	0.09	1.38	0.27	10.34
White goosefoot	2.45	**0.24**	**4.66**	**1.59**	14.13

Phosphate fertilizers are the most common source of trace metal contamination in soil, particularly Cd, which occurs naturally as an impurity in phosphate rocks (Ihesinachi and Eresiya 2014). Nonetheless, in plants and animals, Cd is a non-essential element. Cd accumulation in plants reduces crop output by interfering with nutrient uptake, obstructing respiratory enzymes, metabolic pathway, photosynthesis, and altering antioxidant metabolism (Ihesinachi and Eresiya 2014). Cd accumulates irreversibly in the lungs, hepatic, and kidney of humans (Osundiya et al. 2014). The kidneys and liver produce metallothionein, a Cd-inducible protein that protects cells by tightly interacting with the harmful Cd ion. Long-term exposure to Cd, on the other hand, has been linked to malignancies of the prostate, kidney, and ovary (Kulkarni, Delbari, and Mahajan 2014). The amounts of Ni, Cd, Cr, and Pb in some GLV purchased from the chosen markets were found to be higher than the WHO safe limits for human utilization, posing a health risk to the consumer. Market 1, in Lucknow has the highest quantities of Ni, Cd, Cr, Pb, and Cu in the GLV. Ni, Cu, and Cd concentrations were lowest in the Jankipurum market, Cr in the Kathuta chauraha market, and Pb in the Vithauli crossing market.

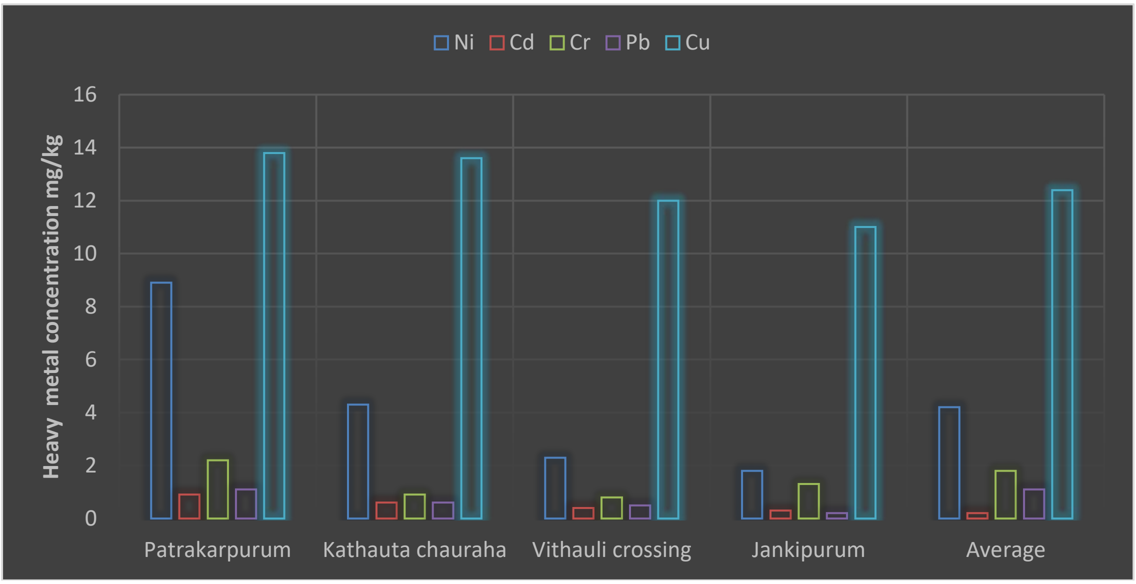

Figure 1: *Heavy metal contents in combined GLV from chosen market sites.*

For the examined heavy metals, the GLV obtained from Lucknow and Kathuta chauraha marketplaces reported the highest amounts. Increased transportation activities may contribute to the deposition of heavy metals in roadside habitats as a result of fast industrialization and urbanization in the aforementioned locations. Cu, Zn, Cd, Ni, Cr, and Pb are common metal contaminants caused by traffic. Fuel combustion, road friction, lubricating oil, tyre and brake wear, and other factors all contribute to heavy metal emissions from vehicles. Heavy metals can be incorporated into the plant tissues of GLV that are marketed at roadsides by air deposit or vehicle runoff.

The heavy metals identified in mixed GLV samples obtained from the four market sites in the Lucknow area, however, showed no significant differences (p>0.05). The current study was the second to examine heavy metals in GLV obtained from Lucknow market sites. However, multiple studies have found that GLV grown in some sections of Lucknow is contaminated with heavy metals (Kulkarni et al. 2014). Reference (Chang et al. 2014) tested GLV from Sedawatta, Welewatta, and Kotuwila in Colombo District, o f Sri Lanka's Wellampitiya Area and discovered significant levels of Cd, Pb, Zn, Cu, and Ni, all of which above WHO's permitted limits. They found 0.590.44 mg/ kg dry weight for Cd, 110.6 mg/ kg dry weight for Cu, 130.9 mg/ kg dry weight for Ni, 83.3 mg/ kg dry weight for Pb, and 4020 mg/ kg dry weight for Zn in GLV. These levels are greater than the mean Cd, Pb, Ni, and Cu values are obtained for the GLV collected from Lucknow area market locations during the present study. Several similar research to analyses the heavy metal pollution of fruits and veggies collected from market sites have been undertaken in various regions of the world.

As per Shakya and Khwaounjoo (Chang et al. 2014), GLV obtained from several market sites in the Kathmandu Area in India includes Pb and Cd levels greater than the WHO max acceptable range for human ingestion, and average percentages of all metals examined in GLV are in the order Zn>Pb>Cd. According to Ref. (Abbas et al. 2010), the amounts of Pb, Cd, Fe, Cr, and Cu in four distinct samples of GLV purchased from Katsina Central Market contain Cu, Zn, Fe, and Pb that are within the WHO safety guidelines. Cu (0.483 mg/kg)>Zn (0.268 mg/kg)>Fe (0.260 mg/kg)>Pb (0.095 mg/kg)>Cd have been reported as the metals in the GLV (ND). Pb, Cd, and Cu mean values indicated in their research are lower than those observed in the current experiment. Heavy metal values in leafy vegetables gathered from several markets in Lagos, Nigeria range from 0.090.01 -0.210.06 mg/kg for Pb; 0.030.01 - 0.090.00 mg/kg for Cd; 0.020.00 - 0.070.00 mg/kg for Cu; and 0.050.04 to 0.240.01 mg/kg for Ni, as shown by (Asdeo and Loonker 2011).

These figures are significantly lower than those found in the current study. The Pb levels of five randomly chosen leafy vegetable samples from two private markets in Metro Manila, Philippines, were examined in Ref. (Gupta et al. 2014), and it was discovered that the majority of leafy vegetables are polluted with Pb. The levels, however, were well below the permissible levels. However, the majority of GLV acquired from the four market sites during this study had a Pb concentration that above the WHO safe limit. The levels of Pb and Cd in vegetables taken from four fresh marketplaces in Surut Thani, Thailand, are shown in reference (Jayasinghe, Jayawardena, and Pathiratne 2005). Even while the Pb levels in the veggies were within the Ministry of Public Health of Thailand's guidelines, the Cd levels were greater than the maximum permitted limits set by the Australia-New Zealand, Codex, China, and European Union guidelines. In the current investigation, both Pb and Cd contents in the GLV acquired from the four marketplaces exceeded WHO permitted limits. The values of heavy metals (mg/kg) in certain Green Leafy Vegetables taken from Sulaimani, Kurdistan-Iraq, range from 6.118 to 339.646 for Fe, 0.041 to 0.247 for Cr, 0.000 to 0.027 for Cd, 0.196 to 0.301 for Cu, 0.037 to 0.503 for Ni, and 0.690 to 2.016 for Zn, according to reference (Jayasinghe et al. 2005). These figures are also significantly lower than those found in the current study.

The average quantities of toxic substances in five types of GLV were also found to have risen in the order of Cd<PbCr<Ni<Cu, according to the findings.

Table 5: Average Heavy Metal Concentrations Discovered in GLV Obtained

	Ni	Cd	Cr	Pb	Cu
Average	*4.25*	*0.37*	*1.79*	*0.74*	*12.61*
Maximum	*15.89*	*0.97*	*5.05*	*1.59*	*18.44*
Minimum	*0.71*	*0.07*	*0.18*	*0.18*	*7.05*
WHO/FAO	*$4*	*0.2*	*2.3*	*0.3*	*40*

White goosefoot leaves had the greatest ability to accumulate Ni, Cd, Pb, and Cu of the 5 kinds of GLV studied from the chosen markets, while Spinach had the highest amount of Cr. The least levels of Cd and Cr were found in Malbar spinach, Ni and Cu in Chaulai, and Pb in Spinach, according to the findings. Significant differences in Pb (P = 0.006, one-way ANOVA) and Cu (P = 0.011, one-way ANOVA) levels were measured were observed were found between the test vegetables in this study, but not in Ni, Cd, or Cr levels.

Rathnayake, Premarathna, and Ariyaratne in 2004 examined market & field specimens of amaranth and cabbage from Zanzibar, Tanzania, and discovered that amaranth has much higher amounts of Zn, Fe, Cr, and Mn than cabbage, whereas cabbage had considerably higher levels of, Ni, and Pb.

Furthermore, Pb and Cd levels were higher than the WHO/FAO permissible limits. Heavy metal concentrations in leafy vegetables from chosen markets in Guyana were studied in ref. (Al-Sharbati, El-Burghthy, and Sudani 1998) Cd, Pb, Cu, Zn, Co, Ni, Mn, and Fe were measured in leafy vegetables from marketplace sites (Brassica oleracea, Brassica

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chinensis, Basella alba, and Lactuca sativa). The results show considerable disparities in elemental levels across the vegetables studied, with Cd, Co, and Mn levels exceeding the acceptable limits in all vegetables and Pb and Fe levels exceeding the safe limits in some. Cu and Zn levels in all of the vegetables were kept within safe ranges. Cu levels were not exceeded in any of the GLVs examined in the current investigation.

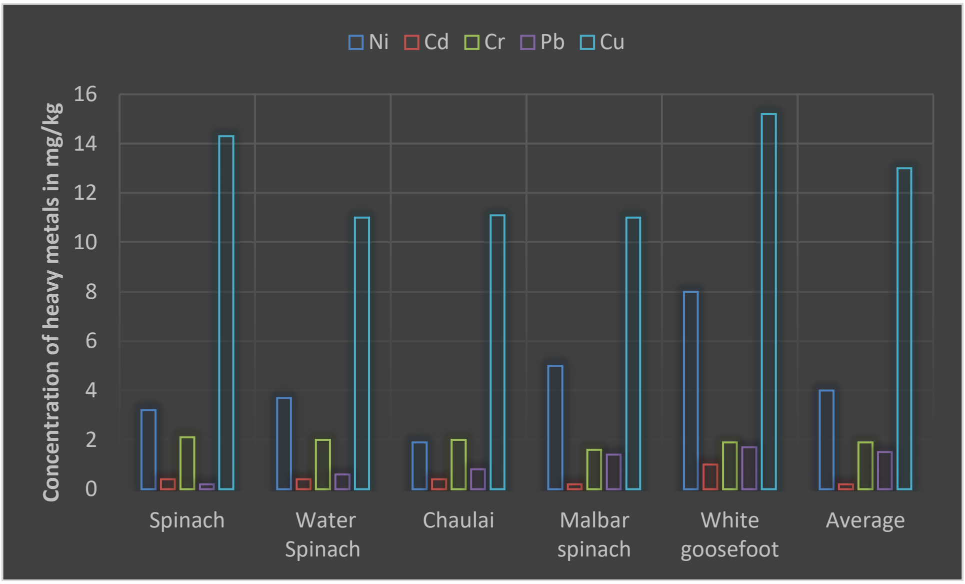

Figure 2: *Heavy metal levels in selected leafy green vegetables from diverse market sites*

Based to a preliminary study, the GLV offered at the chosen market sites was primarily obtained from fields in and around the Lucknow area (Patrakrpurum, Vithauli crossing, Jankipurum). The high quantities of heavy metals detected in GLV could be linked to polluted soil, water sources, fertilizer, and pesticides, or to metals accumulation on plant surfaces due to atmospheric deposition during production, shipping, and marketing (Kanife et al. 2012). Species of plants, growth phase, kind of soil & metal species, soil quality, weather, and surroundings all influence heavy metal assimilation by crops.

Plant species, growth phase, type of soil and metal species, soil condition, weather, and environment are all factors that influence heavy metal uptake by crops (Elbagermi, Edwards, and Alajtal 2012). Furthermore, air depositions and vegetable marketing systems play a key influence in raising heavy metal levels in GLV, posing possible health risks to consumers (Doherty et al. 2011).

4. CONCLUSION :

According to the findings of this study, Ni, Cd, Cr, and Pb contents in several green leafy vegetables acquired from selected marketplaces were beyond WHO permitted limits for human consumption. Cu levels in all GLVs collected, however, were found to be below the maximum allowed range. Long-term ingestion of heavy metal-contaminated GLV could pose a number of health risks to humans. As a result, regular heavy metal monitoring in GLV is critical to avoid excessive accumulation of these metals in the human food chain.

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