PJVMS
Pertanika Journal of Veterinary Medicine and Science
Research Article

Potential Livestock Toxicological Risks from Contaminated *Eichhornia crassipes* in Urban Drainages around Universiti Putra Malaysia

Mohammed Yusuf Adana[1,2], Chee Kong Yap[1*], Rosimah Nulit[1] and Shamarina Shohaimi[1]

ABSTRACT

The distribution, dietary exposure and toxicological risk of copper, nickel and zinc in leaf and petiole tissues of Eichhornia crassipes consumed by sheep were evaluated. The metal concentrations from the analyses varied between tissues with Zn recording the highest accumulation and significant compartmentalization which shows a greater concentration in petioles (67.69±31.6mg/kg) than leaves with 39.93±10.9mg/kg and a $p < 0.001$. In contrast, Cu indicated $p = 0.41$ and Ni $p=0.22$ and this showed no significant tissue differences. The Estimated Daily Intake (EDI) from the result increased proportionally with exposure, with Zn recording the highest intake values. The Target Hazard Quotient (THQ) analysis identified Ni as the principal contributor to toxicological risk, exceeding the safety threshold (THQ > 1) under higher exposure scenarios, whereas Cu and Zn remained below critical limits. The spatial analysis detected no significant differences among sampling sites with $p > 0.05$. A Principal Component Analysis further explained 73.86% of the total variance with Cu and Zn strongly associated with 56.04% as PC1 and Ni primarily represented 17.82% for PC2. Cu and Zn posed minimal immediate risk, whereas Ni may present a long-term concern under elevated exposure, highlighting ruminant-focused risk evaluation in grazing ecosystems through the value of tissue-specific assessment.

Keywords: E. crassipes; Copper; Nickel; Zinc; Estimated daily intake; Target hazard quotient.

Pertanika Journal of Veterinary Medicine and Science; https://doi.org/10.47836/pjvms.1.5.

INTRODUCTION

Anthropization has resulted into the increase of heavy metal contamination in our aquatic ecosystems which has become a major environmental concern. Heavy metals are non-biodegradable, persist in the environment and accumulate in the environment posing risks to ecosystem health and food safety. Aquatic macrophytes mediate the fate of these contaminants through metal uptake and sequestration, where heavy metals such as cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb) and zinc (Zn) are accumulated by Eichhornia crassipes due to its morphological characteristics (Purty et al., 2020; Thapa et al., 2016).

E. crassipes high metal uptake capacity has made it a good candidate for phytoremediation of contaminated aquatic systems. The effectiveness of E. crassipes as a low-cost and environmentally sustainable remediation option has been reported with substantial metal bioaccumulation particularly in the roots, highlighting the species' resilience under heavy metal stress (Mahamood et al., 2023; Aurnab et al., 2024; Yalcin et al., 2025).

Despite these advantages, the accumulation of heavy metals also raises concerns regarding the use of E. crassipes as livestock feed, with reports from developing countries showing seasonal shortages where water hyacinth is used as a supplementary feed with positive responses reported in sheep and goats due to the plant's availability and nutritional value (Fanta et al., 2024). However, plants harvested from contaminated water bodies may introduce accumulated metals into livestock through dietary exposure.

Research has shown that chronic ingestion of contaminated forage has been associated with metal bioaccumulation in livestock organs, causing cellular imbalance with potentials for food safety concerns through contaminated animal products (Faix et al., 2005; Mustafa et al., 2023; El-Ghareeb et al., 2019).

Although numerous studies have examined the phytoremediation potential of E. crassipes, relatively few have investigated tissue-specific metal partitioning and its implications for livestock exposure. In particular, information on the distribution of heavy metals between leaves and petioles, and the resulting dietary risks to sheep based on established exposure indices such as Estimated Daily Intake (EDI) and Target Hazard Quotient (THQ) remains limited. This knowledge gap is especially important in regions where harvested water hyacinth may inadvertently enter livestock feeding systems.

Therefore, this study investigated the partitioning of Cu, Ni and Zn between the leaves and petioles of Eichhornia crassipes and evaluated the associated dietary risks to sheep. Specifically, metal concentrations were quantified, tissue-specific accumulation patterns were assessed and potential non-carcinogenic risks were evaluated using dietary exposure indices. The findings may provide an integrated perspective on the benefits of E. crassipes for phytoremediation while highlighting its potential role as a pathway for heavy metal transfer into grazing livestock.

MATERIALS AND METHODS

Study Area and Sampling Sites

The study was conducted within Universiti Putra Malaysia (UPM) and the surrounding urban area of Serdang, Selangor, encompassing drainage systems with diverse hydrological land-use and anthropogenic characteristics.

Table 1. *Details of sampling sites*

Site	Date	Sampling site	Latitude	Longitude
1	12/07/2025	Persiaran Mardi- UPM	2°59′52.25″ N	101° 42′ 12.80″ E.

1 Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
2 Department of Environmental Management, Nasarawa State University, PMB 1022 Keffi, Nasarawa State, Nigeria
Email: gs66839@student.upm.edu.my; rosimahn@upm.edu.my; shamarina@upm.edu.my
*Corresponding author: yapchee@upm.edu.my
Received: 16 March 2026 Accepted: 15 June 2026 Published: 30 June 2026

Site	Date	Sampling site	Latitude	Longitude
2	16/06/2025	Near UPM train station	3° 00′ 29.8″ N	101.042′26.1″E
3	09/06/2025	Jalan Universiti 1	2°59′54.01″ N	101°42′17.46″ E
4	18/06/2025	Jalan Pongamia 2	3°00′01.83″ N	101°42′14.76″ E
5	16/06/2025	Persiaran Asam Jawa	3°00′24.25″ N	101°42′25.47″ E
6	11/06/2025	Serdang	3°00′30.2″ N	101°42′29.3″ E
7	09/06/2025	Near Pintu 7, UPM	3°00′30.10″ N	101°42′26.34″ E
8	10/06/2025	Near Pintu 4, UPM	2°59′53.98″ N	101°42′20.38″ E
9	11/06/2025	Near Serdang	3°00′31.0″ N	101°42′33.6″ E
10	11/06/2025	Near Petaling, Selangor	3°00′33.1″ N	101°42′35.5″ E
11	12/07/2025	Jalan Alpha	2°59′51.13″ N	101°42′32.40″ E
12	14/07/2025	Near Jalan Bouganvilla	2°59′53.94″ N	101°42′20.43″ E
13	14/07/2025	Jalan Asam Jawa	2°59′54.49″ N	101°42′15.47″ E
14	16/07/2025	Jabatan Fizik	3°00′02.27″ N	101°42′24.70″ E
15	16/07/2025	DKA/DKB	3°00′00.46″ N	101°42′24.52″ E
16	17/07/2025	Near Iputra	3°00′07.35″ N	101°42′33.95″ E
17	19/07/2025	Faculty of Educational studies	3°00′10.8″ N	101°42′38.9″ E
18	20/07/2025	Jabatan Muzik	2°59′57.1″ N	101°42′11.16″ E
19	24/07/2025	Lorong Sapucaya	3°00′24.80″ N	101°42′24.31″ E
20	25/07/2025	Fakulti Perubatan Veterinar	3°00′23.73″ N	101°42′21.27″ E

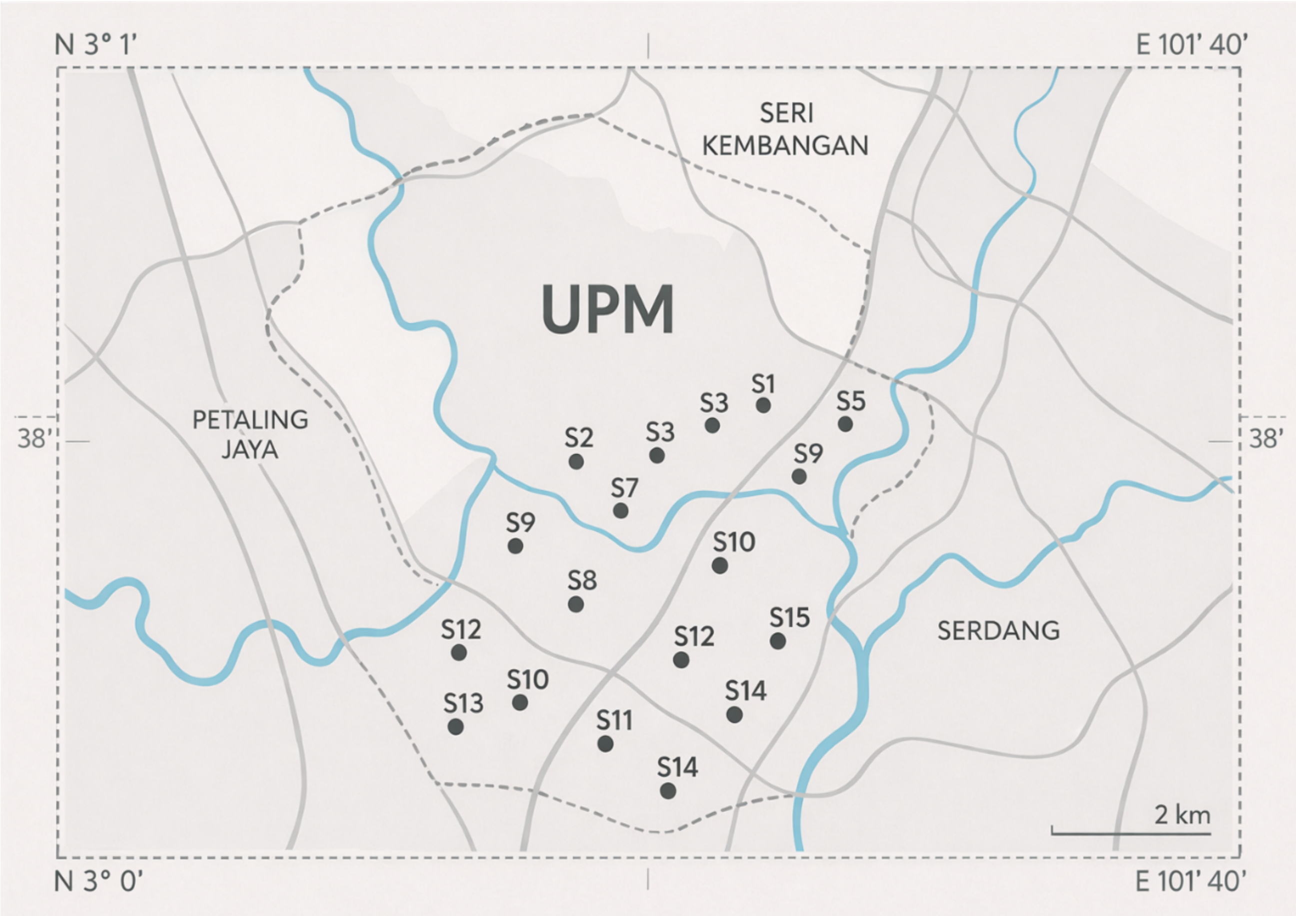

Figure 1. Map of the study area

Sampling Design and Collection

Five mature *Eichhornia crassipes* plants were randomly collected from each of the 20 study sites between June and October 2025 following protocols adapted from the U.S. EPA (n.d.) and Kosmelj (1998). We identified the mature plants by fully expanded leaf rosettes and intact root systems, where the plants were carefully uprooted using our hand and sometimes scoop net, then rinsed on-site to remove debris before being transported to the laboratory for analysis. Furthermore, to evaluate the influence of human activities on metal accumulation from the 20 sampling location, the sites were grouped into four anthropogenic land-use zones such as high-Traffic/Transit covering 4 sites, the off-campus urban with 6 sites, then academic core and agro-veterinary both with 5 sites each. During reconnaissance survey, the drainage channels appear to have been hydrologically connected within the regional catchment, though the sampling sites are widely separated allowing each to capture localized heavy metal inputs before downstream mixing (Kiyemba et al., 2023; Thamaga & Dube, 2019). Thus, each site was treated as an independent sampling unit.

Sample Preparation

The collected plant samples upon reaching the laboratory were thoroughly washed with tap and later distilled water, then cut into leaves and petioles to assess tissue-specific metal compartmentalization (Mohamad, 2010). The samples were oven-dried at 60°C to constant weight (Wong et al., 2021), finely ground using an electric grinder, stored in labelled plastic bags at room temperature until acid digestion and metal analysis.

Sample Treatment and Digestion

Approximately 0.5g of oven-dried *E. crassipes tissues* were digested with 5mL concentrated HNO_3 (69%, AnalaR grade, BDH) following "the University of Wisconsin Soil & Plant Analysis Laboratory (2005)" protocol. The samples were pre-digested at 40°C for 1hour and then digested at 140°C for 3hours (Yap et al., 2022). After cooling, the digests were diluted to 40mL with double deionized water and later filtered through Whatman No. 1 filter paper and stored at 4°C until heavy metal analysis.

Metal determination in Plant samples

The Cu, Ni and Zn concentrations in the samples were determined using an air-acetylene flame atomic absorption spectrophotometer (FAAS; PerkinElmer AAnalyst 800, CT, USA). Calibration was performed using multi-level standards prepared from 10,000 ppm MERCK Titrisol stock solutions. The metal concentrations were expressed on a dry-weight basis.

Quality Control and Quality Assurance

All the glassware used and the non-metallic apparatus were soaked in 5%HNO_3 for 72hours after washing with laboratory-grade detergent (Decon 90). The metal equipment were similarly cleaned to minimize contamination. Digested samples were analyzed in triplicate alongside procedural blanks/calibration standards and certified reference material (SRM 1572) to ensure analytical precision and quality control. At certain intervals the calibration standards were analyzed after every 5–10 samples to verify instrument stability. The FAAS detection limits were 0.010mg/L for Cu and Ni and 0.007mg/L for Zn equivalent to method detection limits of 0.80mg/kg for Cu and Ni and 0.56mg/kg for Zn in dry weight. However, the procedural blanks were negligible relative to sample concentrations, whereas the duplicate and spike recovery analyses were not performed but the calibration curves showed satisfactory linearity throughout the analysis.

The analytical accuracy assessed using SRM 1572 yielded recoveries of 84.6–111.6% suggesting the reliability of the digestion and FAAS procedures.

Table 2. *Comparison of measured Cu, Ni and Zn concentrations in mg/g dry weight with the certified values of SRM 1572 (Citrus Leaves) was used for analytical quality control.*

	Certified Value	Measured Value	Recovery (%)
Cu	16.5±1	13.97 ± 0.09	84.67
Ni	0.6±0.3	0.67 ± 0.06	111.67
Zn	29± 2	26.41 ± 1.09	91.07

Statistical analysis

Descriptive statistics for Estimated Daily Intake (EDI) and Target Hazard Quotient (THQ) of Cu, Ni and Zn in *Eichhornia crassipes* tissues were analyzed using KaleidaGraph (Version 3.08, Synergy Software, Eden Prairie, MN, USA) (Yap et al., 2021), while multivariate analyses were performed using Microsoft Excel.

Estimated Daily Intake (EDI) and Risk exposure

$$\mathrm{EDI} = \frac{C \times IR \times IR_f}{BW}$$

Where:

EDI = "estimated daily intake" of the contaminant (mg/kg/body weight/day).

C = concentration of the contaminant in the feed or plant material (mg/kg).

IR = total daily feed ingestion rate (kg/day).

IR_f = inclusion rate of the contaminated feed in the diet (fraction of total diet).

BW = average body weight of the animal (kg).

The Estimated Daily Intake (EDI) is a widely used metric for estimating dietary contaminant exposure and is calculated as the product of contaminant concentration and ingestion rate that is normalized by body weight (EDI = C × IR / BW) (USEPA, 1989; EFSA, 2015). To better represent animal exposure, an inclusion rate factor (IR_e) was incorporated to account for the proportion of the diet derived from contaminated forage rather than assuming complete dietary substitution. The study adopted a body weight of 20.78 kg reported by Fanta et al. (2024) for an Ethiopian Doyogena sheep fed *E. crassipes* at 50% and 75% dietary inclusion levels because it is a representative of sheep commonly reared in tropical smallholder systems.

Target Hazard Quotient (THQ)

$$\mathrm{THQ} = \frac{\mathrm{EDI}}{\mathrm{TRV}}$$

Where:

THQ = target hazard quotient (unitless).

The Target Hazard Quotient for non-carcinogenic risk was calculated as the ratio of Estimated Daily Intake to the Toxicity Reference Value (TRV) where THQ < 1 indicates negligible non-carcinogenic risk and THQ > 1 suggests potential health concern requiring mitigation (ATSDR, n.d.; U.S. EPA, 2025). From our literature search, sheep-specific TRVs are unavailable; therefore, surrogate values recommended by the USEPA Region 9 Biological Technical Assistance Group (BTAG) were adopted with the following values of 2.67, 0.133 and 9.6 mg/kg body weight/day for Cu, Ni and Zn respectively (USEPA, 2002/2009). Sheep are particularly susceptible to chronic Cu accumulation and toxicosis, therefore, the results were interpreted cautiously (Borobia et al., 2022; Srinivasan et al., 2021).

Target Livestock Species

We focused on sheep as the representative livestock because of their relevance in local grazing systems and direct exposure to contaminated forage. We avoided other domestic and wild animals due to uncertainties arising from species-specific differences in feeding, behaviour, metabolism and toxicokinetics. The assessment represents a receptor-specific risk screening rather than a multi-species ecological risk evaluation.

Daily Feed Intake and Dry Matter Intake

The daily dry matter intake (DMI) was estimated as 3% of body weight from the midpoint of the recommended 2–4% BW/day range for sheep under typical management conditions (University of Arkansas Cooperative Extension Service, 2021; NRC, 2007). For a reference sheep weighing 20.78kg, this corresponds to a DMI of 0.60kg DM/day.

Inclusion Rate Scenarios and Their Biological Justification

Three inclusion rate scenarios were used such as 50%, 75% and 100% to represent a gradient from a realistic supplementary feeding condition to a theoretical worst-case exposure. The 100% was added because an increase in water hyacinth in diet can significantly improve the size and weight of animals (Mani, 2019). Although, 10–20% inclusion level is the ideal representative portions that is most agronomically conservative and commonly recommended threshold (Hadgu et al., 2019).

RESULTS

This section presents the distribution patterns of “Cu, Ni and Zn” in leaf and petiole tissues. The estimated dietary exposure and associated risk indices in sheep was further supported by inferential and multivariate statistical analyses. A detailed breakdown is available in the supplementary materials (Table SM1-SM6).

Table 3. *Cu, Ni and Zn concentrations in petioles and leaves of E. crassipes*

Site	Petiole Cu mg/kg	Leaf Cu mg/kg	Petiole Ni mg/kg	Leaf Ni mg/kg	Petiole Zn mg/kg	Leaf Zn mg/kg
S1	8.27	12.06	3.8	6.55	59.01	45.0
S2	5.69	9.28	5.32	5.47	61.52	37.41
S3	5.38	7.27	4.32	5.14	60.25	30.64
S4	4.88	8.53	4.03	5.3	48.82	32.84
S5	4.35	8.82	4.75	5.69	57.37	37.99
S6	5.13	7.86	4.93	5.38	35.38	33.51
S7	3.89	7.62	5.47	6.03	40.64	41.18
S8	3.37	7.11	6.73	5.73	39.81	31.84
S9	4.4	6.47	4.74	6.47	33.89	26.78
S10	1.39	3.29	4.62	5.58	35.81	35.41
S11	16.13	12.04	6.39	5.62	112.16	50.31
S12	16.74	12.27	6.33	5.81	109.21	49.43
S13	17.14	13.7	6.07	5.92	115.89	56.42
S14	9.54	7.06	5.87	5.06	127.72	71.78
S15	7.63	8.7	5.	5.28	94.20	41.89
S16	8.79	7.64	5.52	5.78	68.44	39.17
S17	5.24	2.98	4.87	4.36	23.43	28.85
S18	9.85	6.49	5.38	5.59	75.23	37.2
S19	11.36	11.73	5.53	5.01	83.0	35.83
S20	11.77	12.36	4.64	4.67	72.1	35.12

Heavy metal concentrations in leaf and petiole tissues

The concentrations of Cu, Ni and Zn varied between leaf and petiole tissues across all sampling sites (Fig. 2). Copper values ranged from 2.98–13.7mg/kg in the leaves and 1.4–17.14mg/kg in petioles showing site-dependent variation and Nickel values were relatively narrow and comparable ranges in leaves with 4.36–6.55mg/kg and petioles 3.80–6.74mg/kg respectively. Zinc recorded the highest concentrations ranging from 26.78–71.78mg/kg in leaves and 23.43–127.72mg/kg. The overall data showed that Cu tissue distribution has higher variation, Ni remained relatively uniform between tissues whereas Zn showed much variability in petioles.

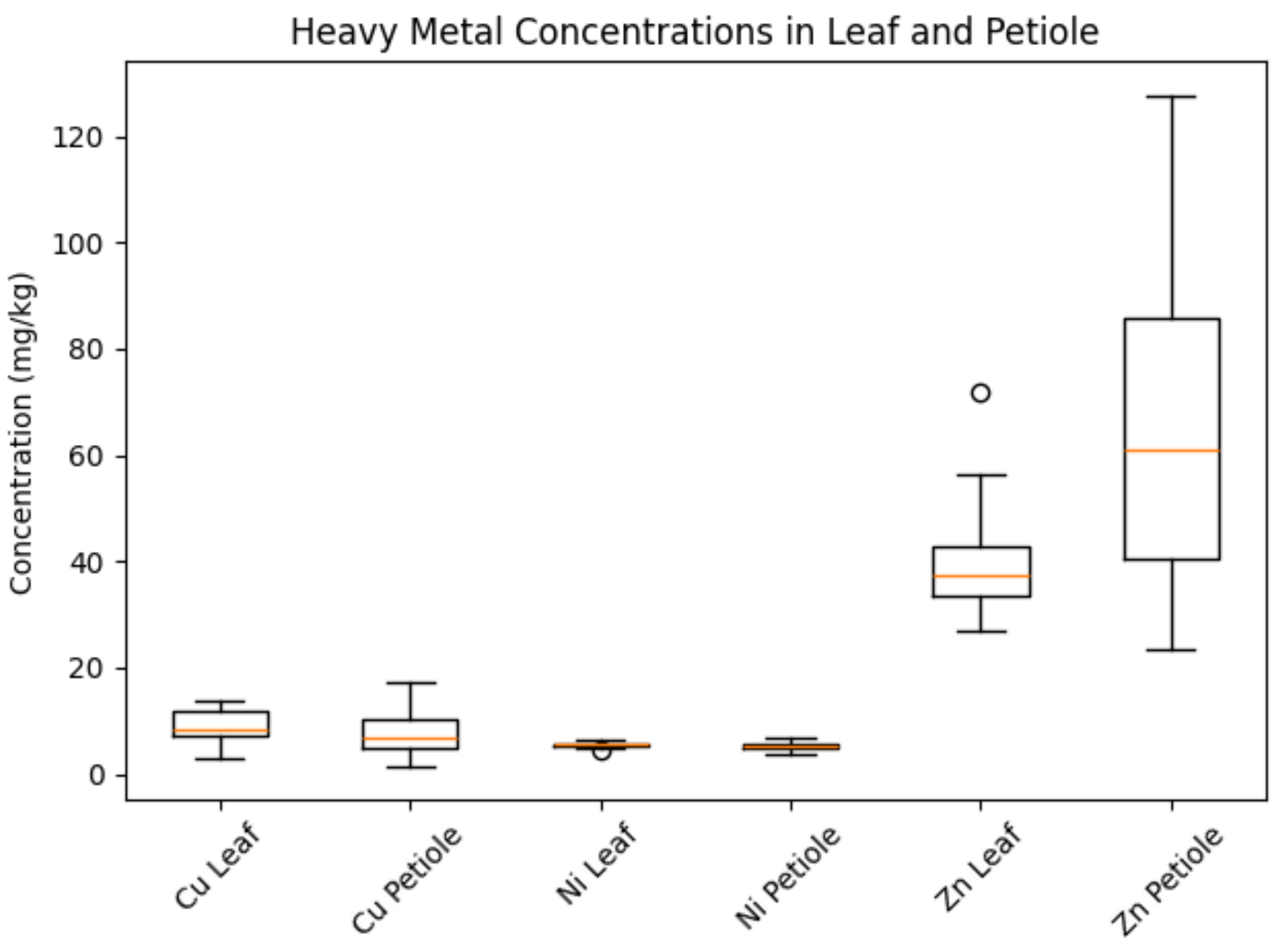

Figure 2. *Cu, Ni and Zn metal concentrations in petiole and leaf.*

Estimated Daily Intake (EDI) across exposure scenarios

The EDI from this analysis showed increased values with exposure level at 50%, 75% and 100% inclusion rate for all the three metals in both plant parts. The values for Cu EDI ranged from 0.02mg/kg/day to 0.47mg/kg/day in petiole, and from 0.05mg/kg/day to 0.4mg/kg/day in leaf tissues across exposure levels, indicating the wide margin in the leaf concentration while nickel EDI ranged from 0.06mg/kg/day to 0.19mg/kg/day in petiole and from 0.06mg/kg/day to 0.19mg/kg/day in leaf tissues. Zinc EDI on the other hand showed the highest values among the analyzed metals ranging from 0.49mg/kg/day to 3.69mg/kg/day in petiole and from 0.39 mg/kg/day to 2.07mg/kg/day in leaf tissues indicating a sharp contrast with the Cu and Ni.

Target Hazard Quotient (THQ) across metals, plant parts, and exposure levels

THQ values followed a similar increasing trend with exposure level across Cu, Ni and Zn within the plant parts. For Cu, THQ values in petiole ranged from 0.01 to 0.19 and leaf THQ ranged from 0.02 to 0.15, Nickel showed higher THQ values relative to Cu and Zn with petiole THQ ranging from 0.41 to 1.46 and leaf THQ ranging from 0.47 to 1.42 while Zinc THQ values were lower compared to Ni but higher than Cu, with petiole values ranging from 0.04 to 0.38 and leaf values ranging from 0.04 to 0.24.

Data Distribution and Normality Assessment

Prior to inferential testing, the data distributions for Cu, Ni and Zn concentrations across both tissues were evaluated using the Shapiro-Wilk test (see table 4). The concentrations for leaf Cu p-value = 0.203, petiole Ni p = 0.942, leaf Ni p = 0.929 and Petiole Zn p-value = 0.208 which conformed to normal distributions, whereas, petiole Cu with p = 0.05 and leaf Zn p-value = 0.011 exhibited significant deviations from normality, necessitating the application of non-parametric equivalents for subsequent comparative analyses.

Table 4. *Shapiro-Wilk normality profiles for heavy metal concentrations in petiole and leaf tissues.*

Tissue Variable	Test Statistic (*W*)	*p*-value	Distribution Status
Petiole Cu	0.904	0.0498	Non-Normal (Skewed)
Leaf Cu	0.936	0.2030	Normal
Petiole Ni	0.981	0.9417	Normal
Leaf Ni	0.980	0.9295	Normal
Petiole Zn	0.937	0.2076	Normal
Leaf Zn	0.867	0.0106	Non-Normal (Skewed)

Heavy Metal Accumulation and Tissue Partitioning

Tissue partitioning between the plant petioles and leaves suggest a distinct elemental-specific behaviours as seen in table 5. From the data, copper and nickel concentrations indicated uniform distribution across both target plant part and the mean copper levels in the petiole was 8.1mg/kg and statistically indistinguishable from leaf concentrations with 8.7mg/kg Wilcoxon (W)=82, p=0.41. Similarly, no significant variations were observed for nickel partitioning between petioles with 5.2mg/kg and leaves 5.52mg/kg, and a paired t-test t=-1.28, p=0.218. In contrast, the mean zinc concentration in the petiole is 67.7mg/kg and was nearly double that quantified in the leaves of 39.9mg/kg, and in conformity the zinc accumulation showed a highly likely significant tissue compartmentalization with Wilcoxon (W) = 6, p < 0.001.

Table 5. *Comparative analysis of Cu, Ni and Zn between petiole and leaf tissues.*

Metal	Petiole Mean (mg/kg)	Leaf Mean (mg/kg)	Statistical Test	Value	*p*-value
Cu	8.05±4.70	8.66±3.03	Wilcoxon Signed-Rank(*W*)	82.00	0.4091
Ni	5.25±0.76	5.52±0.56	Paired *t*-test(*t*)	-1.28	0.2175
Zn	67.69±31.63	39.93±10.97	Wilcoxon Signed-Rank(*W*)	6.00	< 0.001

Spatial Variation Across Sites Clusters

The spatial distribution of the metals across the four predetermined site clusters was evaluated to identify localized pooling of contamination (see table 6). The univariate spatial variance models from the analysis indicated that concentrations of individual metals did not cross the strict threshold of statistical significance among the clusters where Petiole Cu p = 0.19, Petiole Ni p = 0.65, Leaf Ni, p = 0.39, Petiole Zn p = 0.59 and Leaf Zn p = 0.79. However, Leaf Cu content displayed a notable borderline shift across the site groupings with p = 0.05.

Sites like Jalan Bouganvilla and Lorong Sapucaya, which emerged as a distinct copper hotspot, with mean petiole and leaf concentrations of 10.6 mg/kg and 11.4 mg/kg, respectively. Jabatan Fizik acted primarily as a zinc repository, registering the highest tissue concentration of 77.8 mg/kg for Petiole Zn and 43.8 mg/kg of Zn in the Leaf.

Table 6. *Cu, Ni and Zn concentrations*

Cluster Group	PetioleCu (mg/kg)	LeafCu (mg/kg)	PetioleNi (mg/kg)	LeafNi (mg/kg)	PetioleZn (mg/kg)	LeafZn (mg/kg)
Cluster 1	4.58±1.04	7.82±1.01	5.46±0.99	5.59±0.37	50.56±11.75	35.26±4.88
Cluster 2	8.09±6.64	8.70±3.66	5.25±0.76	5.78±0.4	65.08±40.54	40.0±10.15
Cluster 3	8.21±1.87	6.57±2.14	5.47±0.39	5.21±0.54	77.8±38.64	43.78±16.32
Cluster 4	10.6±4.41	11.39±1.63	4.87±1.01	5.47±0.71	74.43±23.36	39.64±5.61

Multivariate Analysis and Elemental Co-Associations

The result of the multivariate analysis showed that PC1 and PC2 accounted for 56% and 17.8%, which identified co-contamination patterns among the measured variables. The first component was dominated by positive loadings for Petiole Zn with 0.518, Petiole Cu=0.496, Leaf Zn value = 0.445, and Leaf Cu with 0.409, indicating a common trace metal contamination pattern. Furthermore, the principal component analysis result was supported by strong positive correlations between Cu concentrations in Petiole and Zn in Petiole with r_s = 0.839, and Petiole Zn and Leaf Zn as r_s = 0.774. In contrast, the second component was primarily associated with Leaf Ni = 0.911, with weak or negative loadings for Cu and Zn, suggesting that Ni distribution is influenced by distinct environmental or biological processes as seen in table 7.

Table 7. *Principal Components Loadings.*

Measured Parameter	PC1 Loading (56.04% Variance)	PC2 Loading (17.82% Variance)
Petiole Zn	0.518	-0.140
Petiole Cu	0.496	-0.028
Leaf Zn	0.445	-0.053
Leaf Cu	0.409	0.322
Petiole Ni	0.342	-0.207
Leaf Ni	0.054	0.911

DISCUSSION

Tissue-metal partitioning and plant physiological controls

The build-up of zinc in petioles compared to leaves shows a clear internal separation. This shows that zinc moves through the vascular system but is kept in structure parts to stop leaf damage and keep the body working properly. This specific movement matches what El Sappah et al. (2024) Found, where plants move metals as a way to deal with stress. On the hand copper and nickel were spread the same in petioles and leaves. Because copper reacts a lot and nickel doesn't help much in the body both need control. Their even spread shows management instead of storing more in one place.

Exposure assessment and toxicological implications

Even though zinc had the Estimated Daily Intake values, it is not as toxic and has higher safe levels, which explains why it has less overall risk. As the exposure situations got worse EDI values went up in a predictable way. When looking at the real risk using Target Hazard Quotients a bigger difference appears.

Nickel always had the long-term health risks with several THQ values going over the important line of one. Copper and zinc stayed below that line in all situations. This matches risk assessment methods, which say that any Target Hazard Quotient over one means a clear risk of long-term health problems (Jangher et al., 2026; Deka et al. 2023).

Metabolic Physiology for Sheep

Considering the digestive system of sheep as ruminants. The rumen changes how much metal is available through reactions where trace metals like copper and zinc can mix with organic material making them hard to absorb in the intestines (Loh et al. 2025). Copper in sheep is especially hard to manage because it works with molybdenum and sulfur which make thiomolybdates in the rumen. This reduces how copper the body takes in causing a shortage even when the diet has enough or when the metal builds up over time leading to liver issues and poison showing how close ruminants are to being too little or too much (López Alonso, 2020).

Compared to animals with one stomach, where absorption happens quickly in the gut sheep have more changeable absorption. So, the EDI-based records might show exposure, than what really happens in the body, but they still help with understanding long-term build-up risks.

Oral bioavailability of plant bound metals

The concentrations found were total concentrations and do not represent the bioavailable fraction. In plants, metals can be bound to other compounds such as fibre and organic ligands which reduces their solubility and their intestinal absorption, and according to Zhu et al., (2023), their bioavailability is also affected by environmental factors.

In ruminants, the retention of trace metals is low, ranging from less than 30% of the ingested amount, and the rest is excreted. This suggests that the actual metal exposure in sheep is lower than it may seem, although chronic exposure may still be possible (Hassan & Albayati, 2025).

Mineral interactions and co-contamination patterns

The multivariate analysis revealed that Cu and Zn were positively correlated with each other, suggesting a common source. At the same time, these two metals were antagonistic with each other because high levels of Zn could interfere with Cu intake and lead to copper deficiency in sheep. Moreover, Cu was antagonistic with Mo and S found in the rumen (López Alonso, 2020). Therefore, it can be suggested that the Cu levels are controlled by the interactions with other metals in the animal's body.

In turn, the analysis revealed that Ni was independent from Cu and Zn, indicating the differences in the contamination mechanism and the lack of interactions with the latter two metals. It could mean that Ni is controlled by other processes than Cu and Zn, making its presence in sheep diet dangerous for health because of the absence of homeostasis mechanisms.

Feed safety standards and implications for sheep

Compared to the established feed safety standard, the obtained concentrations were found to be lower than the maximum tolerable levels (MTL) of 15 mg Cu/kg DM for sheep and 300 mg Zn/kg in the same species, which are much higher than the similar values for cattle, which is explained by their particular sensibility to this element (Clarkson & Kendall, 2023). Thus, the detected levels can be considered as safe for the animals in terms of Cu and Zn intake. However, considering the possibility of chronic poisoning development due to the bioaccumulation of this element in the liver, caution should be exercised when interpreting the results. In turn, the MTL for Ni in sheep remains unclear, but, given the high THQ, it can be regarded as the most dangerous contaminant for the studied species in terms of exposure.

Spatial and multivariate interpretation

The absence of statistically significant spatial variation suggests diffuse contamination rather than strong point-source pollution. Nonetheless, the identification of localized hotspots may indicate possible environmental heterogeneity in contamination inputs, and the PCA results further support the existence of a shared Cu–Zn contamination pathway and a distinct Ni pathway, likely reflecting differences in geochemical origin or anthropogenic sources.

CONCLUSION

Assessing the dietary safety of livestock was the primary goal of the conducted research, which was aimed at estimating the non-carcinogenic risks for animals feeding on *Eichhornia crassipes*. The study highlighted the differences in the distribution of Cu, Ni and Zn between leaves and petioles of the plant species, as well as the associated health hazards. As per the findings reported, the highest concentration of each metal was detected in petioles (Zn: 23.4–127.7 mg/kg; Cu: 3–13.7 mg/kg; Ni: 4.4–6.6 mg/kg) and leaves (Zn: 26.8–71.78 mg/kg; Cu: 1.4–17.1 mg/kg; Ni: 3.8–6.7 mg/kg). The intake of metals for all sites gradually increased, reaching the maximum level of 3.69 mg/kg/day for Zn, 0.47 mg/kg/day for Cu, and 0.19 mg/kg/day for Ni. Nevertheless, the highest non-carcinogenic risk was found for Nickel as the highest threshold hazard index (THQ) was detected, which exceeds the acceptable level, unlike Cu (≤0.19) and Zn ≤0.38.

Therefore, it was concluded that the dietary risk assessment for ruminants feeding on *E. crassipes* requires considering the characteristics of their nutrition, which will ensure sustainable urban grazing systems.

The information discovered may prove the danger of the level of heavy metals in *E. crassipes*, which can be fatal to animals that consume this plant. For example, the presence of a high concentration of Zn in petioles indicates an assessment of the potential threat in a particular part of the plant. In turn, the study highlights Ni as the main metal responsible for causing non-carcinogenic toxicity. Therefore, checking animal feed collected from urbanized drainages is an essential activity to avoid the hazards posed by heavy metals. The study demonstrates the joint assessment of the metal content in *E. crassipes* and its impact on the diet of animals. However, the analysis takes into account the peculiarities of the physiology of ruminants, and this fact can serve as the basis for further research.

Estimating the potential dietary exposure, this study used total concentrations of copper, nickel, and zinc in *Eichhornia crassipes*. Because we did not measure metal speciation or true bioavailability, the resulting EDI and THQ values reflect a worst-case potential exposure rather than the actual amount absorbed by the sheep. Furthermore, the risk assessment was based on standard assumptions for feed intake, body weight, and toxicological reference values, which may not reflect differences in grazing behaviour, breed, physiological status, or management practices. In addition, the results reflect conditions during the sampling period and may not capture seasonal variation or localized spatial differences.

Future studies should use multi-factor approaches to better track heavy metal risks in animal grazing fields.

ACKNOWLEDGEMENT

Not Applicable

ETHIC APPROVAL

Not Applicable

CONFLICT OF INTEREST

The authors declare no competing interests

FUNDING

The support for this research was provided by the Nigerian Tertiary Education Trust Fund, under award TETF/UNI/KEFFI/TSAS/2022. The funder had no role in study design and completion.

AUTHOR CONTRIBUTION

Mohammed Yusuf Adana: Conceptualization, methodology, investigation, formal analysis, data curation, writing of the original draft. Chee Kong Yap: Supervision, validation, review and editing. Rosimah Nulit: Review and editing. Shamarina Shohaimi: Review and editing.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article and its supplementary information files

AI-ASSISTED WRITING DISCLOSURE

The authors declare that generative artificial intelligence tool was used slightly in the preparation of this manuscript using ChatGPT (OpenAI Free Version), which was employed to assist with language refinement, however, all scientific contents are the responsibility of the authors.

ABBREVIATIONS

Term	Definition
BW	Body Weight
Cu	Copper
C	Concentration.
DMI	Dry Matter Intake
EDI	Estimated Daily Intake
FAAS	Flame Atomic Absorption Spectrometry
IR	Ingestion Rate
IR_f	Inclusion Rate of Feed
N	Nickel
THQ	Target Hazard Quotient

REFERENCES

Agency for Toxic Substances and Disease Registry (ATSDR). (n.d.). *Calculating hazard quotients and cancer risk estimates*. https://www.atsdr.cdc.gov/pha-guidance.

Aurnab, I. T., Uddin, M. J., & Kabir, A. (2024). “Risk assessment of four toxic heavy metals in terrestrial and aquatic ecosystems around BSCIC tannery industrial estate of Savar, Dhaka, Bangladesh. *Environmental Science and Pollution Research, 31*, 16426-16443”.

Borobia, M., Villanueva-Saz, S., De Arcaute, M. R., Fernández, A., Verde, M., González, J. M., Navarro, T., Benito, A., Arnal, J. L., De Las Heras, M., & Ortín, A. (2022). “Copper Poisoning, a Deadly Hazard for Sheep”. *Animals: an Open Access Journal from MDPI, 12*. https://doi.org/10.3390/ani12182388

Clarkson, A., & Kendall, N. (2023). *How and why should we determine mineral status in ruminants?* Improve Veterinary Practice.

Deka, A. K., Kumar, K. J., & Basumatary, S. (2023). “Monitoring strategies for heavy metals in foods and beverages: Limitations for human health risks”. In *Heavy Metals – Recent Advances*. IntechOpen.

EFSA Panel on Contaminants in the Food Chain (CONTAM). (2015). “Scientific Opinion on the risks to animal and public health and the environment related to the presence of nickel in feed”. *EFSA Journal*, *13*(4), 4074.

El-Ghareeb, W. R., Darwish, W. S., & Meligy, A. M. A. (2019). “Metal contents in the edible tissues of camel and sheep: Human dietary intake and risk assessment in Saudi Arabia”. *Japanese Journal of Veterinary Research, 67*(2), 61-70.

El-Sappah, A. H., Zhu, Y. Z. Y., Huang, Q., Chen, B. C., Soaud, S. A., Abd Elhamid, M. A., Yan, K., Li, J., & El-Tarabily, K. A. (2024). “Plants' molecular behavior to heavy metals: From criticality to toxicity”. *Frontiers in Plant Science, 15*.

Faix, S., Faixova, Z., Boldizarova, K., & Javorsky, P. (2005). “The effect of long-term high heavy metal intake on lipid peroxidation of gastrointestinal tissue in sheep”. *Veterinarni Medicina, 50*(9), 401-405. https://doi.org/10.17221/5639-VETMED

Fanta, Y., Kechero, Y., & Yemane, N. (2024). “Nutritional response to water hyacinth (*Eichhornia crassipes*) challenges via blood biochemical profiles in goats and sheep”. *Heliyon, 10*(2), e24764.

Hadgu, G. Z., et al. (2019). “Effect of feeding different inclusion rates of water hyacinth [Eichhornia crassipes (Mart.) Solms] on the body weight gain of growing dairy bull calves”. International Journal of Research – GRANTHAALAYAH, 7(6). https://doi.org/10.29121/granthaalayah.v7.i6.2019.2341

Hassan, S. F., & Albayati, H. J. M. (2025). “Micromineral additives (zinc, selenium, copper) and their physiological roles in ruminant nutrition”. *American Journal of Biology and Natural Sciences*.

Jangher, A., Mahara, B. M., & Abboud, H. D. (2026). Quantification of “heavy metals” in marketed fish and evaluation of health risks via EDI and THQ. *Scientific Journal of Basic Sciences*.

Kiyemba, H., Barasa, B., Asaba, J., Makoba Gudoyi, P., & Akello, G. (2023). “Water hyacinth's extent and its implication on water quality in Lake Victoria, Uganda”. *The Scientific World Journal*, *2023*, 1-14. https://doi.org/10.1155/2023/4947272

Kosmelj, K. (1998). “Composite samples in environmental studies”. *Zbornik Biotehniške fakultete Univerze v Ljubljani*, 71, 75–82. [agris.fao.org]

Loh, H. Y., Thomas, T., Miller, L., Thorndyke, M., Alves, J. B., & Engle, T. (2025). “Impact of trace mineral sources on digestion and ruminal fermentation in ruminants”. *Engormix*.

López-Alonso, M. (2020). Copper supplementation: A challenge in cattle. *Animals, 10*(10), 1890.

Mahamood, M., Khan, F. R., Zahir, F., Alhewairini, S. S., Alghanim, K. A., & Al-Misned, F. (2023). *Bagarius bagarius* and “*Eichhornia crassipes* are suitable bioindicators of heavy metal pollution, toxicity, and risk assessment”. *Scientific Reports, 13*, 3869. https://doi.org/10.1038/s41598-023-30987-6

Mani, A. M. (2019). “Utilization leaf meal of water hyacinth (*Eichhornia crassipes*) as a replacement protein source for growing Awassi lambs”.

Mohamad, H. H. (2010). Uptake of cadmium and zinc from synthetic effluent by water hyacinth (*Eichhornia crassipes*). *EnvironmentAsia*, *6*(5), 41-47.

Mustafa, R. M., Hamadamin, R. R., Saleh, M. Q., Yousif, O. H., Ahmed, A. H., & Hassan, R. K. (2023). “Assessment of heavy metals as an emerging environmental pollutant in liver organs of sheep and goats from Erbil, Kurdistan Region of Iraq”. *Zanco Journal of Pure and Applied Sciences, 35*(6), 38-47.

NRC. (2007). Nutrient Requirements of Small Ruminants. National Academies Press.

Purty, R. S., Jha, A. N., & Chatterjee, S. (2020). “Presence of heavy metal in water (Yamuna River), soil and vegetables in Delhi and to examine the effect of phyto-accumulating capacity of *Eichhornia crassipes”*. *Plant Cell Biotechnology and Molecular Biology, 21*(3-4), 22-36.

Srinivasan, P., Madheswaran, R., Kumar, R., Balasubramaniam, G., Balachandran, P., Sasikala, M., Arulmozhi, A., & Nithiya, P. (2021). “Spontaneously Occurring Chronic Copper Toxicosis in Pattanam Breed of Sheep”. Indian Journal of Animal Research. https://doi.org/10.18805/ijar.b-4407

Thamaga, K. H., & Dube, T. (2019). “Understanding seasonal dynamics of invasive water hyacinth (*Eichhornia crassipes*) in the Greater Letaba river system using Sentinel-2 satellite data”. *GIScience & Remote Sensing*, *56*(8), 1355-1377. https://doi.org/10.1080/15481603.2019.1646988

Thapa, G., Das, D., Gunupuru, L. R., & Tang, B. (2016). “Endurance assessment of *Eichhornia crassipes* (Mart.) Solms in heavy metal contaminated site: A case study. *Cogent Environmental Science”, 2*(1), 1219083. https://doi.org/10.1080/23311843.2016.1219083

“U.S. Environmental Protection Agency (EPA). (n.d.). *Selecting a sampling design”*. Retrieved from https://www.epa.gov/quality/selecting-sampling-design

U.S. Environmental Protection Agency (U.S. EPA). (2025). *Regional Screening Levels (RSLs): Generic tables*. https://www.epa.gov/risk/regional-screening-levels-rsls-generic-tables.

U.S. EPA Region 9 Biological Technical Assistance Group (BTAG). (2002/2009). *Recommended toxicity reference values (TRVs) for mammals and birds*. California DTSC. https://dtsc.ca.gov/wp-content/uploads/sites/31/2018/01/Eco_Btag-mammal-bird-TRV-table-1.p

United States Environmental Protection Agency (USEPA) (1989) Risk Assessment Guidance for Superfund Volume I: Human Health Evaluation Manual (Part A). Washington DC.

University of Arkansas Cooperative Extension Service. (2021). Basic nutrition of small ruminants. https://www.uaex.uada.edu/farm-ranch/animals-forages/sheep-goats/nutrition.aspx

University of Wisconsin Soil & Plant Analysis Laboratory. (2005). "*Analysis of major, minor and trace elements in plant tissue samples with ICP-OES and ICP-MS"*. University of Wisconsin–Madison.

Wong, K. W., Yap, C. K., Yaacob, A., Nulit, R., Omar, H., Aris, A. Z., Sharifinia, M., Bakhtiari, A. R., Al-Shami, S. A., Saleem, M., & Okamura, H. (2021). "Bioaccumulation of zinc in edible tropical vegetables in Peninsular Malaysia and its human health risk assessment based on various ethnicities in Malaysia". *Environmental Science and Pollution Research, 28*(29), 39110–39125. https://doi.org/10.1007/s11356-021-13361-3

Yalcin, I. E., Altay, V., & Ozturk, M. (2025). Phytoremediation potential and ecophysiological features of water hyacinth (*Eichhornia crassipes*): A case study from Orontes River, Türkiye. *Journal of Environmental Science and Health, Part A, 60*(1), 1-13.

Yap, C. K., Yaacob, A., Tan, W. S., Al-Mutairi, K. A., Cheng, W. H., Wong, K. W., Edward, F. B., Ismail, M. S., You, C. F., Chew, W., Nulit, R., Ibrahim, M. H., Amin, B., & Sharifinia, M. (2022). Potentially Toxic Metals in the High-Biomass Non-Hyperaccumulating Plant Amaranthus viridis: Human Health Risks and Phytoremediation Potentials. Biology, 11(3). https://doi.org/10.3390/biology11030389

Yap, C.K.; Chew, W.; Al-Mutairi, K.A.; Al-Shami, S.A.; Nulit, R.; Ibrahim, M.H.; Wong, K.W.; Bakhtiari, A.R.; Sharifinia, M.; Cheng, W.H.; et al. (2021). Invasive Weed Asystasia gangetica as a Potential Biomonitor and a Phytoremediator of Potentially Toxic Metals: A Case Study in Peninsular Malaysia. Int. J. Environ. Res. Public Health, 18, 4682. https://doi.org/10.3390/ ijerph18094682.

Zhu, Q., Ji, J., Tang, X., Wang, C., & Sun, H. (2023). Bioavailability assessment of heavy metals and organic pollutants in water and soil using DGT: A review. *Applied Sciences*, *13*(17), 9760.