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RESEARCH ARTICLE

Assessment of heavy metals in *Ipomoea aquatica* Forssk. and potential effects on human health

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Abstract

In wetlands areas, macrophytes indicate the extent of bioavailable heavy metal contamination, and particular species of aquatic vascular plants are employed to evaluate the bioavailable fraction of heavy metal pollution. However, heavy metal bioavailability and absorption by aquatic plants depend on various environmental factors. This study aims to determine the presence of heavy metals such as lithium (Li), chromium (Cr), and nickel (Ni) in *Ipomoea aquatica* Forssk collected from the Lamphelpat wetland in Imphal West District of Manipur, India. It also focused on assessing the associated health risk with prolonged consumption of *I. aquatica* referring to the Estimated Daily Intake (EDI) of the concerned heavy metals, the Target Hazard Quotient (THQ), and the Hazard Index (HI). A total of 24 composite samples were collected across the three seasons in 2022-2023 and analyzed them for the heavy metals using ICP-MS. The results showed that concentrations (mean±SD) of nickel (4.032±0.830) and chromium (3.448±1.267) in the collected samples were higher than the permissible limit of 1mg/kg set by the FSSAI for leafy vegetables. Concentration of studied heavy metal in *I. aquatica* were in the order of Ni > Cr > Li. According to THQs and HI, consumption of the examined species is considered unsafe for human health, as the THQ value of Cr is >1. The overall HI for both men and women is also >1. The gender-wise estimation of THQs indicates a greater impact on the women population compared to the male.

Keywords: Health hazards; Heavy metals; *Ipomoea aquatica*; Lamphelpat wetland

1. Introduction

More than 500 plant species are recognized for their significant ability to bioaccumulate heavy metals. Researcher have utilized these plants as hyperaccumulators for phytoremediation (Sarma et al., 2011). However, plants with high concentrations of heavy metals pose serious health risks to humans (Singh et al., 2013); hyperaccumulating plants, especially those found near urban waste disposal sites, may be unsuitable for consumption. *Ipomoea aquatica* Forssk. belonging to Convolvulaceae family is one of such plants, which is also known as water spinach, is selected for assessing heavy metals and its potential human health effects. *I. aquatica* grows well above 24°C and flourishes in tropical and subtropical climatic conditions (Göthberg et al., 2002). *I. aquatica* has a high growth rate, attaining up to 10 cm daily (NRC, 1976). It thrives on saturated soils and near aquatic habitats. Its roots emerge from the nodes and anchor themselves in moist soil or mud, while its elongated, hollow, vine-like stems may either extend along the ground or float over the surface of water. The leaves are arranged alternately, typically oblong-lanceolate or narrowly triangular in shape. They measure 5-10 cm in length and 2-6 cm in width, featuring a hastate base and an acute apex. Petiole lengths are 6-10 cm long. The flowers are purplish-white, solitary, or few in cymes. Sepals are subequal, and the length is 6-8 mm long, oblong-lanceolate, membranous, and glabrous. Corollas are funnel-shaped, about 5 cm in length, and exhibit a colour range from pale purple to nearly white. Stamens comprised unequal filaments which are hairy at the base. The ovary is glabrous, and the capsule is globose; there are 4 or 2 seeds, which are minutely pubescent (IBP, 2016).

Small ponds and often inundated areas are ideal environments for the growth and propagation of *I. aquatica*. It is native to Southeast Asia, India, and southern China, capable of growing in both wild and cultivated habitats. It is cultivated in eutrophic, shallow ponds and canals or in former rice fields prone to flooding, demonstrating efficacy in improving water quality in eutrophic water bodies (Jha et al., 2016). However, *I. aquatica* is considered an invasive species on islands in the Pacific and Indian Oceans, as well as in Cuba and the USA (California and Florida). It is the second most problematic plant species in the Philippines, where it frequently dominates freshwater marginal regions. It is classified as a Federal Noxious Weed in the United States (Dueñas-López, 2023).

The different parts of the *I. aquatica*, including young shoot, floating stems (upper 40-50 cm), the petioles, and leaves are considered delectable vegetables. People of Manipur and other states consumed it in both cooked and raw forms. It is a highly nutritious and rich source of vitamins A, C, and iron. Manvar and Desai (2013) reported health benefits of *I. aquatica* having rich source of vitamins, minerals, proteins, fibers, carotenes and flavonoids. It is used in Ayurvedic, folk, Siddha, and Traditional Chinese Medicine. Edible aquatic plants have nutritional and medicinal benefits but can accumulate toxic heavy metals when grown in polluted waters (Rai, 1996). Heavy metals such as lithium (Li), chromium (Cr), and nickel (Ni) do not have any essential function, but they are detrimental, even in small quantities, to plants, animals, and humans, and accumulate within them due to their prolonged biological half-life. The amount of heavy metals absorbed by plants differs among species. The absorption capacity also varies according to the metal type. Various environmental factors affect the bioavailability and absorption of heavy metals by plants in aquatic habitats, including pH, temperature, organic matter, and redox potential. Macrophytes signify the degree of bioavailable metal contamination in the environment, and specific species of aquatic vascular plants are utilised to assess the bioavailable proportion of heavy-metal pollution.

Previous studies have reported the accumulation of heavy metal in four aquatic plant species, namely, *Oreochromis niloticus, Barbonymus gonionotus, Rasbora tornieri* and *Anabas testudineus* (Intamat et al., 2017; Sriuttha et al., 2017). Research on additional edible plant species often consumed by indigenous populations is lacking. Heavy metal contamination in food crops has been studied extensively; however, investigations on edible aquatic plants remain largely overlooked. A study by Rai et al (1996) indicates significant metal accumulation in *I. aquatica* from the Eastern Ghats. However, there are currently no studies on bioaccumulation of heavy metals through the food chain in *I.*

aquatica and the resulting health effects in the wetlands of Imphal Valley, Manipur. Investigating the heavy metal concentrations in plants is crucial for assessing their safety as future food sources. The study is also relevant due to the impending reliance of humans on aquatic plants for food and forage, particularly concerning health implications.

2. Methods and materials

2.1. Study Site

The study was conducted in a lake known as Lamphelpat, which lies at 24.82°5067''N and 93.90°8987''E, is a semiurban wetland located in the Lamphel Subdivision of the Imphal West district of Manipur. The wetland is approximately 3 km from the capital city, Imphal, and situated at an altitude of 780 m above Mean Sea Level (MSL). The total area is 5.32 km^2; however, the core portion that retains water throughout the year measures only 2.9 km^2. Lamphelpat is one of the most important and last remaining wetlands of Imphal Valley, historically intertwined with the culture and ethos of Manipur. Lamphelpat provies various economically important plants species supporting the livelihood of local people, *I. aquatica* is one among the various plant species. This lake also serves as the natural habitat of "Kombirei," *Iris laevigata* Fisch. an endangered plant that is associated with the social and cultural ethos of the Meitei community. Lamphelpat renders various ecological and economic services to the nearby inhabitants. It also aids in flood mitigation and moderate temperature. However, the area is highly fragmented and degraded due to various anthropogenic activities. Infrastructural development, siltation, illegal encroachment, discharging of hospitals and domestic effluents, and landfilling are primary causes of its degradation. The area also experienced significant dumping of municipal solid wastes. The pollution level in Lamphelpat is compounded by run-off from the Nambul River, the most polluted river in the Imphal Valley of Manipur, as well as sewerage from the nearby locality. The wetland readily assimilates detrimental chemicals from waste disposal sites, which may contain lithium (Li), chromium (Cr), and nickel (Ni) degrading water quality; hence these heavy metals were selected for the study. *I. aquatica* is a perennial aquatic vascular herbaceous plant that thrives in both semi-aquatic or terrestrial habitats. It remains dormant during the dry season but initiates vigorous growth with the onset of pre-monsoon during the April and May months and proliferates extensively, exhibiting high in abundance, during the monsoon season until August and September (Saikia et al., 2023).

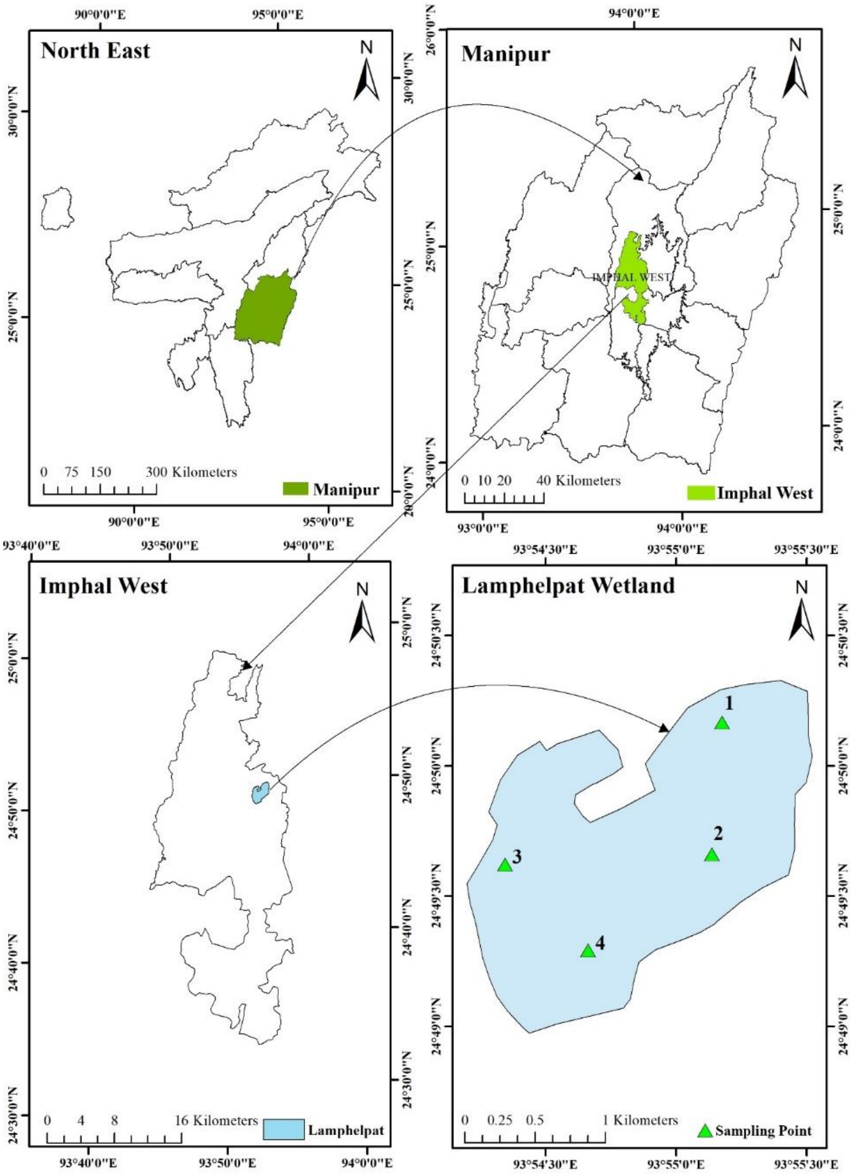

Figure 1. Map of study site (Lamphelpat) of Manipur showing sampling points.

2.2. Sample collection

Sampling was conducted seasonally across two years (2022-2023), considering three seasons, i.e., pre-monsoon, monsoon, and post-monsoon. Plant samples *I. aquatica* were collected from four sampling points of the study site (Figure 1), taking into account the accessibility and representation of the study area. The apical or terminal part of *I. aquatica*, with 25-30 cm length including both stem and foliage, were collected from five individuals from the 4 different sampling points. The collected five replicates were made into a composite sample. A total of 24 composite samples were collected across the three seasons during 2022 to 2023, and analysis of the heavy metals like lithium (Li), chromium (Cr), and nickel (Ni) was conducted using Inductively coupled plasma mass spectrometry (ICP-MS).

2.3. Sample preparation and analysis

The collected samples were first washed with tap water and then with double distilled water. The excess water was wiped off with tissue paper, and the fresh weight was recorded. The material was air dried for 15 hours, followed by drying in a hot air oven at 70°C until a constant weight was obtained. The dried samples were ground in an agate mortar and pestle (Figure 2) and stored in a polyethene bag for further analysis. Digestion was done using a modified version of the United States Environmental Protection Agency (USEPA) method 3052 (USEPA, 1996). Accurate sample weights of 0.25 g dry weight were digested in a mixture of 5 ml conc. nitric acid, 2 ml ultrapure water, and 1 ml conc. hydrogen peroxide in a closed PTFE vessel. Sample digestion was carried out using a microwave-assisted system (Multiwave 3000, Anton Paar GmbH) at 1400 W, with a ramp time of 15 minutes and a hold time of 10 minutes. After digestion, the digestate volume was made up to 50 ml using MiliQ water (18.3 MΩ-cm, Millipore Sigma, USA) and subsequently filtered through a 0.2μm pore size PVDF syringe filter (Whatman Corp.). Quality assurance was carried out by incorporating analytical blanks and standard reference materials (SRM) in each digestion, which were prepared in the same manner as to the samples. The digested sample solution was analysed for heavy metals like lithium (Li), chromium (Cr) and nickel (Ni) using ICP-MS (iCAP RQ, ThermoFisher Scientific).

2.4. Quality control and quality assurance

Blank solutions and calibration standards at concentrations of 12.5, 25, 50, 100, and 200 μg/l or ppb were prepared gravimetrically by adding the appropriate quantity of a multi-elemental stock solution (Inorganic Ventures™ IV stock-4) directly into a solution containing 2% m/m HNO_3 (65% HNO_3 Optima™ grade, Thermo Fisher Scientific). All solutions were prepared in sterile vials. This acid matrix was selected to reflect a sample preparation procedure that is broadly appropriate to various industries and applications (Tomoko, 2017).

2.5. Human health risk assessment

The Estimated Daily Intake (EDI) of the concerned heavy metals, the Target Hazard Quotient (THQ), and the Hazard Index (HI) were calculated to assess the health risk associated with the prolonged consumption of *Ipomoea aquatica* from the study sites (USEPA, 2007). The USEPA (1989) assumes that the ingested and absorbed doses are considered equivalent in these calculations.

EDI is an index that quantifies the amount of heavy metals transferred from plants or other organisms to humans (Chamannejadian et al., 2013). According to Song et al (2011), EDI depends on the metal concentrations in the plants, the daily consumption rate, and the average body weight of consumers.

$$EDI=\frac{Cm \times IR}{BW}$$

Where Cm = concentration of metal in *Ipomoea aquatica*, IR = ingestion rate, which is assumed to be 18.33 g/day per adult (Vanitha et al., 2013), and BW = body weight of consumer, taken as 65 kg for an adult men while 55 kg for a adult women (ICMR-NIN, 2020). The THQ estimates the non-carcinogenic risk level due to metal exposure (Elhaddad et al., 2022; Javed and Usmani, 2016). THQ is a dimensionless value and is calculated as per USEPA (2011).

$$THQ = \frac{EF \times ED \times IR \times Cm \times 10^{-3}}{RFD \times BW \times AT}$$

Where EF is exposure frequency (365 days/year), ED is exposure duration (67.3 years), which is taken to be the average life expectancy of an Indian (WHO, 2024), RFD is the oral reference dose (mg/kg/day), AT is the average exposure time (365 days/year × life span), and IR, Cm, and BW are the same as given in EDI. RFD is the oral reference dose (mg/kg/day), and AT is the average exposure time (365 days/year × life span). The corresponding oral reference doses for Li, Cr, and Ni were 0.002, 0.0003, and 0.91 mg/kg/day (USEPA, 1993). While a THQ value of 1 or higher suggests a possible health risk to the exposed population, a value of less than 1 indicates that the exposed population is less likely to suffer negative consequences as a result of metal contamination (Wang et al., 2005; Janadeleh and Kameli 2017; Islam et al., 2016; Yacoub et al., 2021). Lastly, the sum of all individual THQs was used to calculate HI, which is the total potential non-carcinogenic health risk presented by all the heavy metals evaluated together (USEPA, 2011; Javed and Usmani, 2016).

$$HI = \sum_{i=1}^{n} THQi$$

Here, i stands for each metal, and n is the total number of heavy metals. When the HI value is more than 1, it suggests that there may be serious health consequences for such heavy metals (Huang et al., 2008).

2.6. Data analysis

The results have been presented as mean ± standard deviation (SD) values. The Kolmogorov-Smirnov test was conducted to check the normality of the data. Analysis of Variance (ANOVA) was performed to check the significant differences in the heavy metal concentrations during the different seasons. The required calculations and graphical presentations involved in the analysis of the experimental data and the statistical tests, like descriptive statistics, independent sample, and one way ANOVA were done using IBM SPSS Statistics-27 and MS-Excel.

3. Results

3.1. Concentration of heavy metals in Ipomoea aquatica

The estimated mean concentrations values (mean±SD) of two year (2022-2023) of the selected heavy metals in the *I. aquatica*, along with the respective permissible limits prescribed by regulatory bodies, are presented in Table 1. The concentration of heavy metals in *I.aquatica* was in the order of Ni > Cr > Li. The recorded lithium (Li) concentration was 0.263±0.067 mg/kg dry weight, with a minimum value of 0.133 mg/kg and a maximum value of 0.395 mg/kg. The highest concentration (0.388±0.089) was recorded during the monsoon, while the lowest concentration (0.201±0.065) occurred in the pre-monsoon period (Figure 3A). However, no statistically significant differences were observed in Li concentration among the sampling seasons ($p>0.05$) as shown in Table 2. The mean concentration of chromium (Cr) was 3.448±1.268 mg/kg, ranging between 1.196 and 5.843 mg/kg. It surpassed the FSSAI permissible limit of 1 mg/kg, indicating that prolonged use of *I. aquatica* from the studied site may pose potential health risks. The three sampling seasons did not show statistically significant ($p>0.05$) difference in Cr concentration (Table 2). The highest concentration of chromium, having 4.313±1.150 mg/kg was recorded in post-monsoon, while pre-monsoon exhibited a minimum concentration of 2.779±1.063mg/kg (Figure 3B). The mean concentration of nickel (Ni) was 4.032±0.830 mg/kg, with a minimum concentration of 2.956±0.963 mg/kg which was recorded during the monsoon and a maximum concentration of 5.576±0.717 mg/kg in post-monsoon (Figure 3C). For Ni, there were significant differences (F=8.04; $p<0.01$) in the concentration across sampling seasons (Table 2). The mean concentration of Ni in *I. aquatica* exceeds the FSSAI-mandated threshold of 1 mg/kg in green vegetables. Estimates of high concentration of Ni and Cr above the FSSAI threshold limits indicates that these elements are contaminating the water and sediments at the study site at high level.

Figure 2. *Ipomoea aquatica* Forssk, sample plant (A), Sample grinding (A), Preparation for sample digestion (C) and Microwave digester (D).

3.2. Health risk assessment of heavy metals in Ipomoea aquatica

The health risks associated with the consumption of *I. aquatica* from the wetland regarding heavy metal contamination have been assessed using EDI, THQ, and HI (USEPA, 2012). Table 3 presents the EDI, THQ, and HI values of the selected heavy metals. The EDI (mg/kg-day BW) for men of Li, Cr, and Ni were found to be 0.080, 0.976, and 1.137, respectively. In women, the values were 0.095, 1.149, and 1.344, respectively. The differences in the EDI values between men and women arise because EDI is inversely proportional to the consumer's body weight. The EDI values were compared with the recommended daily dose.

The mean THQ values per metal were registered in the following order: Cr > Li >Ni for both men and women (Table 3). The THQs for men for Li, Cr, and Ni are found to be 0.040, 3.241, and 0.001, respectively, while for women they are 0.034, 2.717, and 0.001. Cr has the highest THQs value, with 3.241 and 2.717 for men and women, respectively. However, there are no significant differences ($p> 0.05$) in THQ values across the genders. Both men and women have a THQ for Cr >1 for, which indicates a significant likelihood of non-carcinogenic health risks to the exposed population due to long-term consumption of *I. aquatica* from the studied site. The overall hazard index for men and women were found to be 3.283 and 2.752, respectively.

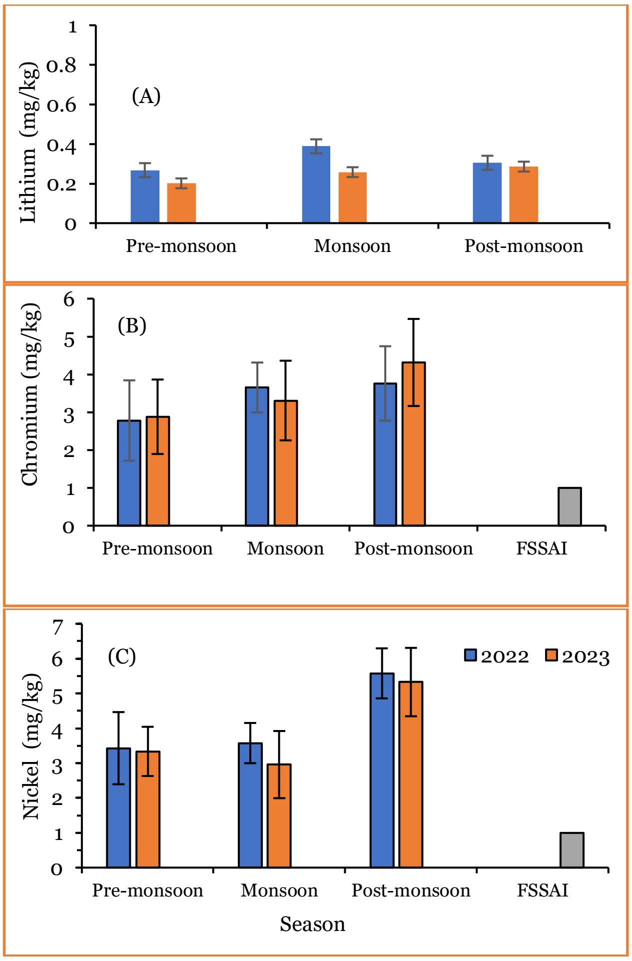

Figure 3. Concentration of different heavy metals, lithium (A), chromium (B) and nickel (C) in *I. aquatica* estimated in three seasons during the study period (2022 and 2023).

Table 1. Mean concentration of heavy metals in *Ipomoea aquatica,* along with the FSSAI (2011) permissible limit for leafy vegetables.

Heavy metals	Mean ± SD (mg/kg)	Min (mg/kg)	Max (mg/kg)	FSSAI (mg/kg)
Lithium (Li)	0.2634±0.06681	0.133	0.40	-
Chromium (Cr)	3.448±1.3	1.196	5.843	1
Nickel (Ni)	4.0329±1.285	1.60	4.0329	1

Table 2. One-way ANOVA test showing any significant difference in heavy metals concentration of the *Ipomoea aquatica* between the sampling seasons.

Heavy metals	Pre-monsoon	Monsoon	Post-monsoon	F- value	Significance
Li	0.2634±0.06681	0.322±0.23	0.295±0.06	0.741	0.491
Cr	2.828±1.6	3.479±0.94	4.03675±1.13	1.626	0.224
Ni	4.335±1.94	3.265±0.8	6.326±1.36	8.04	0.003**

** Significant at $p < 0.01$

Table 3. Gender-wise health risk assessment of heavy metals in *Ipomoea aquatica*

Heavy metals	Estimated daily intake (EDI)		Target hazard quotient (THQ)		Hazard index (HI)	
	Men	Women	Men	Women	Men	Women
Li	0.080	0.095	0.040	0.034		
Cr	0.972	1.149	3.241	2.717	3.283	2.752
Ni	1.137	1.344	0.001	0.001		

4. Discussion and conclusion

I. aquatica were collected from the Lamphelpat, a semi-urban wetland of the Imphal West district of Manipur, and the presence of heavy metals in it was investigated. Concentration of heavy metals in *I. aquatica* was found to be in the order of Ni > Cr > Li. The wetland may be contaminated with heavy metals because of the unsorted municipal solid wastes that is dumped around the edges of it. These wastes include organic waste, plastic waste, electronic waste, medical waste, sanitary waste, and industrial waste. The heavy metals then get into the plants that grow there. This unsegregated mixture of biological and hazardous waste dump at this wetland poses extraordinary toxicity, and together with the subsequent degradation of land and water ecosystems in the surrounding area, is unparalleled. On the other hand, leachate from municipal solid waste dumping sites is the main source of harmful chemicals in nearby wetlands and waterways. Heavy metals, alien nutrients, ammonia, and carbon in this leachate penetrate the water body and disintegrate with the wetland hydrology. This turns wetland water into a hazardous interface for aquatic biodiversity (Baruah and Goswami, 2022). Kapil (2008) revealed that areas of the Deepor Beel that were in close vicinity of the municipal garbage dumping site had abnormally high values of the chemical parameters and constituents.

The concentration of chromium in *I. aquatica*, which was measured to be 3.448±1.267mg/kg, was found to be higher than the FSSAI-approved limit of 1 mg/kg. This indicates that the consumption of *I. aquatica* from the current studied site could potentially lead to adverse health effects if consumed for an extended period of time. Likewise, the concentration of nickel (4.032±0.830 mg/kg) *I. aquatica*, is found to be higher than the threshold value of 1 mg/kg in green vegetables as given by FSSAI (2011) for leafy vegetables. Prolonged exposure to Cr (VI) compounds increases the risk of developing cancer in the sinuses, nasal cavity, and lungs. It may also result in severe dermatitis and predominantly painless skin ulcerations. Certain research indicates that prolonged, low-dose exposure to Cr (VI) may lead to reversible renal tubular injury (Wu et al., 2020; Sharma et al., 1978). Cr (VI) compounds can cause liver problems that vary from mild to severe. Cardiovascular collapse can occur after consuming a lethal dose of chromate. Hematological toxicity can occur from oral exposure to Cr (VI) compounds. The potential effects of human chromium on reproduction have not been sufficiently studied. Research indicates that compounds containing Cr (VI) are carcinogenic to animals. It causes chromosomal abnormalities, gene mutation, sister chromatid exchanges, and DNA damage in various targets, including human and animal cells both in vitro (Yu, 2008). Cr is one of the most difficult metals to remove from the water because aquatic macrophytes do not require this element for any physiological purpose (Mishra and Tripathi, 2008), but for *Ipomoea aquatica*, which is a floating as well as rooted plant, it can accumulate greater Cr from the sediment medium, which is the storehouse of this metal. The bioaccumulation properties of metals may vary depending on the plant species and exposure level. Duman et al (2009) reported that *Nasturtium officinale* (watercress) tends to accumulate appreciable amounts of Cd, Cr, and Co in different concentrations.

According to Kumkrong et al (2021), Li concentration in plants typically ranges between 0.2 to 30 mg/kg which is in tandem with the current study. It has also been reported that lithium concentrations in plant foodstuffs vary widely from 0.01 ppm (dry basis) in bananas to 55 ppm in oats (Shacklette et al., 1978). The estimated concentration of Li in the present study is 0.133 mg/kg to 0.395 mg/kg, which found to be comparatively lower level. In the present study, the estimated daily intake (EDI) of Nickel (Ni) is higher in women (1.344) than that of men (1.137) indicating a higher risk to women. Alinaghi et al (2019) and Thyssen et al (2007) reported that females are more susceptible to Ni allergic responses than males. Depending on the dose and length of exposure, as an immunotoxic and carcinogenic agent, Ni can cause a variety of health effects, such as contact dermatitis, cardiovascular disease, asthma, lung fibrosis, and respiratory tract cancer (Genchi et al., 2020; Guo et al., 2020; Leonard and Jacquet, 1984).

Seasonal variability influences the bioavailability and bioaccumulation of heavy metals in aquatic biota, resulting in varying levels of risk for human health. Similarly to our findings, several studies have reported increased heavy metal concentrations in aquatic biota during the dry season due to reduced water dilution and heightened inputs from agricultural runoff and the seeping of leachate (Jara-Marini et al., 2020; Pedro et al., 2016). The values for HI for both men (3.283) and women (2.752) were >1, indicating potential adverse health effects if the *I. aquatica* from this studied site continues to be consumed over an extended period.

The heavy metal accumulation in the plant tissue was due to polluted water as a result of discharge of untreated municipal solid waste, hospitals and household discharges, etc. The edible plant, namely *Ipomoea aquatica*, grown in Lamphelpat near the municipal solid waste dumping site, exhibited heavy metal contamination. The mean concentration and human health indices, such as EDI, THQ, and HI values, indicate a potential source of non-carcinogenic and carcinogenic risks to human health of local people. Local inhabitants frequently consume edible plants from this study site, especially *I. aquatica*, which exhibits a significant THQ value for chromium. Thus, effective leachate treatment and management for the municipal landfill and effluents from nearby localities is necessary to reduce the human health risks associated with heavy metal exposure.

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Author's contribution

All authors contributed to the study conception and design. Phurailatpam Surjit Sharma, under the guidance of Prof. Ashalata Devi, performed the material preparation and data collection, and conducted the analysis. The manuscript was written by Phurailatpam Surjit Sharma and Prof. Ashalata Devi. All authors read and approved the final manuscript.

Declaration of conflict of interest

The authors declare no conflict of interest.

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