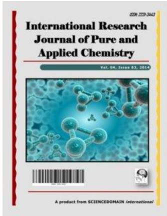

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Assay of Heavy Metals in Water Hyacinth (*Eichhornia crassipes*) Growing in River Benue, Nigeria and Its Safety as Food

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Authors' contributions

This work was carried out in collaboration between the three authors. Author LAN designed the study, wrote the protocol and produced the first draft of the manuscript. Author OAO executed the bench and field work, preformed the statistical analysis and managed the analyses of the study. Author ISE managed the literature searches and did the proof reading at all stages. All three authors read and approved the final manuscript.

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ABSTRACT

Laboratory tests have established that some aquatic plants are heavy metal hyper-accumulators. To estimate the extent of this phenomenon on the field, an assessment of heavy metals (Fe, Mn, Zn, Cu, Cd, Cr, and Pb) content of water hyacinth (*Eichhornia crassipes*) freely growing in river Benue at Makurdi, Central Nigeria, was done using atomic absorption spectrophotometry (AAS). Heavy metal loading of the river water was also analyzed. The result showed the mean concentration of heavy metals in *E. crassipes* samples as: Fe, 103±95.7 mg/kg, Mn, 28.5±19.7 mg/kg, Zn, 34.0±21.0 mg/kg, Cu, 3.94±3.4 mg/kg, Cd 1.00±0.3 mg/kg, Cr 3.78±0.2 mg/kg and Pb 42.9±24.8 mg/kg. The load of heavy metals in water hyacinth leaves samples increased in the order Cd<Cr<Cu<Mn<Zn<Pb<Fe, this is similar to the order observed for the water samples (Cd<Cr<Cu<Pb<Zn< Mn<Fe). The concentrations of Fe, Mn, Zn and Cu were below permissible

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limits while Cd, Cr and Pb exceeded their permissible limits for vegetable samples set by Codex Alimentarius Commission. Considering the possible utilization of water hyacinth in nutrition (as table vegetable and animal feed) and industry, there is need to exercise caution in this regard and continuously monitor the heavy metal content of this plant growing in river Benue.

Keywords: Water hyacinth (Eichhornia crassipes); heavy metals; atomic absorption spectrophotometry; Benue.

1. INTRODUCTION

Agricultural industries and practices, industrial effluents, hydrocarbons from oil refineries and radionuclide are veritable sources of water pollution in many parts of the world [1]. Water is one of the most critical resources. The importance of water resources, particularly surface water (rivers), in meeting the water needs of humans, animals and industries makes it pertinent that they are protected from contamination. When municipal, industrial, and agricultural wastes enter into water, biological and chemical contaminants including heavy metals also find their way into the water. Although some of these metals are essential as micronutrients, their high concentration in the food chain causes toxicity and environmental impacts and endanger aquatic ecosystems and their users [2]. Heavy metals entering the aquatic ecosystem originate from different sources such as decay of plants and vegetation, atmospheric particulates, discharge of domestic and municipal wastes, etc [3].

It has become necessary to focus attention on heavy metals in Nigeria as there are reports of recent issues of heavy metals pollution from different parts of the country [4]. The Zamfara lead poisoning, the worst heavy metals incidence in Nigerian records, claimed the lives of over 500 children within seven months in 2010. Illegal miners from seven villages of Bukkuyum and Gummi local governments in Zamfara state brought rocks containing gold ore into the villages from small-scale mining operations ignorant that the ore also contained extremely high levels of lead. The ore was crushed inside village residences, spreading lead dust throughout the community soil, air and water. By December 2010, the tragedy had affected 3,600 children, with speculations that 180 villages covering around 30,000 people were in danger. Several other cases of heavy metals pollution were reported from different parts of Nigeria in 2010, 2011 and 2015 [5-9].

River Benue, a freshwater flowing through Nigeria, is the second largest river in the country. The river originates from the Adamawa mountains of Cameroun, some bounding the Nigerian frontier and flows eastward through the Nigerian territory before joining the River Niger at Lokoja, Kogi state, Nigeria [10]. The Benue river has features of a matured river with extensive achieved plains stretching for several kilometres. The greater part of this plain is flooded during the rainy season and forms breeding ground for many fish species, most especially when it's bank is full. The area of river Benue is 129,000 ha, and there can be as much as 25 m difference between the high and low water levels. Irrigation farming is carried out on the river bank, whereby agrochemical runoff gets into the river and has potential to impact on the waters. The Wadata sampling point is along the Wadata market refuse dump site. People are usually at this site carrying out their domestic activities such as washing clothes and cooking utensils, bathing, and defecating. There is a nearby abattoir and water from the river is used to wash dung, debris and blood from slaughtered animals. River Benue is therefore open to untreated municipal sewage, agrochemical runoff, abattoir wastewater, and leachates of solid wastes around it [11].

Plants have the ability to accumulate nonessential metals such as Cd and Pb, and this ability could be harnessed to remove pollutant metals from the environment. About 400 plant species have been identified as metal hyper-accumulators [12]. Water hyacinth is one of four aquatic plants reported to phytoremediate Cd, Cr, Co, Ni and Pb [13]. Water hyacinth (*Eichhornia crassipes* (Mart.) Solms) is known as a voracious weed, which causes blocks in waterways and disturbs aquatic life. However, the appetite for nutrients and explosive growth rate of this floating macrophyte has been put to use in cleaning up municipal and agriculture wastewater [14].

Many laboratory studies have documented the phytoremediation potential of *E. crassipes* [1,13,15-17], but field tests in freshwater to confirm these findings are few and far between.

In many parts of the world, there is growing interest in the use of *E. crassipes* in nutrition and food industry [18-20]. The aim of this work is to analyze the concentration of Fe, Mn, Zn, Cu, Cd, Cr and Pb in *E. crassipes* growing in the River Benue and compare these with Codex Alimentarius Commission (CAC) limits for vegetables. This is with regard to its suitability and safety for human consumption and use as animal feed. This study also compares the level of these seven heavy metals in *Eichhornia crassipes* with the concentration of these metals in river water.

2. EXPERIMENTAL DETAILS

2.1 Study Site

The sampling site, depicted in Fig. 1, is located within latitude 9º4'N to longitude 7º29'E in Benue state, Central Nigeria. Five sampling stations covering a distance of 4km were selected; New bridge/Old bridge area, water-works/Trade centre, St. Joseph's Technical College, John Holt area and Wadata. The choice of site was based on proximity to the dumping sites for refuse, metal works, motor mechanic garage, water traffic, human activities such as washing, discharge of human waste, buying and selling of fresh fish, cooked food, and soft drinks and the cultivation of leafy vegetables and fluted pumpkin (*Telferia occidentalis*) by farmers.

2.2 Reagents and Chemicals

All reagents/chemicals used in this work were of analytical grade. The bench work was carried out in the Centre for Agrochemical Technology laboratory of the University of Agriculture, Makurdi, Benue State, Nigeria. Stock solutions were prepared according to standard methods [13]. All glass ware and plastic containers used were thoroughly washed with detergent solution followed by 20% (v/v) HNO_3 and then rinsed with tap water and finally with distilled water. Atomic absorption spectrophotometer Shimadzu AA 6800 was used.

Standard solutions were prepared by taking 10 ml of each of the stock solutions and diluting to 1 litre with de-ionized water (1 ml of solution contains 10 μg). The standards were used to prepare a sub set of standard solutions, and these were used to generate values for the working curves (calibration curve) [13]. The use of blank (a solution containing the solvent and all of the reagents used in analysis, without the sample to be analyzed), and internal standard reference materials were employed in the process of quality assurance to ascertain the reliability of resulting data.

2.3 Sample Collection and Preservation

The water hyacinth plants were collected manually, packed in polythene bags and transported to the laboratory where they were washed using a sequence of tap water and distilled water. They were then cut into smaller pieces using a stainless steel knife rinsed with distilled water. The water hyacinth samples were air-dried, and stored in nylon bags [21]. Water samples were collected from the river at a depth of about 15 cm below the water surface using plastic bottles with screw caps. The bottles were treated with nitric acid and rinsed with distilled water before use. The water samples were acidified with 5 ml of nitric acid to prevent degradation of the metals by micro-organisms.

The *Eichhornia crassipes* samples were labeled WHS1, WHS2, WHS3, WHS4 and WHS5 and water samples labeled WS1 to 5.

(a) WHS represents Water Hyacinth Sample.
(b) 1= New/old bridge area, 2 = Water works/trade center, 3 = St. Joseph Technical college, 4 = John Holt area, 5 = Wadata

2.4 Ash Content

A crucible was washed, oven dried and weighed. Four (4) grams of dried samples was put into the crucible, the weight of the sample plus crucible was measured and recorded. The sample was placed in a furnace set at 550℃ for 4 hours after which the sample was removed from the furnace, cooled and reweighed to 0.001 g [22].

The ash content was calculated by simple difference and recorded in % ±0.1.

2.5 Moisture Content

About 2 g of fresh sample was weighed into a previously weighed crucible, and transferred into an oven set at 105℃. This was allowed to dry to constant weight, which took 24 hours. At the end of the 24 hours, the crucible plus sample was removed from the oven and transferred to desiccators, cooled for ten minutes and weighed.

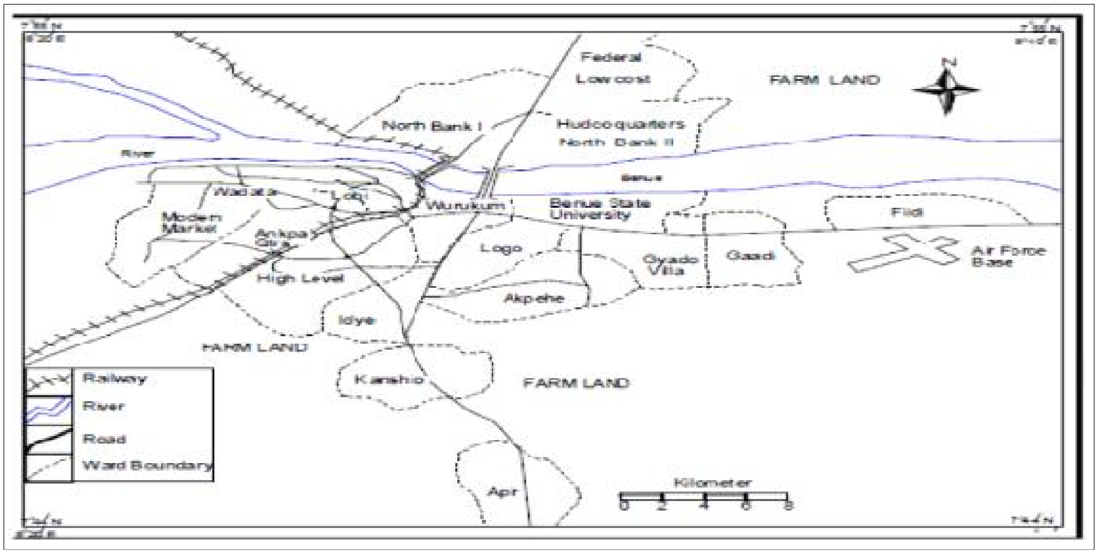

Fig. 1. Map of study site

The moisture content of the sample was thus determined using equation 1 [23].

$$moisture\ content\ = \frac{W_1 - W_3}{W_1 - W_0} \times 100\% \qquad (1)$$

Where weight of empty crucible = W_o; weight of crucible + sample = W_1; weight of crucible + oven dried sample = W_3.

2.6 Sample Digestion

4g of dried *E. crassipes* sample was placed in a crucible and ashed in a furnace at 550°C for about 5 hours. The ashed plant sample was placed in a 250 mL beaker, 5 mL nitric acid added and heated gently at 80°C on a hot plate until brown fumes disappeared, 5 ml of de-ionized water was added and heated until a clear solution was formed. The solution was transferred into a 100 mL volumetric flask by filtration through Whatman filter paper No 2, and the volume was made to the mark with deionised water. This solution was used for elemental analysis [24].

2.7 Statistical Analysis

The minimum, maximum, range, mean, standard deviation, standard error, variance as well as the student T-test values of the concentrations of heavy metals in water hyacinth were determined. Analysis of variance of heavy metal concentrations between *E. crassipes* and water samples were conducted. SPSS-16 and Microsoft Excel (Windows 2007) was employed in statistical analysis.

3. RESULTS AND DISCUSSION

3.1 Ash Content

The percentage ash content of water hyacinth leaves, reported in Table 1, ranged from 7.9% to 12.9% with a mean value of 9.34±2.1%. This is in conformity with the value of 16.79% obtained by Okoye et al. [19].

3.2 Moisture Content

The percentage (%) moisture content of water hyacinth leaves reported in Table 1 ranged from 86.5% to 92.5% with a mean value of 90.3±2.3%, in agreement with the value of 85.15% reported in Okoye et al. [19].

Table 1. Ash and moisture content of *E. crassipes* leaves

Samples	Moisture (%)	Ash (%)
WHS1	90.5	9.0
WHS2	91.3	7.9
WHS3	92.5	7.9
WHS4	86.5	12.9
WHS5	90.6	9.0

3.3 Heavy Metal Content of Water Hyacinth Leaves

Iron: The level of Fe concentration in water hyacinth samples from the river at Bridge/Old Bridge (273 mg/kg), St Joseph's Technical College (80.4 mg/kg), Water Works / Trade Centre (56.3 mg/kg), John Holt New (56.3 mg/kg), and Wadata (48.1 mg/kg) were high. The Fe content of water hyacinth plants in the study is due to the absorption from water and the atmosphere. The mean concentration of Fe in water hyacinth leaves, 103±95.7 mg/kg was greater than the maximum value 0.57 mg/kg obtained at Mwazan Region in Tanzania reported in Kisamo [24] and 8.10 mg/kg at Olege Lagoon, Lagos reported in Ndimele and Jimoh [21], but less than the permissible limit (426 mg/L) set by CAC [25].

The minimum and maximum concentrations of the heavy metals investigated in this study, their ranges and standard deviations are displayed in Table 3.

Manganese: The high concentration of Mn in water hyacinth samples from the river at John Holt (48.5 mg/kg), Water Works / Trade Centre (47.1 mg/kg), Wadata (29.3 mg/kg), St Joseph Technical (12.1 mg/kg), and New Bridge/Old Bridge (5.3 mg/kg) may be due to its entry into the environment through discarded steel items. Manganese is highest at the John Holt and Water Works / Trade Centre points because more industrial activities take place in those areas. Electronic appliances such as radios, computers and dry cells contribute large quantities of manganese to the environment. Potassium permanganate is used as a disinfectant and a deodorizer in water purification and in pharmaceutical products. Manganese sulfate is an important ingredient in some kinds of fertilizers. The mean concentration of Mn in water hyacinth leaves, 28.5±19.7 mg/kg was greater than the maximum value mg/kg at Mwazan Region in Tanzania [24], 0.1811 mg/L reported for fish in river Benue [26] and 8.10 mg/kg at Olege Lagoon, Lagos [21], but less than the limit (500 mg/kg) set by CAC [25].

Zinc: There is an observed high level of Zn in water hyacinth samples at John Holt (58.8 mg/kg), New Bridge/Old Bridge (37.8 mg/kg), Water Works / Trade Centre (37.8 mg/kg), St Joseph's Technical College (36.3 mg/kg), and Wadata (0.427 mg/kg). This may be probably due to its absorption from water and the environment. Coal burning releases zinc into the atmosphere. The mean concentration of Zn in water hyacinth leaves (34.0±21.0 mg/kg) was less than the maximum value of 105 mg/kg recorded at Mwazan Region in Tanzania [24], and 709 mg/kg at Pariyej Community Reserve, Gujarat, India [27]. It is however, greater than 8.10 mg/kg at recorded for Olege Lagoon, Lagos [21], 0.079 mg/L for fish in river Benue [26] and less than the limit (99.4 mg/kg) set by CAC [25].

Copper: The concentration of Cu in *E. crassipes* samples at New Bridge/Old Bridge (6.03 mg/kg), St Joseph Technical (4.75 mg/kg), John Holt (4.33 mg/kg), Water Works / Trade Centre (3.99 mg/kg), and Wadata (0.598 mg/kg) in the study occurs from excessive use of fertilizers and fungicides. Inorganic copper pesticides and fungicides are still in use in Nigeria. Copper ranges between 1 - 300 mg/Kg in phosphate fertilizers, 2 – 172 mg/Kg in farmyard manure and 13-3580 mg/Kg in compost manure. The mean concentration of Cu (3.94±2.02 mg/kg) was less than the maximum value 27.6 mg/kg at Mwazan Region in Tanzania [24], and less than the limit (73.3 mg/kg) set by CAC [25]. Copper concentration is highest at the New bridge/Old bridge sample point since that is the urban area of the study. Copper may also be introduced into water via the leaching of copper pipes by acidic or alkaline water which may in turn introduce copper into the river sediments due to the waste water disposal practices.

Cadmium: The Cd concentration content of water hyacinth samples recorded at New Bridge/Old Bridge (1.27 mg/kg), Water Works / Trade Center (1.24 mg/kg), John Holt (1.17 mg/kg), St Joseph's Technical College (0.654 mg/kg), and Wadata (0.671 mg/kg) in the study is due to the fact that Cadmium occurs naturally in low levels in the environment and is also used in batteries, pigments and metal coatings. Volcanic activity, industrial processes such as smelting or electroplating and the addition of fertilizers are veritable ways that increase concentration of Cd in the environment. The mean concentration of Cd in water hyacinth leaves (1.00±0.312 mg/kg) was less than the maximum value 2.24 mg/kg at Mwazan Region in Tanzania [24], but greater than the limit (0.1 mg/kg) set by CAC [25].

Cadmium absorption occurs in the intestines and lungs of animals. It is a cumulative toxicant and carcinogen that affects kidneys, generates various toxic effects in the body, disturbs bone metabolism and deforms the reproductive tract as well as the endocrine system [28]. Occupational human exposure has been correlated with lung cancer. Cadmium exposure, during human pregnancy, leads to reduced birth weights and premature birth. Ingesting very high levels severely irritates the stomach, leading to vomiting and diarrhoea. Long-term exposure to lower levels leads to a build up in the kidneys and possible kidney disease, lung damage, and fragile bones. There are several morpho-pathological changes in the kidneys due to long-term exposure to cadmium [29].

Chromium: The Cr concentration of *E. crassipes* samples at St Joseph's Technical College site (9.36 mg/kg), Wadata (2.69 mg/kg), Water Works/Trade Centre (2.12 mg/kg), New Bridge/Old Bridge (1.94 mg/kg) and John Holt (0.741 mg/kg) in the study is due to the absorption from water and the atmosphere. The mean concentration of Cr in water hyacinth leaves (3.78±3.42 mg/kg) was less than the maximum value of 105 mg/kg at Mwazan Region in Tanzania reported [24], but greater than the limit (0.1 mg/kg) set by CAC [25]. Toxic effects of Cr includes vomiting and persisting diarrhoea, hemorrhagic diathesis, epitasis, convulsions, perforations of the nasal septum, skin ulceration "chrome holes," loss of the sense of smell, acute irritative dermatitis or allergic eczematous dermatitis, cancer of the respiratory organs and bronchial asthma [30].

Lead: The Pb concentration of *E. crassipes* samples at John Holt (72.4 mg/kg), Wadata (65.1 mg/kg), St Joseph's Technical College site (32.4 mg/kg), New Bridge/Old Bridge (31.0 mg/kg), and Water Works / Trade Centre (13.9 mg/kg) in the study may be due to its use in batteries, solder, paints, ammunition, devices to shield against x-rays and most consumer electronic items. Lead-based paint and leaded fuel are still in use in Nigeria. The mean concentration of Pb in water hyacinth leaves (43.0±24.8 mg/kg) was greater than the maximum value 9.81 mg/kg at Mwazan Region in Tanzania reported in Kisamo [24], 0.30 mg/kg at Olege Lagoon, Lagos reported by Ndimele and Jimoh [21] and greater than the limit of 2 mg/kg set by CAC [25].

Lead can severely affect the central nervous system. Overt signs of acute intoxication include dullness, restlessness, irritability, poor attention span, headaches, muscle tremor, hallucinations, and loss of memory. Signs of chronic lead toxicity include tiredness, sleeplessness, irritability, headaches, joint pain, and gastrointestinal symptoms [31].

The mean concentrations of heavy metals in *E. crassipes* for all the sample stations are displayed in Fig. 2. The observed trend of heavy metal concentration is in the order Cd<Cr<Cu<Mn<Zn<Pb<Fe. The heavy metal load in water samples is shown on Table 4, revealing that these concentrations increase in the order Cd<Cr<Cu<Pb<Zn<Mn<Fe.

Table 2. Heavy metals concentration (mg/kg) in *E. crassipes* leaves at each sample site

Sample	Heavy metals						
	Fe	Mn	Zn	Cu	Cd	Cr	Pb
WHS1	273	5.32	37.8	6.03	1.28	1.94	31.1
WHS2	56.3	47.1	36.8	3.99	1.24	2.12	13.9
WHS3	80.4	12.1	36.3	4.74	0.654	9.36	32.4
WHS4	56.3	48.5	58.8	4.33	1.17	0.741	72.4
WHS5	48.1	29.3	0.427	0.598	0.671	2.69	65.08

WHS represents water hyacinth sample. 1= New/Old bridge, 2= Water works/Trade Centre, 3= St. Joseph's Technical College, 4= John Holt area, 5= Wadata

Table 3. Statistics of heavy metal concentrations (mg/kg) in water hyacinth

Heavy metal	N	Range	Min	Max	Std. error	Std. deviation	Variance
Fe	5	225	48.1	273	42.8	95.7	9163
Mn	5	43.2	5.32	48.5	8.81	19.7	388.3
Zn	5	58.4	0.427	58.8	9.40	21.0	442
Cu	5	5.43	0.598	6.03	0.903	2.02	4.08
Cd	5	0.620	0.654	1.27	0.139	0.312	0.097
Cr	5	8.62	0.741	9.36	1.53	3.42	11.7
Pb	5	58.5	13.9	72.4	11.1	24.8	614

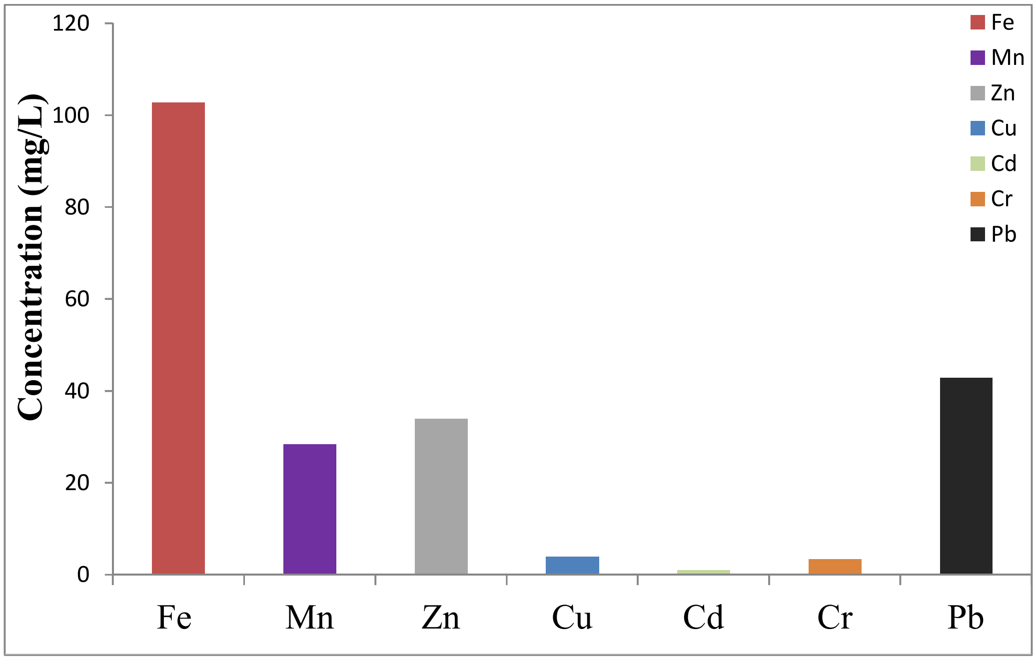

Fig. 2. Mean concentration of heavy metals in *E. crassipes* samples

Table 4. Heavy metals concentration in water

Sample	Heavy metals (mg/L)						
	Fe	Mn	Zn	Cu	Cd	Cr	Pb
WS1	0.011	0.038	0.007	0.001	0.001	0.005	0.125
WS2	0.096	0.014	0.002	0.001	0.003	0.006	0.006
WS3	0.151	0.065	0.004	0.001	0.004	0.021	0.154
WS4	0.808	0.047	0.011	0.002	0.002	0.000	0.148
WS5	0.773	0.033	0.003	0.0004	0.001	0.000	0.040
WS6(control)	0.302	0.014	0.002	0.002	0.002	0.000	0.190

The trend of heavy metals concentration in *E. crassipes* is similar to that observed in the water samples from the sample points. Analysis of variance for heavy metal concentrations between water hyacinth and water (not displayed) shows that Fe, Mn, Zn, Cu, Cd, and Pb were not significant at $p>0.05$, while the concentration of Cr was significant at $p<0.05$.

3.4 T-test Analyses of Heavy Metals Concentration

T-test analyses were carried out and the T-values were determined along with the significance (Table 5). The significance was set at $P = 0.05$. Significant T values show that the concentrations of heavy metals in the samples are significantly different. The result shows that the T-test values (T=2.40, N=5, P=0.074) for Fe and (T=0.20, N=5, P=0.092) for Cr were not significant indicating that the concentration of Fe and Cr in the samples were not significantly different. The T-test values for the other heavy metals (Mn, Zn, Cu, Cd and Pb) were significant at $P = 0.05$.

Table 5. T-test values of heavy metals in *E. crassipes* leaves samples

Heavy metals	T-test values	Significance (*P*-values)
Fe	2.40	0.074
Mn	3.23*	0.032
Zn	3.62*	0.022
Cu	4.36*	0.012
Cd	7.18*	0.002
Cr	2.20	0.092
Pb	3.88*	0.018

(a) Significance set at P = 0.05 (b) Values with asterisks (*) are significant

4. CONCLUSION

The study shows that the investigated heavy metals, Fe, Mn, Zn, Cu, Cd, Cr and Pb were present in all *E. crassipes* leaves samples. The load of heavy metals in samples increased in the order Cd<Cr<Cu<Mn<Zn<Pb<Fe, similar to the observed order in water samples (Cd<Cr<Cu<Pb<Zn<Mn<Fe). The level of Fe, Mn, Zn, and Cu in water hyacinth leaves makes them a good source of animal and plant nutrients which are essential for proper growth and development since they were less than the permissible limit in vegetables set by CAC. However, Cd, Cr and Pb concentrations were higher than the CAC limits and thus constitute a source of concern to the health of man and animals. It was necessary to compare the concentration of these metals with the CAC levels because water hyacinth leaves are used as a carotene-rich table vegetable in Taiwan, and the Javanese sometimes cook and eat the green parts and inflorescence [32]. These leaves are considered for food in countries with high rates of under nutrition and malnutrition [20]. This study confirmed that water hyacinth growing on the south bank of river Benue is a veritable source of these heavy metals and might not be safe for consumption.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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