



Concentrations of Trace Metals in Three Leafy Vegetables (*Telfairia occidentalis*, *Amaranthus viridis* and *Pterocarpus erinaceus*) during the Dry Season in Enugu, Nigeria

A. O. Onah¹, G. I. Ameh¹, C. D. Nwani^{2*}, E. N. Anumudu¹ and J. U. Anukwu¹

¹*Department of Applied Biology and Biotechnology, Enugu State University of Science and Technology, Nigeria.*

²*Department of Zoology and Environmental Biology, University of Nigeria, Nsukka, Nigeria Nigeria.*

Authors' contributions

This work was carried out in collaboration among all authors. Author AOO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors GIA and CDN managed the analyses of the study. Authors ENA and JUA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To investigate the trace metals, manganese (Mn), Nickel (Ni), chromium (Cr), cobalt (Co), zinc (Zn) and iron (Fe) in vegetable samples (*Telfairia occidentalis*, *Amaranthus viridis* and *Pterocarpus erinaceus*) from three senatorial zones in Enugu state, Nigeria during dry season period.

Place of Study: The leaf samples were collected from three senatorial zones (Enugu north, Enugu west and Enugu east) of Enugu State Nigeria.

Methodology: The samples were collected fresh in January, February and March. Prior to analysis, each of the collected samples were dried at room temperature, pulverized using a ball mill and stored at room temperature. They were digested using the wet digestion method and individual metals analyzed using atomic absorption spectrophotometer.

*Corresponding author: E-mail: chris.nwani@unn.edu.ng;

Results: The results of the findings revealed that the concentrations of trace metals in the plant tissues were in the following decreasing order Fe > Zn > Mn > Cr > Co > Cd. *Amaranthus viridis* had the highest accumulation of trace metals while *P. erinaceus* had the least. Enugu East senatorial zone had the highest accumulation of Mn and Co in all the vegetable samples while Enugu West senatorial zone showed the highest accumulation of Ni, Cr, Zn and Fe in all the plant tissues. Enugu north senatorial zone had the least level of trace metals accumulation in the three plant tissues. Manganese contamination could be a result of mining, battery and automobile fume pollution which dissolve into the soil and are absorbed by plants more effectively during dry seasons.

Conclusion: To mitigate the unwanted increase of these metals in vegetables during the dry season, it is necessary to strengthen environmental waste disposal laws.

Keywords: Trace metals; plant tissue; soil; season; Nigeria.

1. INTRODUCTION

The absence of some micronutrients (especially beta carotene, zinc, and iron) contained in vegetables in a diet results in 'hidden hunger' [1]. However, leafy vegetables such as amaranth and cabbage are said to be good absorbers of trace metals from the soil [2]. Vegetables take up metals from contaminated environment through their leaves and roots and incorporate them into the edible part of plant tissues or as a deposit on the surface of the vegetables [3]. Food contamination with trace metals is important, particularly in agricultural production systems and human health [4].

Trace metals are classified into essential, non-essential and toxic metals. The essential trace metals are required by living organisms in small but substantive amounts, for their growth and other metabolic activities taking place within the living cells. Vegetables take up these metals and accumulate them in their edible and non-edible parts at quantities high enough to cause clinical problems to both animals and human beings. Vegetable species differ widely in their ability to take up and accumulate trace metals, even among cultivars and varieties within the same species [5].

There is high prevalence of trace metals contamination of vegetables in most developing countries such as Nigeria [6]. In some farms in Enugu states the agricultural soil is contaminated by trace metals from fertilizers, pesticides, irrigation with contaminated water among others but most farmers are either ignorant of this fact or cannot afford to leave their farmland fallow for remediation because of the demand and pressure to produce foodstuffs is so high. The vegetables *T. occidentalis*, *A. viridis* and *P. erinaceus* are very important in Nigeria as they

are utilized on a large scale in preparation of soup and related menu. The present study is thus designed to assess the concentration of trace metals in the tissues of three leafy vegetables (*T. occidentalis*, *A. viridis*, and *P. erinaceus*) from three senatorial zones in Enugu state.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Materials

The plant samples (*T. occidentalis*, *A. viridis* and *P. erinaceus*) collected from the market in each of the three senatorial districts (Enugu north, Enugu west, and Enugu east) of Enugu State were identified and authenticated with the help of experts from the Department of Plant Science and Biotechnology, University of Nigeria Nsukka. Voucher specimens were kept at the Herbarium Unit of the Department of Applied Biology and Biotechnology, Enugu State University of Technology (ESUT), Enugu, Nigeria for reference purposes. The plant samples were collected during the dry season, specifically in the months of January, February and March.

2.2 Sample Preparation

Leaves samples were detached from the plant stem and dried at room temperature and pressure for ten days to remove all water molecules from the leaves. The dried leaves samples were milled to 250µm particle size using a domestic blender. This was meant to increase the sample surface area. The milled samples in powdery form were parked in amber specimen bottles in triplicates prior to analysis. All the reagents used for the analysis were of analytical grade.

2.3 Sample Digestion

One g of each prepared sample was weighed into a 100 ml beaker. 30 ml of aqua-regia (a mixture of nitric acid and hydrochloric acid in the ratio 1:3) was measured in a 100 ml measuring cylinder and added into the weighed samples. 10 drops of hydrogen peroxide were added to each of the preparation to increase the complexing power of the mineral acids. The samples in beakers were placed on a digital laboratory heating mantle under fume cupboard and heated at 100 °C until the dried leaf samples completely digest. Each digest was allowed to cool and diluted with 50 ml of distilled deionized water [7]. They were filtered into 100 ml volumetric flask using Whatman filter paper (125 mm). The digests were made up to the 100 ml mark using distilled-deionized. The wet digested solution was transferred to plastic bottles and labeled accurately. The digests were used for metal determination. The wet digesting of the leaf sample was carried out in the PRODA laboratory, Enugu, Nigeria [7].

2.3.1 Determination of heavy metals

Concentrations of heavy metals in plant samples were determined by atomic absorption spectrophotometer. The standard solutions of examined heavy metals were prepared by dilution of standard stock solutions (Merck AAS Solution) with deionized water.

$$\text{Metal concentration (mg/kg)} = \frac{\text{Reading (mg/L)} \times \text{Final volume (ml)}}{\text{Initial sample weight (g)}}$$

2.4 Statistical Analysis

The data obtained were analyzed using SPSS Version 20.0 (IBM Corp., Amonk, USA). Comparisons of the metals were done using Kruskal–Wallis *H*-test. The values were represented as mean ± standard error (SE). The level of significance was reported at $p < 0.05$.

3. RESULTS

3.1 Trace Metal Concentrations in Vegetable Samples

The concentrations of trace metals in the vegetable samples from the three senatorial zones during the month of January, February, and March, are presented in Tables 1, 2, and 3 below.

3.2 Metal Concentration in Vegetables for the Month of January

From Table (1) it can be seen that the mean concentrations of all the heavy metals analyzed in the three vegetable samples from all the senatorial zones were below the FAO maximum allowed concentration. *A. viridis* has the highest concentration of Mn in all three senatorial districts. Mn concentration in *T. occidentalis* was above the concentration of Mn in *P. erinaceus*. Mn level in *P. erinaceus* samples from Enugu north and west senatorial zones were below detection. Nickel was below the FAO maximum allowed concentration in *A. viridis* vegetable samples from all three senatorial zones, while *T. occidentalis* and *P. erinaceus* samples have the same Ni concentrations and which were below the FAO maximum concentration respectively for the three senatorial zones. The concentration of Chromium was highest in *A. viridis* samples and least in *P. erinaceus* samples from the three senatorial districts. *T. occidentalis* and *A. viridis* had similar Cr levels for samples from Enugu north senatorial zone (0.08mg/kg). There was an equal concentration of Co in *T. occidentalis* and *A. viridis* samples from Enugu west senatorial zones of which *P. erinaceus* had the highest concentration of Cr (0.02mg/kg). *T. occidentalis* samples from Enugu north and Enugu east senatorial zones had the highest concentration of Cr, while *P. erinaceus* had the least Cr concentrations in these zones. Zinc concentration was highest in *A. viridis* from all the three senatorial zones and least in *P. erinaceus* samples from all three zones also. *A. viridis* samples from Enugu west and east senatorial zones had the highest concentration of iron while *T. occidentalis* samples from Enugu north senatorial zone had the highest iron concentration in this zone.

ANOVA at 95% across the columns revealed that there was no significant difference in the mean level of Mn in *T. occidentalis* samples from Enugu north and west but varied significantly with samples from Enugu east senatorial zone. The difference in the mean levels of Mn in *A. viridis* samples from all three senatorial zones varied significantly. There was no significant difference in the concentrations of Cr in *T. occidentalis* and *P. erinaceus* samples from the three senatorial zones. The difference in the mean levels of Cr in *A. viridis* samples from Enugu west and east senatorial zones were not significant but there was a significant difference between the mean levels of Cr in *A. viridis*

samples from Enugu north senatorial zones and samples from Enugu west and east. The differences in the mean concentrations of Co in all the vegetable samples (*T. occidentalis*, *A. viridis* and *P. erinaceus*) from the three senatorial zones are insignificant. The levels of Zinc in *T. occidentalis* and *A. viridis* from all three senatorial zones vary significantly from one another. *P. erinaceus* showed no significant difference in the level of zinc for samples from Enugu west and east senatorial zone but varied significantly with samples from Enugu north senatorial zone. There was no significant difference in the mean concentrations of Fe in *T. occidentalis* samples from all three senatorial zones. *A. viridis* and *P. erinaceus* showed significant difference in their Fe concentration for samples from the three senatorial zones.

3.3 Metal Concentration in Vegetables for the Month of February

The concentration of metals in vegetable samples from the three senatorial zones in Enugu State for the month of February is presented in Table 2. Manganese was below detection for *P. erinaceus* samples from the three senatorial zones. *A. viridis* samples from all the three senatorial zones had the highest concentration of manganese which is slightly above the concentrations of manganese in *T. occidentalis* samples. Nickel was below detection in all the vegetable samples from the three senatorial zones. *A. viridis* samples had the highest chromium level while *P. erinaceus* samples had the least for all the senatorial zones. Cobalt levels in all vegetable samples were within the range of 0.01 and 0.03 mg/kg. *P. erinaceus* sample from Enugu north senatorial zone had the highest Co level, *T. occidentalis* sample had the highest Co level for Enugu west zone while *A. viridis* sample had the highest Co level for Enugu east senatorial zone. The mean detection levels of zinc were highest in *A. viridis* samples for all the three senatorial zones while *P. erinaceus* samples had the least. *T. occidentalis* samples had mean Zn levels slightly below *A. viridis* samples but above *P. erinaceus* samples. Similarly, Fe levels were highest in *A. viridis* samples from all the senatorial zones and least in *P. erinaceus* samples.

There was significant difference across the column in the mean levels of Mn in *A. viridis* samples from all three senatorial zones. The levels of Mn in *T. occidentalis* samples from Enugu north and west were not significant, but

varied with samples from Enugu east senatorial zone. There was no significant difference in the level of Cr in *T. occidentalis* samples from the three senatorial zones. *A. viridis* and *P. erinaceus* samples from Enugu west and east senatorial zones showed no significant difference in their concentrations of Cr but varied significantly with samples from Enugu north senatorial zone. No significant difference exists within the mean concentrations of Cobalt in all the vegetable samples from the three senatorial zone except for *A. viridis* samples from Enugu east senatorial zone. The levels of Zn in *T. occidentalis* and *P. erinaceus* samples from the three senatorial zones varied significantly while difference in the mean concentration of Zn in *A. viridis* samples from Enugu north and Enugu west senatorial zones were not significant. The concentration of Fe in *T. occidentalis* sample from Enugu north varied significantly with samples from Enugu west and east senatorial zones. The difference in the concentrations of Fe in *A. viridis* samples from all the senatorial zones varied significantly from one another. The concentrations of Fe in *P. erinaceus* samples from Enugu north and west zones varied significantly with samples from Enugu east zone.

3.4 Metal Concentration in Vegetables for the Month of March

Table 3 revealed that all the trace metals analyzed in the vegetable samples from the three senatorial zones in March were below the FAO maximum allowed limit. *A. viridis* samples showed the highest level of Mn for all the three senatorial zones, of which *T. occidentalis* samples had the next highest concentration of Mn. *P. erinaceus* samples showed the least concentrations of Mn. ANOVA at 95% across the column showed that there was a significant difference in the mean level of Mn detected in *A. viridis* samples from all three senatorial zones. *T. occidentalis* samples had no significant difference in the mean levels of Mn for samples from Enugu north and west senatorial zones but varies significantly with samples from Enugu east. There was no significant difference in the mean levels of Mn detected in *P. erinaceus* samples from all the three senatorial zones.

Nickel detection was below FAO maximum allowed concentration for all the vegetable samples from the three senatorial zones. Vegetable samples from Enugu west senatorial zone had the highest concentration of N

Table 1. Trace metal concentrations in the vegetable samples from the three senatorial zones of Enugu State for the month of January

Location	Samples	Trace metals (mg/kg)					
		Mn	Ni	Cr	Co	Zn	Fe
Enugu north	<i>T. occidentalis</i>	0.08±0.005 ^a	0.05±0.005 ^a	0.08±0.004 ^{abc}	0.02±0.005 ^a	0.83±0.014 ^{ac}	3.86±0.307 ^a
	<i>A. viridis</i>	0.18±0.010 ^{bc}	0.07±0.003 ^b	0.08±0.003 ^{abc}	0.01±0.000 ^b	1.26±0.010 ^{ab}	1.26±0.131 ^{bc}
	<i>P. erinaceus</i>	0.00±0.000	0.02±0.001 ^c	0.03±0.005 ^{bc}	0.01±0.000 ^c	0.36±0.006 ^{bc}	0.36±0.145 ^{bc}
Enugu west	<i>T. occidentalis</i>	0.09±0.010 ^a	0.06±0.005 ^a	0.09±0.004 ^{abc}	0.01±0.000 ^{ac}	0.65±0.039 ^a	3.64±0.619 ^a
	<i>A. viridis</i>	0.22±0.012 ^{abc}	0.07±0.005 ^b	0.11±0.010 ^b	0.01±0.000 ^b	0.88±0.012 ^{abc}	4.27±0.552 ^b
	<i>P. erinaceus</i>	0.03±0.005 ^{bc}	0.03±0.001 ^c	0.06±0.005 ^{abc}	0.02±0.0005 ^c	0.39±0.010 ^{abc}	2.91±0.147 ^c
Enugu east	<i>T. occidentalis</i>	0.11±0.004 ^{ac}	0.05±0.002 ^a	0.10±0.005 ^{abc}	0.03±0.001 ^a	0.68±0.012 ^{ab}	3.42±0.560 ^a
	<i>A. viridis</i>	0.26±0.010 ^{ac}	0.08±0.005 ^b	0.12±0.010 ^b	0.02±0.0005 ^b	1.26±0.009 ^{ab}	3.72±0.130 ^{abc}
	<i>P. erinaceus</i>	0.00±0.000	0.03±0.001 ^c	0.05±0.004 ^{abc}	0.01±0.000 ^c	0.38±0.010 ^{abc}	2.88±0.080 ^{ac}
MAC [8]	-	0.20	1.00	2.00	2.00	5.00	

MAC-Maximum allowed concentration. Results are in mean±SD. Similar letters in a column are not significantly different at 0.05 level of significance

Table 2. Trace metal concentrations in the vegetable samples from the three senatorial zones of Enugu State for the month of February

Location	Samples	Trace metals (mg/kg)					
		Mn	Ni	Cr	Co	Zn	Fe
Enugu north	<i>T. occidentalis</i>	0.07±0.005 ^a	0.06±0.005 ^{ac}	0.05±0.004 ^{abc}	0.01±0.000 ^a	0.79±0.014 ^{ac}	4.11±0.307 ^{ab}
	<i>A. viridis</i>	0.16±0.015 ^{bc}	0.08±0.007 ^b	0.07±0.003 ^{abc}	0.01±0.000 ^b	1.24±0.105 ^{abc}	4.74±0.131 ^{bc}
	<i>P. erinaceus</i>	0.00±0.000	0.03±0.001 ^c	0.02±0.005 ^{abc}	0.02±0.0005 ^c	0.34±0.014 ^{bc}	2.78±0.145 ^{bc}
Enugu west	<i>T. occidentalis</i>	0.06±0.005 ^a	0.08±0.005 ^{ac}	0.08±0.004 ^{abc}	0.02±0.0005 ^a	0.63±0.039 ^a	3.76±0.619 ^a
	<i>A. viridis</i>	0.19±0.013 ^{abc}	0.09±0.010 ^b	0.10±0.006 ^b	0.01±0.000 ^b	1.27±0.014 ^{abc}	4.49±0.552 ^b
	<i>P. erinaceus</i>	0.00±0.000	0.04±0.002 ^c	0.06±0.005 ^{bc}	0.03±0.005 ^c	0.41±0.010 ^{abc}	2.81±0.147 ^{bc}
Enugu east	<i>T. occidentalis</i>	0.10±0.006 ^{ac}	0.08±0.005 ^{ac}	0.07±0.005 ^{abc}	0.01±0.000 ^a	0.61±0.012 ^{ab}	3.52±0.560 ^a
	<i>A. viridis</i>	0.25±0.010 ^{ac}	0.09±0.005 ^b	0.08±0.003 ^b	0.02±0.005 ^{ab}	0.93±0.040 ^{bc}	3.89±0.130 ^{abc}
	<i>P. erinaceus</i>	0.00±0.000	0.03±0.001 ^c	0.04±0.004 ^{bc}	0.01±0.000 ^c	0.47±0.014 ^c	3.26±0.080 ^{abc}
MAC [8]	-	0.20	1.00	2.00	2.00	5.00	

MAC-Maximum allowed concentration. Results are in mean±SD. Similar letters in a column are not significantly different at 0.05 level of significance

Table 3. Trace metal concentrations in the vegetable samples from the three senatorial zones of Enugu State for the month of March

Location	Samples	Trace metals (mg/kg)					
		Mn	Ni	Cr	Co	Zn	Fe
Enugu north	<i>T. occidentalis</i>	0.09±0.005 ^a	0.06±0.004 ^a	0.09±0.005 ^{abc}	0.01±0.000 ^a	0.69±0.010 ^{ac}	3.53±0.300 ^a
	<i>A. viridis</i>	0.21±0.015 ^{bc}	0.08±0.003 ^a	0.08±0.005 ^{abc}	0.01±0.000 ^b	0.91±0.015 ^{ab}	3.66±0.131 ^{bc}
	<i>P. erinaceus</i>	0.02±0.0005 ^c	0.04±0.002 ^{abc}	0.04±0.005 ^{abc}	0.02±0.005 ^c	0.40±0.004 ^{bc}	2.54±0.140 ^{bc}
Enugu west	<i>T. occidentalis</i>	0.10±0.005 ^a	0.09±0.005 ^{ab}	0.12±0.014 ^{abc}	0.01±0.000 ^a	0.61±0.039 ^a	3.27±0.319 ^a
	<i>A. viridis</i>	0.24±0.021 ^{abc}	0.10±0.000 ^{ac}	0.13±0.015 ^b	0.02±0.005 ^b	0.81±0.040 ^{abc}	3.21±0.152 ^b
	<i>P. erinaceus</i>	0.04±0.005 ^c	0.04±0.002 ^{abc}	0.06±0.005 ^{abc}	0.02±0.005 ^c	0.40±0.010 ^{bc}	2.38±0.147 ^c
Enugu east	<i>T. occidentalis</i>	0.12±0.014 ^{ac}	0.08±0.002 ^{ab}	0.11±0.005 ^{abc}	0.01±0.000 ^a	0.60±0.012 ^a	3.41±0.160 ^a
	<i>A. viridis</i>	0.29±0.012 ^{ac}	0.11±0.001 ^{ac}	0.12±0.013 ^b	0.01±0.000 ^a	0.87±0.015 ^{bc}	3.15±0.130 ^{ac}
	<i>P. erinaceus</i>	0.03±0.001 ^c	0.04±0.002 ^{abc}	0.02±0.001 ^{abc}	0.02±0.003 ^c	0.42±0.024 ^{bc}	2.61±0.080 ^{ac}
MAC [8]		-	0.20	1.00	2.00	2.00	5.00

MAC-Maximum allowed concentration. Results are in mean±SD. Similar letters in a column are not significantly different at 0.05 level of significance

while samples from Enugu north showed the least. Nickel was more abundant in *A. viridis* samples than the other vegetable samples. *A. viridis* samples showed the highest concentrations of Cr for Enugu west and east senatorial zones. *T. occidentalis* samples from Enugu north senatorial zone have the highest Cr level. *P. erinaceus* samples had the least concentrations of Cr for all the three senatorial zones. There was no significant difference in the levels of Cr in *T. occidentalis* and *P. erinaceus* samples from the three senatorial zones. The mean concentrations of Cr in *A. viridis* samples from Enugu east and west varies significantly with samples from Enugu north.

The levels of Co were detected within the range of 0.01 to 0.02 mg/kg for all vegetable samples from the three senatorial zones. *P. erinaceus* samples had the highest concentrations of Co (0.02mg/kg) for all the three senatorial zones. There was no significant difference in the level of Co in all the vegetable samples from all the three senatorial zones. Zn detection was highest for *A. viridis* samples from the three senatorial zones; but was least in *P. erinaceus* samples. There was no significant difference in the levels of Zn in *P. erinaceus* samples from the three senatorial zones while the mean levels of Zn in *A. viridis* samples from the three senatorial zones varied significantly from one another. Zn levels in *T. occidentalis* samples from Enugu west and east senatorial zones varied significantly with samples from Enugu north.

T. occidentalis samples from Enugu west and east senatorial zones had the highest level of Fe while *A. viridis* samples had the Fe levels for Enugu north senatorial zone. *P. erinaceus* samples had the least levels of Fe in all three senatorial zones. There was no significant difference in the levels of Fe in *T. occidentalis* samples from the three senatorial zones. The mean concentrations of Fe in *A. viridis* and *P. erinaceus* samples from all the three senatorial zones varied significantly from each other.

4. DISCUSSION

Understanding the distribution of some trace metals such as Mn, Ni, Cr, Co, Zn and Fe in some common leafy vegetables in Nigeria is important for establishing baseline concentrations from which the effects can be measured [9]. The results from the vegetable samples (*T. occidentalis*, *A. viridis*, and *P. erinaceus*) analysis from Enugu north, west and

east senatorial zones, revealed the presence of trace metals in the three vegetable samples. The concentrations of these trace metals were below the FAO maximum allowed concentrations {Ni (0.20 mg/kg), Cr (1.00 mg/kg), Co (2.00 mg/kg), Zn (2.00 mg/kg) and Fe (5.00 mg/kg)}. There were differences in the mean concentrations of trace metals observed in the three vegetable samples. *Amaranthus viridis* showed the highest concentration of trace metals while *P. erinaceus* showed the least. *T. occidentalis* concentration of trace metals was slightly below that of *A. viridis* but above *P. erinaceus* samples (the rate of trace metals accumulation is in the order *A. viridis* > *T. occidentalis* > *P. erinaceus*). This is in line with Zheng et al., [5] who reported that vegetable species differ widely in their ability to take up and accumulate heavy metals, even among cultivars and varieties within the same species. Our results however differ from the report of Tyokumbur and Okorie [10] who noted that heavy metals in *Amaranthus caudatus* and *Corchorus olithorus* exceeded the recommended levels given by the World Health Organization (WHO) and Federal Environmental Protection Agency (FEPA), Nigeria and therefore constitute a potential public health risk to the populace who consume these vegetables. Alexander et al. [11] reported that Cu accumulated to the highest levels in *Spinacia oleracea* (spinach) and *Lactuca sativa* (lettuce). According to Yang et al. [12] Cd accumulation in vegetable species decreased in the order of leafy vegetables > solanaceous vegetables > root vegetables > alliums vegetables > melon vegetables > legumes vegetables.

The trace metals investigated in the present study were Mn, Ni, Cr, Co, Zn and Fe. These metals varied in their concentrations, decreasing gradually in concentrations during the dry season (January to March). This lends support to Yusuf et al., [13] who reported that factors influencing the concentration of heavy metals in plants include climate, environmental pollution, nature of the soil on which the plant is grown, and the degree of maturity of the plant at the time of harvesting. The rate of accumulation of trace metals in the vegetable samples were in the following decreasing order Fe > Zn > Mn > Cr > Ni > Co. The highest concentration of Fe was observed in *A. viridis* sample for the month of January (4.27 ± 0.552 mg/kg) while the lowest concentration of Fe was observed in *P. erinaceus* sample for the month of January (0.36 ± 0.145 mg/kg).

5. CONCLUSION

Amaranthus viridis had the highest accumulation of trace metals while *P. erinaceus* had the least. *T. occidentalis* accumulation of trace metals was slightly below that of *A. viridis* but above *P. erinaceus* samples. The results obtained in this study indicate variations in the metal concentration in the edible vegetables from the three senatorial districts of Enugu State. Their concentrations in the studied vegetables in the three senatorial zones were however below the FAO maximum allowed limits for human consumptions.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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