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Heavy metal constituent of medicinal plants: a case study of *Moringa oleifera* Lam. from selected areas in Accra, Ghana

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Abstract

Background: Anthropogenic activities release metals into the environment that could be absorbed by plants and assimilate into herbal medicines.

Objective: This study sought to correlate the outcome of anthropogenic activities that release metals into the environment with levels of metals in medicinal plants using the leaves of *Moringa oleifera* Lam. *(M. oleifera)* as a case study.

Methods: Leaves of *M. oleifera* and soil around sampled plants were collected from three locations in the Accra Metropolis: a commercial area, an area with dense vehicular traffic, and a quasi-natural habitat. Samples were analyzed with Energy Dispersive X-ray Fluorescence (ED-XRF) spectroscopy for the presence and levels of arsenic (As), cobalt (Co), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb), vanadium (V) and zinc (Zn). Statistical analyses were performed and the correlation between metal concentrations in leaves and soil was assessed using Spearman's correlation coefficient.

Results: Levels of metals in the leaves and soil samples varied with the site of sample collection. Levels of metals in leaves were significantly lower than permissible limits ($p < 0.05$) in herbal medicines. Translocation factor (TF) estimates indicated that the *M. oleifera* leaves exclude the metals under investigation irrespective of the site of collection. However, the TF > 1 in the case of Zn from the quasi-natural habitat signifying bioaccumulation in the leaves. Except for Co, the total concentration of each metal in the soil was not correlated with its concentration in the leaves.

Conclusion: The concentration of sampled metals in the leaves and soil varied with the site of sampling and potentially with the anthropogenic activities that release metals into that environment.

Keywords: Anthropogenic, herbal medicine, *Moringa oleifera*, soil-plant transfer factor, Ghana

INTRODUCTION

Plants and plant-based medicinal products are the mainstay of primary healthcare for a considerable number of people in sub-Saharan Africa, and most developing countries [1,2]. The over-reliance on herbal medicine for the prevention, management, and treatment of different forms of diseases is attributable to the cheap cost, easy accessibility, cultural acceptability, and perceived lesser side effects compared to orthodox medicines [2]. Plants absorb minerals from the ground for biosynthesis and growth. Different plant species grown on the same soil may contain different levels of the same element. Differences in the bioaccumulation of metals in plant cultivars, age of plants, plant organs, and tissues have also been reported [3,4]. Ghanaian medicinal plants, traditionally harvested crudely and unscientifically from a wide range of locations, are typically immediately made available for public consumption without any appropriate quality assessment. Heavy metal contamination has been reported as one of the possible causes of the adverse effects associated with the

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use of herbal medicines [5-10]. Hence, the quality of the plant source is very essential to minimize possible side effects arising from toxic metals and other pollutants. *Moringa oleifera* Lam. (*M. oleifera*) grows wildly in all the agro-ecological zones of Ghana. It is widely consumed as part of human and animal feed. It has shown antioxidant, antitumor, antimicrobial, anti-inflammatory, cardio-protective, and antidiabetic activity [11,12]. It is rich in the essential elements sodium (Na), magnesium (Mg), aluminium (Al), silicon (Si), phosphorous (P), sulphur (S), potassium (K), calcium (Ca), manganese (Mn), and iron (Fe) [13]. The plant also has a high capacity for toxic metals such as cadmium (Cd), arsenic (As), lead (Pb), and mercury (Hg) [13, 14]. Its high metal absorption capacity has found use in the phytoremediation of metal-polluted soils [15]. It is also used to remove metals and organic pollutants in water purification [16,17].

Plants that grow in metal-polluted soils are expected to have high levels of metals which can compromise the quality of medicinal products from such plants. Once a heavy metal enters the food chain, its concentration increases due to its non-biodegradability and long half-life. Unlike microbial contaminants, boiling does not reduce levels of metal contamination [18,19]. However, careful harvesting, handling, and storage could reduce the levels of metal contamination [20]. The high capacity for such toxic metals makes it imperative to determine the effect of anthropogenic activities on the mineral composition of the various parts of the plant. Hence, this research investigated the effects of anthropogenic activities on the mineral composition of medicinal plants using the leaves of *M. oleifera* as a case study. The leaves were investigated because it is the most commonly utilized part of the plant. The elemental composition of the soil around the plants were also analyzed since the soil is the main source of minerals for the plants.

MATERIALS AND METHODS

Study setting

Samples were collected from three sites in Accra, Ghana, namely Ridge Roundabout (RRA) (Latitude: 5.5594° North; Longitude: 0.1969° West [21]), an area with dense vehicular traffic; the Ghana Geological Survey Authority compound (GSA), (Latitude: 5.5580° North; Longitude: 0.2023° West, [22]) a commercial area; and the Legon Botanical Garden (LBG) (Latitude: 5.6605° North, Longitude: 0.1860° West, [23]), a quasi-natural habitat. These sites are exposed to metal pollutants from anthropogenic activities such as fossil fuel combustion from automobiles, improper waste disposal, run off, and agricultural activities. Specimen of sampled leaves of *M. oleifera* were identified and authenticated by a botanist at the Herbarium of the Department of Plant and Environmental Biology, University of Ghana, Legon, Ghana, (Herbarium Reference Number: PGHB/2019/Mo001), before subsequent laboratory work was done. For the soil sampling, three points were selected within a 20 - 30 cm radius around the trees whose leaves were sampled. Topsoil samples (0 – 10 cm depth) were collected around each plant and analyzed.

Sampling and ED-XRF spectroscopic measurement

All glassware used were acid-washed to avoid contamination from metal impurities. The leaves were washed with deionized water and air-dried under dust-free conditions, for 4 days at ambient temperature. The dried leaves were pulverized with a stainless-steel blender and sieved using aluminum test-sieves with a vibratory electronic sieve shaker of 180 µ mesh size (Retsch GmbH, Haan, Germany). Soil samples were crushed into a coarse powder and air-dried under ambient conditions for two weeks to reduce moisture content below 10%. The dried soil samples were treated similar to the leaves to obtain a fine powder. The pulverized and sieved leaves and soil samples were further dried in an oven at 60 ºC till constant weight before pelletization for analysis. Energy Dispersive X-ray Fluorescence (ED-XRF) spectroscopic analysis was conducted to quantify the levels of metals in *M. oleifera* leaves and the soil samples from the site of growth of the sampled plants. The method as described by Kodom et al. [24] was followed to prepare pellets for the analysis. Instrumentation of the ED-XRF spectrometer and the principle of X-ray Fluorescence spectroscopy (XRF) are described elsewhere [13,25]. A certified reference material was analyzed prior to the sample analysis. Measurements results were reported as mg/kg of the metal in dry leaves or dry soil samples.

Statistical analysis

Data from the three sampling sites were entered into Microsoft Excel and imported into STATA Statistical Software (Version 14, StataCorp LLC, College Station, TX).. Bar charts were plotted to show the distribution of metals in *M. oleifera* leaves and soil samples. To quantify the transport of metals from the soil to the leaves and the bioaccumulation capacity of the leaves, the transfer factor, TF, was calculated as follows:

$$TF = \frac{\text{Concentration of metal in plant tissue}}{\text{Concentration of metal in soil}}$$

The relationship between concentrations of metals in soil versus leaves was assessed with Spearman's rank correlation coefficients (r_s). All statistical analyses were considered significant if the p was < 0.05.

RESULTS

The ED-XRF analysis revealed variable amounts of As, Co, Cr, Cu, Ni, Pb, V, and Zn in both the leaves and the soil.

Accumulation of metals in leaves of *M. oleifera*

The concentrations of elements from the various sampling sites are shown in Figure 1. Except for As, there were varying levels of metals in the *M. oleifera* leaves from the different sampling sites. The LBG had the highest levels of Zn, Cr, and Cu while GSA had lower levels of Cr, Ni and

V compared to the other sampling sites. Arsenic concentration was the same (0.16 mg/kg) in all leaf samples irrespective of the sampling site. This was higher than most results reported in literature e. g. 2.20 × 10^{-3} mg/kg by Asiedu-Gyekye et al. [13] and 0.02 mg/kg by Agboola et al. [4]. Levels of chromium in the leaves [4.60 mg/kg (GSA) to 8.64 mg/kg (LBG)] were also higher than reported values in *M. oleifera* leaves [13, 26]. Levels of cobalt and zinc were lower than reported values [13,27]. However, the levels of lead, copper, and vanadium were within ranges reported in literature [13,26-28]. Table 1 summarizes the concentration of the metals in the leaves and results are compared with permissible limits in herbal medicines. Concentrations of all metals were below levels permissible in herbal medicines [29,30]. The mean metal concentrations as presented in Table 1 were in the decreasing order of Zn (13.48 mg/kg) > Cr (6.75 mg/kg) > Cu (3.68 mg/kg) > V (1.65 mg/kg) > Ni (1.36 mg/kg) > Co (0.96 mg/kg) > Pb (0.37 mg/kg) > As (0.16 mg/kg) in the dried leaf samples.

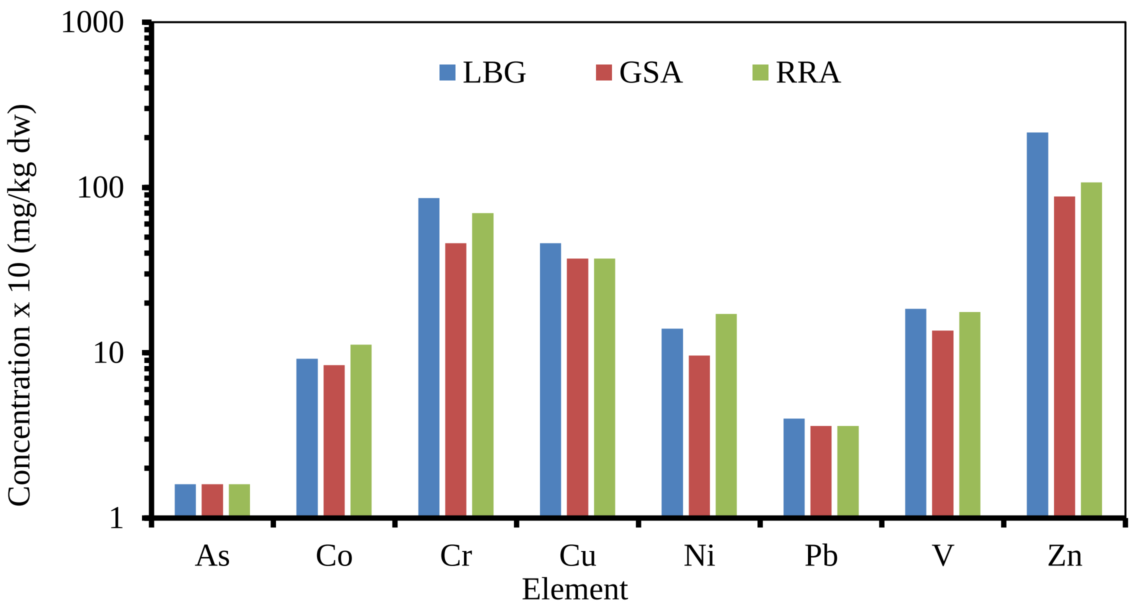

Figure 1. Concentration of metals in dry leaves of *M. oleifera* sampled from three sites in the Accra metropolis. RRA - Ridge Roundabout (heavy vehicular traffic), GSA - Geological Survey Authority compound (commercial), LBG - Legon Botanical Gardens, University of Ghana (quasi-natural habitat).

Table 1. Concentration (in mg/kg dry weight) of metals in leaf samples of *M. olifeira* from three sampling sites in the Accra metropolis compared with maximum permissible limits in herbal medicines

Ele	Minimum (mg/kg)	Maximum (mg/kg)	Mean (mg/kg)	SD	Permissible limit (mg/kg)
As	0.16	0.16	0.16	0	5 [29]
Co	0.84	1.12	0.96	0.14	Not available
Cr	4.60	8.64	6.75	2.03	25 [30]
Cu	2.72	4.60	3.68	0.94	100 [30]
Ni	0.96	1.72	1.36	0.38	25 [30]
Pb	0.36	0.40	0.37	0.02	10 [29]
V	1.36	1.84	1.65	0.26	Not available
Zn	8.80	20.92	13.48	6.51	150 [30]

Ele, element; SD, standard deviation

Levels of metals in the soil

Figure 2 shows the concentration of metals in dry soil samples. Levels of Cr were highest and As were the lowest in the dry soil samples from the three study sites. The concentrations of Zn and Pb were also high in the RRA site as compared to LBG and GSA sites. The concentrations of Ni and V were relatively high in GSA and LBG respectively, compared to the other study locations.

Transfer factor

The transfer factor, a measure of the bioaccumulation capacity of the leaves for metals, was determined (Figure 3). The results indicated that of all the metals detected in leaves of *M. oleifera*, bioaccumulation was greatest for Zn and Cu at all the study sites. Bioaccumulation capacity was both metal and site dependent.

Correlation between metal concentration in leaves and soil

Table 2 shows pairwise correlation between concentrations of metals in soil and leaves. Cobalt demonstrated a significant correlation between concentration in the soil, as compared to the concentration found in the leaves (r_s = 1.00, p < 0.001). Although none of the other metals exhibited a correlation between its concentration in soil versus the leaves, there was a positive correlation between Co concentrations in leaves, or soil, and As concentrations in the soil which was statistically significant (r_s = 1.00, p < 0.001). The levels of Pb in leaves were significantly and positively correlated with the concentration of Cu in the

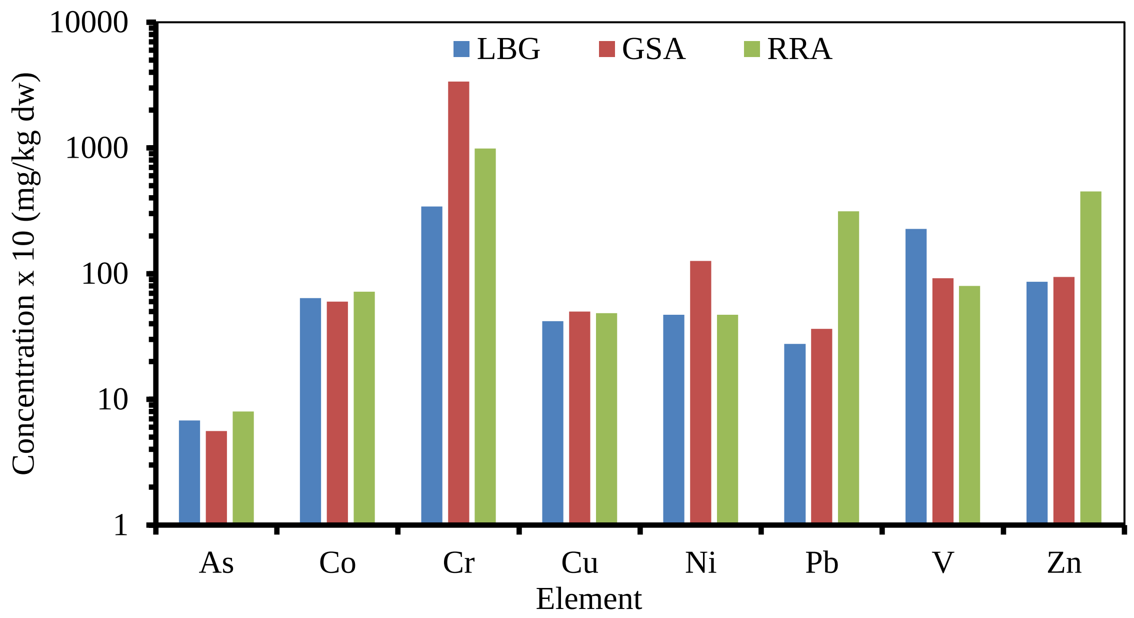

Figure 2. Levels of metals (mg/kg of dry soil samples) from three sites in the Accra metropolis where *M. oleifera* leaves were sampled. RRA - Ridge Roundabout (heavy vehicular traffic), GSA - Geological Survey Authority compound (commercial), LBG - Legon Botanical Gardens, University of Ghana (quasi-natural habitat).

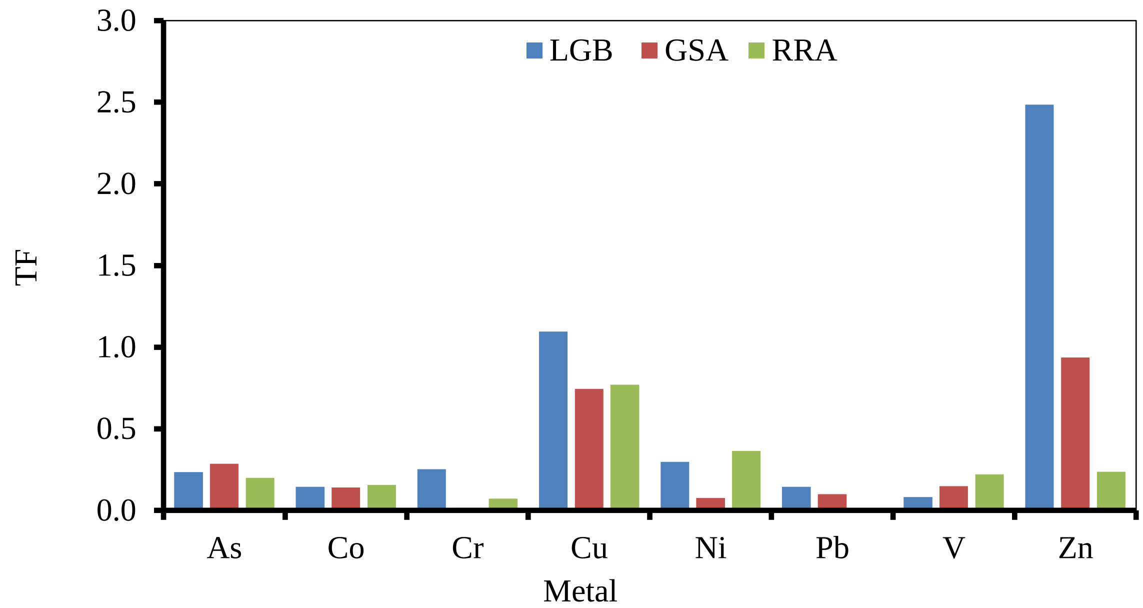

Figure 3: Transfer factor index values of metals detected in leaves and soil. RRA - Ridge Roundabout (heavy vehicular traffic), GSA - Geological Survey Authority compound (commercial), LBG - Legon Botanical Gardens, University of Ghana (quasi-natural habitat).

Table 2. Pairwise correlation between metal concentration in leaves and soil by Spearman's correlation coefficient

Ele	$[As]_l$	$[As]_s$	$[Co]_l$	$[Co]_s$	$[Cr]_l$	$[Cr]_s$	$[Cu]_l$	$[Cu]_s$	$[Ni]_l$	$[Ni]_s$	$[Pb]_l$	$[Pb]_s$	$[V]_l$	$[V]_s$	$[Zn]_l$	$[Zn]_s$
$[As]_l$	-															
$[As]_s$	-	1.000														
$[Co]_l$	-	1.000*	1.000													
$[Co]_s$	-	1.000*	1.000*	1.000												
$[Cr]_l$	-	0.500	0.500	0.500	1.000											
$[Cr]_s$	-	-0.500	-0.500	-0.500	-1.000	1.000										
$[Cu]_l$	-	0.000	0.000	0.000	0.866	-0.866	1.000									
$[Cu]_s$	-	-0.500	-0.050	-0.500	-1.000	1.000*	-0.866	1.000								
$[Ni]_l$	-	1.000*	1.000*	1.000*	0.500	-0.500	0.000	-0.500	1.000							
$[Ni]_s$	-	-0.866	-0.866	-0.866	-0.866	0.866	-0.500	0.866	-0.866	1.000						
$[Pb]_l$	-	0.000	0.000	0.000	0.866	-0.866	1.000*	-0.866	0.000	-0.500	1.000					
$[Pb]_s$	-	0.500	0.500	0.500	-0.500	0.500	-0.866	0.500	0.500	0.000	-0.866	1.000				
$[V]_l$	-	0.500	0. 500	0.500	1.000*	-1.000	0.866	-1.000	0.500	-0.866	0.866	-0.500	1.000			
$[V]_s$	-	-0.500	-0.500	-0.500	0.500	-0.500	0.866	-0.500	-0.500	0.000	0.866	-1.000	0.500	1.000		
$[Zn]_l$	-	0.500	0.500	0.500	1.000*	-1.000	0.866	-1.000	0.500	-0.866	0.866	-0.500	1.000*	0.500	1.000	
$[Zn]_s$	-	0.500	0.500	0.500	-0.500	0.500	-0.866	0.500	0.500	0.000	-0.866	1.000*	-0.500	-1.000	-0.500	1.000

Ele, element; is significant at $p < 0.001$. $[Q]_s$ is concentration of metal Q in soil, $[Q]_l$ is concentration of metal Q in leaves

leaves of the *M. oleifera* plant ($r_s = 1.00$, $p < 0.001$). The concentration of V in *M. oleifera* leaves, was found to be positively and significantly correlated with the concentration of Cr in the leaves of *M. oleifera* but not in the soil where *M. oleifera* grew ($r_s = 1.00$, $p < 0.001$). A significant positive correlation was also observed between the concentration of Zn and Pb in the soil ($r_s = 1.00$, $p < 0.001$).

DISCUSSION

A large section of the world's population relies on herbal medicines for treatment of diseases [1,2]. Plant-based medicines are generally harvested from the wild. However, over exploitation, indiscriminate harvesting, and increased demand due to population growth has led to shortage in supply of herbal medicines from the wild [31,32]. This has led to increased cultivation of medicinal plants in urban areas and cities where pollution from anthropogenic activities is also high. Anthropogenic activities such as fossil fuel combustion, indiscriminate and improper disposal of municipal wastes, sewage sludge, agrochemical application, refuse incineration, landfills, corrosion of metallic waste, and runoff from erosion of nearby contaminated sites contribute significantly to the levels of metal pollutants in the soil [33]. Essential and toxic metals absorbed by the plants that grow in these metal-polluted soils end up being consumed by mankind. The health impact of these metals is a source of major public health concern since no proper quality assurance is generally conducted on herbal medicines before use. The findings as presented in Figure 1 and Table 1 revealed varying concentrations of Co, Cr, Cu, Ni, Pb, V, and Zn in the *M. oleifera* leaves from the different sampling sites. Leaves from the quasi-natural habitat, LBG, had higher levels of Zn, Cr and Cu compared to the other sampling sites. The LBG, being a quasi-natural habitat, was expected to be the pseudo-control site. However, increased levels of metals were observed in soil samples from LBG which may be ascribed to apparent indiscriminate and improper waste disposal at the site (Figure 2). The commercial area, GSA, had lower levels of Cr, Ni and V compared to the other sampling sites. The overarching factor that determines the levels of metals in plants is the concentration of the metals in the soil on which the plant grows [33].

Plants cultivated on metal-polluted soils tend to have higher concentrations of the metals. Comparing metals in the soil from the various sampling sites revealed little differences in the levels of As, Co, and Cu. However, levels of Cr, Pb, and Zn were significantly higher for soil samples from the heavy vehicular traffic area, RRA. The high vehicular traffic at RRA discharges large volumes of automobile engine exhaust into the environment. Automobile exhaust releases Pb, Cr and Zn into the environment. Though, leaded gasoline is no more used in Ghana, old use has discharged Pb into the environment over a period. GSA, a commercial area, also had relatively higher levels of Cr and Ni. A refuse dump close to the site of sample collection coupled with the improper and indiscriminate domestic waste disposal and incineration, discharge of pesticides from nearby vegetable gardens due to runoff are the possible sources of the higher levels of Cr and Ni [30]. The intermediate levels of Pb and Zn are attributable to the light vehicular traffic in GSA. The transfer factor calculations revealed that except for Cu and Zn from LBG, values for all metals were below 1 in all samples irrespective of their site of collection even though their levels were high in the soil samples (Figure 3). The low TF values (TF < 1) indicates poor metal absorption and poor accumulation by the leaves of *M. oleifera*. The high TF values (TF > 1) in the case of Cu and Zn indicates that *M. oleifera* is a bioaccumulator for Cu and Zn and can be a hyperaccumulator for Zn depending on the site of collection. Bioaccumulation of Zn, Cu, Cr, and Pb was highest in LBG and reduced in the commercial (GSA) and high traffic density (RRA) areas. It can be deduced from the results that the accumulation and retention of metals especially Zn, Cu, Cr, and Pb by the leaves of *M. oleifera* are location dependent. The results also indicate that the leaf of *M. oleifera* is not a bioaccumulator for As, Co, Cr, Ni, Pb, and V irrespective of the site of collection. This is contrary to the established fact that the leaves of *M. oleifera* hyperaccumulate these metals [13-17]. However, the concentrations in the leaves can be high if samples are not collected from appropriate sites.

Pairwise correlation revealed that metal concentrations in *M. oleifera* leaves did not typically correlate linearly with the concentration of the metal in the soil (Table 2). This could be attributed to the fact that the ED-XRF analysis measured the total concentration of the metals in the soil which can be different from the bioavailable concentration of the metal in the soil depending on the soil characteristics such as pH and organic matter content [31]. Also, if a substantial amount of the metals is absorbed from the atmosphere through the leaves instead of the roots, the correlation between soil concentration and leaves concentration will not be linear. Lastly, if the metal preferentially bioaccumulates in other parts of the plant, it will lead to a non-linear correlation. From Table 2, only Co demonstrated a significant linear correlation between concentration in the soil as compared to the concentration found in the leaves ($r_s = 1.00$, $p < 0.001$). This strong positive correlation suggests that as the concentration of Co in the soil increases, the levels of Co in the leaves will be elevated accordingly and this correlation is statistically significant. Although none of the other metals exhibited a linear correlation between its concentration in soil versus the leaves, there was a strong positive correlation between Co concentrations in leaves or soil and As concentrations in the soil, which was statistically significant ($r_s = 1.00$, $p < 0.001$). Similarly, the levels of Pb in leaves were significantly and positively correlated with the concentration of Cu in the leaves of the *M. oleifera* plant ($r_s = 1.00$, $p < 0.001$).

The concentration of V in *M. oleifera* leaves was also found to be positively and significantly correlated with the concentration of Cr in the leaves of *M. oleifera* but not in the soil where *M. oleifera* grew ($r_s = 1.00$, $p < 0.001$). The negative correlation between V in leaves and Cr in soil as well as Cu in soil implies that as the concentration of Cr or Cu in the soil increases, the amount of V decreases in the *M. oleifera* leaves, although this relationship did not reach statistical significance. The results also indicate a non-significant and negative correlation between the concentration of Cu in the soil and the concentration of Zn in the leaves of *M. oleifera* ($r_s = -1.00$, $p > 0.05$). Thus, it is probable that a lower concentration of Cu in the soil enhances Zn uptake and accumulation in the leaves. This inverse correlation could be attributed to a competition for uptake sites in the roots of the plant. At low concentration of Cu in the soil, Zn, is selectively absorbed, hence the higher leaf concentration of Zn. A significant positive correlation was also observed between the concentration of Zn and Pb in the soil ($r_s = 1.00$, $p < 0.001$).

Conclusion

The analysis revealed varying concentrations of As, Co, Cr, Cu, Ni, Pb, V, and Zn in the leaves of moringa and the soil. Metal concentrations in the leaves are lower than the permissible limit for herbal medicines. The levels of metals in the soil were generally higher than in the leaves except for Zn and Cu at LBG. Hence, the TF values, a measure of the bioaccumulation capacity of the leaves, were lower than 1 except for Zn and Cu from the quasi-natural habitat, LBG. Thus, *M oleifera* leaves can be classified as excluder for As, Co, Cr, Ni, Pb, and V. On the other hand, the plant can be an accumulator for Cu and Zn depending on the site of collection. Pairwise correlation revealed no correlation between the concentration of metals in the soil and the leaves except for Co. However, strong and statistically significant positive and negative correlations were observed for some metals in the leaves and soil. These findings can be very informative to researchers and herbal medicine practitioners about human activities and its resultant negative effect on the quality of medicinal plants.

The unscientific and indiscriminate collection of medicinal plants can influence their quality. Hence, users of herbal medicines should be wary of potential of heavy metal poisoning. Even at low concentrations, their immediate availability to the public without any appropriate quality assessment could have a potential public health consequence due to bioaccumulation in the body over a long period of usage. This work throws light on the the potential effect of anthropogenic activities and their possible effect on the quality of herbal medicines using *M. oleifera* as a case study. Results from a single plant species it is not broad enough to be representative of the dangers associated with indiscriminate harvesting and use of herbal medicines. Future work should look at a larger collection of medicinal plants thorough soil analysis should be conducted to establish a stronger correlation between soil chemistry and quality of herbal medicines.

DECLARATIONS

Ethical considerations

Not applicable

Consent to publish

All authors agreed to the content of the final paper.

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Competing interests

No potential conflict of interest was reported by the authors.

Author contributions

ML, SFM, NNAO, POB planned the work, collected, and prepared the samples and evaluated the analytical results. AARM and POB performed the XRF measurements under the supervision of DB and they all assisted in the evaluation of the results. AAL carried out the statistical analysis. ML drafted while SFM and AAL technically edited the script. All authors read and contributed to the final manuscript.

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Availability of data

The dataset used and analyzed during the current study is available from the corresponding author upon a reasonable request.

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