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Energy Dispersive X-Ray Fluorescence Spectrometric Study of Compositional Differences in Trace Elements in Dried *Moringa oleifera* Leaves Grown in Two Different Agro-Ecological Locations in Ebonyi State, Nigeria

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ABSTRACT

This study investigated the compositional differences in trace elements in *M. oleifera* leaves grown in two different agro-ecological locations in Ebonyi State, Nigeria. Fresh samples of dried *M. oleifera* leaves were collected and analysed for P, S, K, Ca, Mn, Ti, Cu, Mo, Fe, Zn, Ni, Re, Eu and Pb using Energy Dispersive X-ray fluorescence (ED-XRF) spectrometry, following standard procedures. The results showed varying amounts of the studied elements in the samples from the two locations, with relatively high amounts of Pb. When the mean content of P, K Ca, Mn, Cu, Fe and Zn in the samples where compared to their daily requirements for adults and the expected quantity of *M. oleifera* leaves an adult will eat to achieve the daily requirements, it was concluded that the leaves could serve as veritable source of these elements to local consumers. Levels of significant differences in the concentrations of the trace elements in samples of *M. oleifera* leaves grown in both locations were tested at 95% confidence level (α = 0.05) using *F*- and student's *t*-tests. The outcome showed that although there are compositional differences in the amounts of the elements studied in the samples, these differences are not significant ($t_{exps} < t_{crits}$) at 95% confidence interval.

Keywords: trace elements, daily requirements, *M. oleifera*, ED-XRF spectrometry, Mgbabor, Okposi-Okwu

INTRODUCTION

M. oleifera is native to the sub-Himalayan regions of Northwest India. However, it is now widely distributed throughout world including Africa, Southeast Asia (Valdez-Solana *et al*., 2015). Over the years, parts of *M. oleifera* have been used for medicinal purposes (Sulaiman *et al*., 2008; Choudhury and Sinha, 2015). This is because pharmacological studies have shown that the extracts of the plant have antioxidant (Verma *et al*., 2009), anti-carcinogenic (Jung, 2014), and anti-inflammatory properties (Khalafalla *et al*., 2010). Traditionally, the leaves, fruits, flowers, and immature pods of this tree are edible (Valdez-Solana *et al*., 2015). They are used as a highly nutritive vegetable in many countries, including Nigeria (Oluduro, 2012). They contain trace elements that play an important role in metabolism (Valdez-Solana *et al*., 2015).

Some reports have indicated that the nutrient content of newly harvested *M. oleifera* naturally varies with soil type, climate conditions and plant age. Differences in processing and storage procedures add more variation and the use of different analytical techniques amplifies the variation further (Witt, 2016). In terms of soil, it is also believed that different soil types contain different qualities and quantities of mineral elements whose bioavailability depends on the soil properties (pH, clay and humid complex, and mineralogy, etc) (Valdez-Solana *et al*., 2015). Based on these, this research was carried out to investigate the compositional differences in chemical elements of *M. oleifera* leaves grown in two different agro-ecological locations in Ebonyi State, Nigeria.

MATERIALS AND METHODS

Sample Collection and Preparation

M. oleifera leaves were collected from Mgbabor and Okposi Okwu, both in Ebonyi State South-eastern Nigeria, in October 2015.

The leaves were carefully separated from other plant parts and washed. A set of five samples from each location were randomly collected and analyzed. The samples were air-dried for five days at room temperature, ground into fine powder, sieved (90 μm mesh size) to obtain very fine samples and protected from light until further analysis. The sample materials were then loaded into sample holder and analysed using ED-XRF spectrometer (Thermo Scientific Niton XL3t950).

Statistical Treatment of Data

The data were expressed as means ± standard deviation, and the results were statistically treated using Microsoft Excel Spreadsheet 2007 (Data analysis ToolPak). The *F*-Test Two-Sample for Variances and *t*-Test Two-Sample Assuming Unequal Variances were used according to the procedure reported by Harvey, (2000). The differences were considered statistically significant where $p < 0.05$ at 95% confidence level.

RESULTS AND DISCUSSION

Table 1 and Fig. 1 show the results of the 14 major and minor elements determined in the samples of the *M. oleifera* leaves that were obtained from the two locations. The results are compared to values reported in literature on similar studies. Energy dispersive X-ray (ED-XRF) was used for simultaneous analysis and measurement of the elemental content of the samples. The procedure, which used three-axial geometry, reduced background noise due to radiation polarization. The monochromatic radiations emitted from the X-ray tube were applied to excite the atomic species in the sample (Asiedu-Gyekye, *et al*., 2014).

The ED-XRF elemental analysis showed that the samples contained high contents of Ca (78.63 ± 0.01 and 95.1 ± 0.04 mg/100 g) compared to other essential or major elements. This was closely followed by K (78.93 ± 0.02 and 64.98 ± 0.3 mg/100 g), and then P (5.07 ± 0.84 and 3.9 ± 0.00 mg/100 g). Mo (10.56 ± 0.08 and 9.39 ± 0.07 mg/100 g) and Fe (4.8 ± 0.0 and 4.65 ± 0.07 mg/100 g) were found to be relatively high compared to the levels of Mn (0.48 ± 0.0 and 0.54 ± 0.0 mg/100 g), Cu (0.54 ± 0.02 and 0.48 ± 0.02 mg/100 g), Ti (0.39 ± 0.01 and 0.36 ± 0.01 mg/100 g), Zn (0.09 ± 0.07 and 0.06 ± 0.02 mg/100 g) and Ni (0.09 ± 0.01 and 0.06 ± 0.0 mg/100 g). For the inner transition elements, Re (1.62 ± 0.03 and 2.07 ± 0.02 mg/100 g) was found to be higher than Eu (0.24 ± 0.0 and 0.51 ± 0.02 mg/100 g). All the samples were found to contain high contents of Pb (22.83 ± 0.6 and 24.51 ± 0.9 mg/100 g).

Table 1: Mean concentration (mg/100 g) of trace element in the dried *M. oleifera* leaf powder

Element	**Mgbabor**	**Okposi Okwu**	**Reported in literature** (Valdez-Solana, et al., 2015)
P	5.07 ± 0.84	3.9 ± 0.0	297 ± 149.0**
S	12.36 ± 0.81	10.89 ± 0.06	-
K	78.93 ± 0.02	64.98 ± 0.3	1845 ± 7.0
Ca	78.63 ± 0.01	95.1 ± 0.04	2016.5 ± 22.6
Mn	0.48 ± 0.0	0.54 ± 0.0	79 ± 1.88*
Ti	0.39 ± 0.01	0.36 ± 0.01	-
Cu	0.54 ± 0.02	0.48 ± 0.02	1.03 ± 0.7***
Mo	10.56 ± 0.08	9.39 ± 0.07	0.0056****
Fe	4.8 ± 0.0	4.65 ± 0.07	19.37 ± 6.6
Zn	0.09 ± 0.07	0.06 ± 0.02	1.0 ± 0.7***
Ni	0.09 ± 0.01	0.06 ± 0.0	0.0148****
Re	1.62 ± 0.03	2.07 ± 0.02	-
Eu	0.24 ± 0.0	0.51 ± 0.02	-
Pb	22.83 ± 0.6	24.51 ± 0.9	0.355 ± 0.0

Results are mean ± standard deviation, analyzed individually in triplicate.
*value based on mg/L concentration ± standard error of the mean, reported according to Offor, Ehiri, and Njoku, (2014)
**value as reported according to Witt, (2013)
***values in μg/100 g
****value as reported according to Asiedu-Gyekye *et al*., (2014)

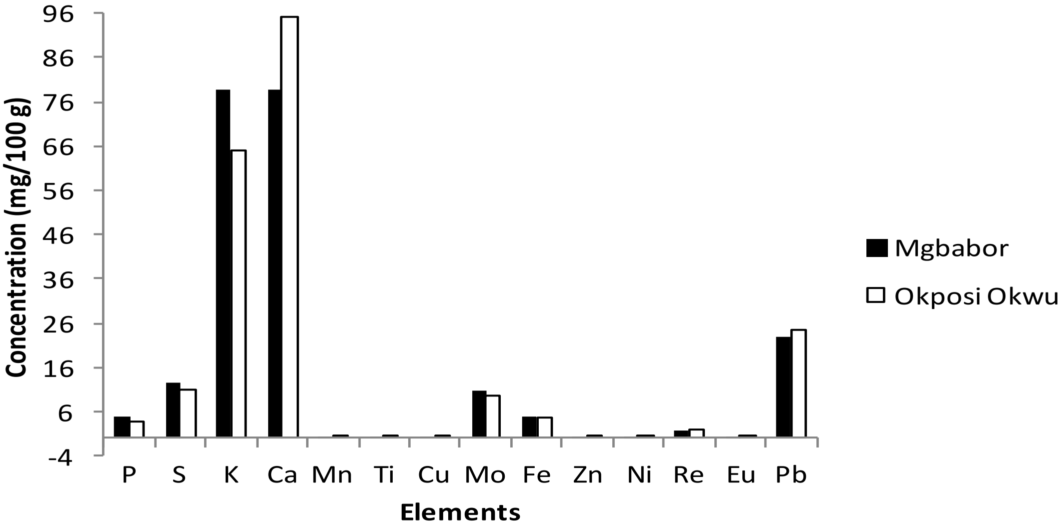

Fig. 1. Concentration of trace elements in dried *M. oleifera* leaf powders from the sampled locations

Chemical elements in soils can occur as constituents of primary rock-forming minerals, minerals formed during weathering, metallic ions adsorbed onto colloidal particles and clays, and part of organic matter (Rose, 1975). Plants cultivated on soils utilize some of the chemical elements for growth and metabolism. Therefore, the main source of the elements in the *M. oleifera* leaves could be attributed to background geochemistry and geochemical cycles of the elements in the environment. Manganese is one of the most abundant metals in the Earth's crust, usually occurring with iron. It is possible that the level of element(s), their oxides or hydroxides in soil could affect the level or uptake of another element(s) by plants. For example, presence of Fe-Mn oxyhydroxides could affect the levels (enrichment) of other elements in environmental samples. This is because metals dissolved in solution adsorb strongly onto Mn oxide surfaces due to their large specific area (Itumoh *et al*., 2015). Mn is widespread in the environment (Heal, 2001). Generally, environmental samples often contain mixtures of other contaminants (Salbu *et al*., 2005), because of varied sources contributing to their enrichment. Pb is known to absorb onto soil constituent surfaces such as clay, oxides, hydroxides and organic matter. This adsorbed Pb often finds its way into plants. The amount of Pb found in the samples of *M. Oleifera* leaves in this study is not surprising because the principal ore of Pb, galena, (PbS), is found abundantly in many parts of Ebonyi State. Abakaliki is one of the well-known Pb-Zn mineralized districts in Africa (Hutchison and Meema, 1987; Itumoh *et al*., 2012). High concentrations of Pb (352 mg/L) were similarly observed in *M. oleifera* leaves cultivated in Oshiri, Ebonyi State (Offor *et al*., 2014).

Several reports (Valdez-Solana *et al*., 2015; Mbaiguinam *et al*., 2014; Asante *et al*., 2014; Moyo *et al*., 2011) have shown that *M. oleifera* leaves could serve as a major source of nutirents and important trace elements to consumers, especially people living in rural areas and low-income families. Table 2 and Fig. 2 show the mean content of three major elements (P, K and Ca) and four micro elements (Mn, Cu, Fe and Zn) determined in samples of *M. oleifera* leaves from the two agro-ecological locations. The Figure and Table also show the daily requirements for adults and the expected quantity of *M. oleifera* leaves an adult will need to consume in order to achieve the daily requirements. Based on the gramme-quantity of *M. oleifera* leaves required per day, the leaves could be regarded as good sources of P, K, Ca, Mn, Cu, Fe and Zn. This is because an adult will need to consume far less than 100 grammes (about 20-25 g) of the leaves per day in order to achieve the daily requirements of these important elements.

Table 2: Mean content of trace elements, daily requirements for adults and expected quantity of leaves needed per day

Element	Mgbabor (mg/100 g)	Okposi-Okwu (mg/100 g)	Daily requirement (mg/day)*	Quantity needed (g/day) Mgbabor	Quantity needed (g/day) Okposi-Okwu
P	5.07	3.9	580 – 1055	11.44 – 20.81	14.87 – 27.05
K	78.93	64.98	4500 – 4700	5.70 – 5.95	6.93 – 7.23
Ca	78.63	95.1	1000 – 1200	1.27 – 1.53	1.05 – 1.26
Mn	0.48	0.54	1.8 – 2.3	0.38 – 0.48	0.33 – 0.43
Cu	0.54	0.48	900	0.13 – 0.17	0.15 – 0.19
Fe	4.8	4.65	18 – 27	0.38 – 0.56	0.39 – 0.58
Zn	0.09	0.06	8 – 11	8.89 – 12.22	13.33 – 18.33

*values as reported according to US Food and Nutrition Board, Institute of Medicine and National Academies (2001)

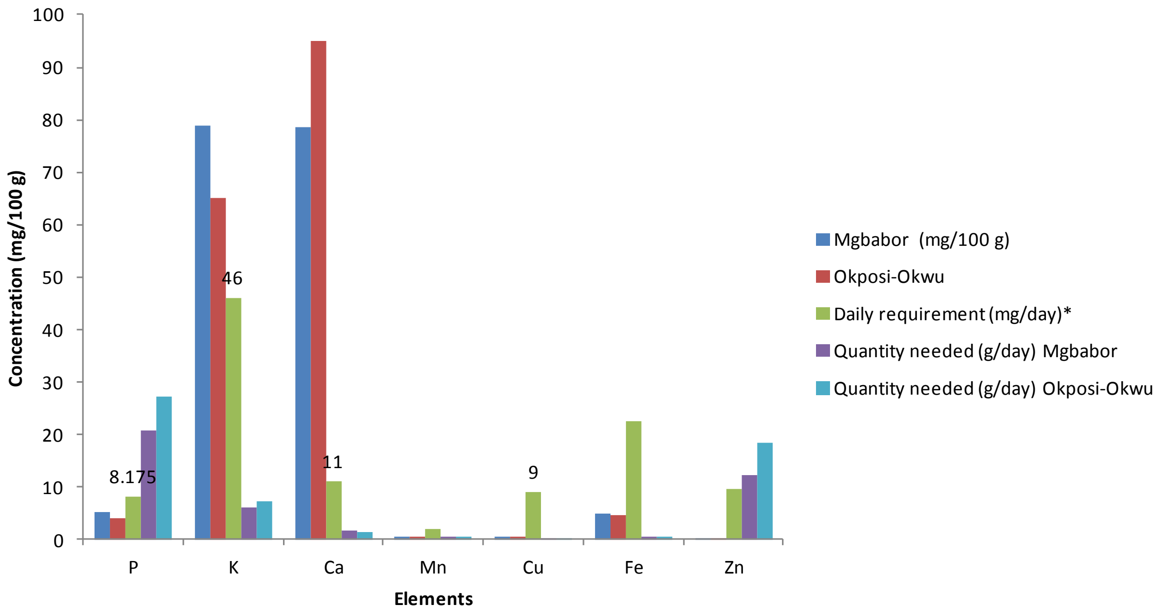

Fig. 2. Mean content of trace elements, daily requirements for adults and expected quantity of leaves needed per day.

*Daily requirements values with labels on the graph (8.175, 46, 11 and 9) are in 100ths.

Possible Cumulative Toxic Effects of Metals

M. oleifera leaves have found everyday use in the treatment of various diseases and are available without a medical prescription, often in the form of an herbal infusion (Valdez-Solana *et al.*, 2015). Caution is required in ways in which the leaves are utilized and consumed by locals. This is because toxic effects of accumulation of some of the elements in consumers are possible. Metals could have toxic effects even at very low concentrations (Salama and Radwan, 2005) or at high concentrations (Stević *et al.*, 2010). Several cases of human diseases, disorders, malfunction and malformation of organs due to metal toxicity are known (Anglin-Brown *et al.*, 1995; Salama and Radwan, 2005; Stoica, 1999). These adverse effects of metals on human beings and animals occur because there is no effective mechanism for elimination metals from the bodies of human beings and animals (Suruchi and Pankaj, 2011). One of the metals found at a very high concentration in both samples was Pb. Pb toxicity could lead to anaemia and impairment of haem biosynthesis, red blood cells; and depression of sperm count (Anglin-Brown *et al.*, 1995). In human body, metals are sulphur-seeking and easily bind to S-CH_3 and S-H (sulphydryl groups) in enzyme proteins. Such "immobilized" enzymes cannot function properly and the affected individual suffers (Okonkwo and Eboatu, 1999). Few toxicological data on rare-earth metals such as Eu are available, in comparison to other metals. However, since chemical properties of rare-earth metals are very similar to other metals, it is plausible that their binding affinities to biomolecules, metabolism, and toxicity in the living system are also very similar (Hirano and Suzuki, 1996).

Significance Tests

The levels of significant differences in the concentration of the elements in the two samples were tested at 95% confidence level ($\alpha = 0.05$) using *F*- and student's *t*-tests. The *F*-test was used to determine whether the variances for the concentrations of the elements in the samples are equal or unequal. The null hypothesis was that the variances are equal, such that *variances of elements in Mgbabor samples = variances of elements in Okposi-Okwu*. The alternative hypothesis was that the variances are not equal, such that *variances of elements in Mgbabor samples ≠ variances of elements in Okposi-Okwu*. Afterwards, an unpaired *t*-test assuming Unequal Variance was computed. The null hypothesis was that there is no difference between the means, *means of elements in Mgbabor samples = means of elements in Okposi-Okwu*, and the alternative hypothesis was that there is a difference between the means, *means of elements in Mgbabor samples ≠ means of elements in Okposi-Okwu*. From our statistical analysis, it was observed that F_{exp}, which is 0.80, is greater than $F(0.05, 13, 11)$, which is 0.31 ($F_{exp} > F_{crit}$). We retain our alternative hypothesis that the variances are not equal at an α value of 0.05 (*variances of elements in Mgbabor samples ≠ variances of elements in Okposi-Okwu samples*). Having found no evidence suggesting equal variances, we then computed an unpaired *t*-test assuming unequal variances. From the unpaired *t*-test, it was found that t_{exp}, which is -0.05, is less than $t(0.05, 22)$, which is 2.10 ($t_{exps} < t_{crits}$). We retain the null hypothesis that there is no evidence of a difference between the means at an α value of 0.05 (*means of elements in Mgbabor samples = means of elements in Okposi-Okwu samples*). The significance tests suggest that although there are compositional differences in the amounts of the elements studied in the samples, these differences are not significant ($t_{exps} < t_{crits}$) at 95% confidence interval.

CONCLUSION

The levels of the elements, P, S, K, Ca, Mn, Ti, Cu, Mo, Fe, Zn, Ni, Re, Eu and Pb, were determined in dried *M. oleifera* leaves samples which were collected from two different agro-ecological locations in Ebonyi State, Nigeria. From the results obtained, it was reasonably thought that the main source of the elements in the samples could be attributed to background geochemistry and geochemical cycles of the elements in the environment. On the basis of daily requirements for adults for P, K Ca, Mn, Cu, Fe and Zn, and the expected quantity of *M. oleifera* leaves an adult will need to consume to achieve the daily requirements, this study concluded that the leaves could serve as veritable source of these elements to local consumers. The study also found that the compositional differences in the amounts of the elements between the two set of samples were not significant at 95% confidence interval. This study further suggested that caution is required in ways in which the leaves are utilized and consumed by locals, because of possible toxic effects of accumulation of some of the elements in consumers, especially Pb which was found at a very high concentration in both samples.

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