Evaluation of heavy metal contamination and toxicological risk of selected *Moringa oleifera* products in Southwest Nigeria

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ABSTRACT

Background: Heavy metals are the main sources of pollution in the environment and its determination is important in enhancing production efficiency in plants.

Objectives: This study determined the concentrations of selected heavy metals, trace and essential elements present in thirteen (13) *Moringa oleifera* products marketed in southwest Nigeria.

Methods: Each *Moringa* sample was digested with a mixture of concentrated nitric acid and sulphuric acid (3:1 v/v) and subjected to standard heavy metal analysis using the atomic absorption spectrometric technique. Calibration curves of absorbance against concentration were plotted to determine unknown metal concentration.

Results: The elements analysed were present in varying concentrations. Cadmium concentrations was between 0.0011 to 0.0030 mg/kg, lead ranged between 0.0010 to 0.0110 mg/kg, while calcium ranged between 145.7 to 957.2mg/kg. Copper ranged between 0.35 to 38.9 mg/kg and that of sodium fell between 105.9 to 947.6 mg/kg. Comparison of these concentration values with the World Health Organisation, WHO/Nigerian National Agency For Food And Drug Administration And Control, NAFDAC standards revealed varying levels of compliance with permissible levels set by these agencies.

Conclusion: Based on present safety and permissible standards, the *Moringa* products selected in the present study were found to be safe for human consumption.

Keywords: *Moringa oleifera* products; Elemental pollution; Atomic absorption spectrometry, AAS; Heavy metals

Évaluation de la contamination par les métaux lourds et du risque toxicologique de certains produits de *Moringa oleifera* dans le sud-ouest du Nigeria

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RÉSUMÉ

Contexte : Les métaux lourds sont les principales sources de pollution de l'environnement et leur détermination est importante pour améliorer l'efficacité de la production dans les usines.

Objectifs : Cette étude a déterminé les concentrations de certains métaux lourds, d'oligo-éléments et éléments essentiels présents dans treize (13) produits de moringa oleifera commercialisés dans le sud-ouest du Nigeria.

Méthodes : Chaque échantillon de Moringa a été digéré avec un mélange d'acide nitrique concentré et d'acide sulfurique (3:1 v/v) et soumis à une analyse standard des métaux lourds en utilisant la technique de spectrométrie d'absorption atomique. Les courbes d'étalonnage de l'absorbance en fonction de la concentration ont été tracées pour déterminer la concentration en métal inconnu.

Résultats : les éléments analysés étaient présents à des concentrations variables. Les concentrations de cadmium se situaient entre 0,0011 et 0,0030 mg/kg, celle du plomb entre 0,0010 et 0,0110 mg/kg, tandis que celle du calcium entre 145,7 et 957,2 mg/kg. Le cuivre se situait entre 0,35 et 38,9 mg/kg et celle du sodium entre 105,9 et 947,6 mg/kg. La comparaison de ces valeurs de concentration avec les normes de l'organisation mondiale de la santé (OMS) et l'agence nationale nigériane pour l'administration et le contrôle des aliments et des médicaments (Nafdac) a révélé des niveaux variables de conformité avec les niveaux admissibles fixés par ces agences.

Conclusion: Lead and cadmium are known cumulative poisons and carcinogens hence, the need to evaluate the levels of their contaminations in teas and food plants. National health agencies should therefore take responsibility in this regard for the safety of consumers especially those who patronise alternative herbal remedies for solution to their ailments.

Conclusion : Le plomb et le cadmium sont des poisons cumulatifs et cancérigènes connus d'où la nécessité d'évaluer les niveaux de leurs contaminations dans les théiers et les plantes alimentaires. Les agences nationales de santé devraient donc assumer leur responsabilité à cet égard pour la sécurité des consommateurs, en particulier ceux qui ont recours à des remèdes alternatifs à base de plantes pour résoudre leurs problèmes de santé.

Mots-clés : Produits de *Moringa oleifera* ; Pollution élémentaire ; Spectrométrie d'absorption atomique, AAS ; Métaux lourds

INTRODUCTION

Moringa oleifera (Fam: *Moringaceae*), which is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh, and Afghanistan[1] presents all parts as edible and have long been consumed by humans. Almost all the different parts of this plant: the root, bark, gum, leaf, fruit (pods), flower, seed and seed oil have been used for the treatment of various ailments in the indigenous medicine of South Asia;[2] aside being a nutritional supplement. *Moringa* leaves and seed have been reported to be a rich source of amino acids, essential minerals, and fatty acids (omega-3 and omega-6 fatty acids), vitamins, fiber, condensed tannins, and polyphenols[3] and other bioactive compounds like flavonoids, glycosides, phenolics and carotenoids and *Moringa* oil.[4,5] These include essential vitamins, carotenes, phytochemicals, protein, ascorbic acid, calcium, zinc and potassium and act as a good source of natural antioxidants; [6] and thus enhance the shelf-life of fat containing foods. It possesses various medicinal properties including antimicrobial,[7,8] antipyretic, antitumor,[9-11] antiulcer, [12] antispasmodic, anti-inflammatory,[13,14] diuretic, cholesterol lowering,[15] antioxidant;[16,17] osteoprotective,[18] anti-hypertensive,[19] antidiabetic [18,20] and hepatoprotective activities.[21] Some studies have also reported on its antisickling[22] nd nutraceutical properties.[23,24]

Moringa-based food and medicinal products could be manufactured and exported to other parts of the world where it does not grow and can be commercialized.[25] A number of these *Moringa* products have found their way into the Nigerian markets as teas or other products aside indigenously produced ones; some of which may be contaminated with heavy metals. These heavy metals could enter the human body by ingestion or inhalation[26] via food, drinking water, soft drinks, hot drinks, other fluids, food, cigarettes, and air or through other sources of contamination.[27] Heavy metals constitute a health risk to consumers since they bio accumulate in the body when ingested and are stored faster than they are broken down or excreted hence, their safety is of major concern. Factors that influence the uptake and bioaccumulation of heavy metals in plants are climate, atmospheric depositions, heavy metal concentration in the soil, nature of soil on which the herbs are grown and the degree of maturity of the plant at the time of harvest.[28] Pharmaceutical industries could also discharge heavy metals alone or with other chemicals into surface and ground waters.[29] Consequently, heavy metals are a threat and poses deleterious effects to human health due to their toxicity and potential damage to human cells and the skeletal system causing osteoporosis, kidney failure and lung and blood cancer.[30,31] The World Health Organization (WHO) being informed by the International Agency for Research on Cancer (IARC), has classified cadmium and its compounds as a Class I carcinogen while lead is classified as a category 2B carcinogen.[32] Hence, the reason for analysing and monitoring the levels of cadmium, lead and other heavy metals in plants or plant products, food and food products including our samples in this study because essential nutrients may become harmful or toxic when they exceed certain levels.

The WHO recommended that qualitative and quantitative analysis of herbal drugs and medicinal plants should be carried out to control their pollution by heavy metals with regards to their maximum permissible limits.[33,34] Hence, the determination of harmful and toxic elements in different *M. oleifera* samples would give direct information on the safety of the products and ascertain if the elements are within the maximum permissible limit stated by the WHO.

Several studies have been carried out on the nutritional and elemental analysis of *M. oleifera*, however little has been done on the elemental contamination of the plant products particularly in Nigeria. Bauer and Tittel,[35] opined that the efficacy and safety of phytotherapy as an integral part of medical treatment should be obligatory and the need of pharmacological, toxicological, and clinical trials is obvious. This is because the efficacy and safety of herbal medicine finished products are directly dependent on the quality and chemistry of medicinal herb raw materials.[36] Also, the increase in the popular use of *M. oleifera* leaves and seeds and their products raise the question about their safety and quality, especially in relation to their heavy metal concentrations.[37] Daily exposure to heavy metals above the permissible limits has been associated with mental retardation, cancer, neuropathy, hepatic dysfunction, and renal failure[38] due to the accumulation of heavy metals in this plant. Consequently, this study seeks to confirm the presence of selected heavy metals in *Moringa* leaf and seed powder products circulating in the Nigerian market and compare the concentration of these elements with permissible limits.

MATERIALS AND METHODS

Collection of *Moringa* products

Thirteen (13) *Moringa* products (samples) were purchased randomly from retail Pharmacy outlets in Sagamu, Ogun State, Nigeria and in Festac Town, Lagos, Nigeria. Products sampled included *Moringa* tea, leaf powder and seed powder. Table 1 describes the characteristics of *Moringa* samples used for the Atomic Absorption Spectrometric (AAS) analysis.

Digestion process

Based on the method described by AOAC International,[39] 0.5 g of each *Moringa* sample was digested using 8 ml of a mixture of concentrated nitric acid and sulphuric acid (3:1, v/v). The mixture was evaporated at 100°C on a hot plate in a fume cupboard until a clear solution was observed. The mixture was left to cool to room temperature. The digested sample was then transferred into a 25 ml volumetric flask and made up to mark with distilled water. Each digested sample was filtered into a sample bottle for AAS analysis.

Elemental analysis

Validated methods for determining Cadmium, Lead, Calcium, Copper, and Sodium were used as found in the Official Methods of Analysis of AOAC International.[39] The Muffle furnace (SM 9080), Atomic Absorption Spectrophotometer (Techcomp AA 6000) located at the Analytical Department, Federal Institute of Industrial Research, Oshodi (FIIRO), Lagos, Nigeria was used for the elemental analysis in the flame configuration mode. The concentrations of selected elements present in the sample were determined by measuring the absorbed radiation of the chemical element of interest when reading the spectra produced when the sample was excited. The elements suspected to be present in each *Moringa* sample were set at specific wavelengths, the analyses commenced, and the concentrations of each element present in the *Moringa* sample in parts-per-million, ppm was obtained. The unknown concentration of the elements in the sample solution was determined from the calibration curves (Absorbance versus Concentration curves obtained using standards of known concentration.) and the calibration curve for copper is displayed as Figure 1 while Table 2 presents the calibration data on regression equations and correlation coefficients while the concentration obtained in each case is presented in Table 3 under results.

RESULTS

Table 1 gives a description of *Moringa* samples used for the AAS Analysis while Figure 1 displays a sample calibration curve for copper.

Table 1: Description of *Moringa s*amples used for AAS analysis

S/N	**Sample Code Name**	**Sample Product**	**Manufacturer's Name**	**Manufactured Date**	**Expiry Date**
1.	SBMT	*Moringa* Tea	Super Blend *Moringa* Tea	20/09/15	19/09/21
2.	SBMLP	*Moringa* Leaf Powder	Super Blend *Moringa* Leaf Powder	2015	2019
3.	SBMSP	*Moringa* Seed Powder	Super Blend *Moringa* Seed Powder	2017	2021
4.	TMSP	*Moringa* Seed Powder	Temef *Moringa* Seed Powder	2016	2021
5.	TMT	*Moringa* Tea	Temef *Moringa* Tea	2015	2020
6.	AJMT	*Moringa* Tea	Auntie Julie's *Moringa* Tea	2017	2021
7.	AJMLP	*Moringa* Leaf Powder	Auntie Julie's *Moringa*	15/08/16	14/08/21
8.	NIMT	*Moringa* Tea	Noble Icon *Moringa* Tea	2016	2020
9.	DFMLP	*Moringa* Leaf Powder	Divine Favour *Moringa* Leaf Powder	2016	2020
10.	DFMSP	*Moringa* Seed Powder	Divine Favour *Moringa* Seed Powder	2016	2020
11.	BMMT	*Moringa* Tea	Bright Morning *Moringa* Tea	2017	2021
12.	MLP	*Moringa* Leaf Powder	Locally Processed *Moringa*	2016	2020
13.	MSP	*Moringa* Seed Powder	Locally Processed *Moringa*	2016	2020

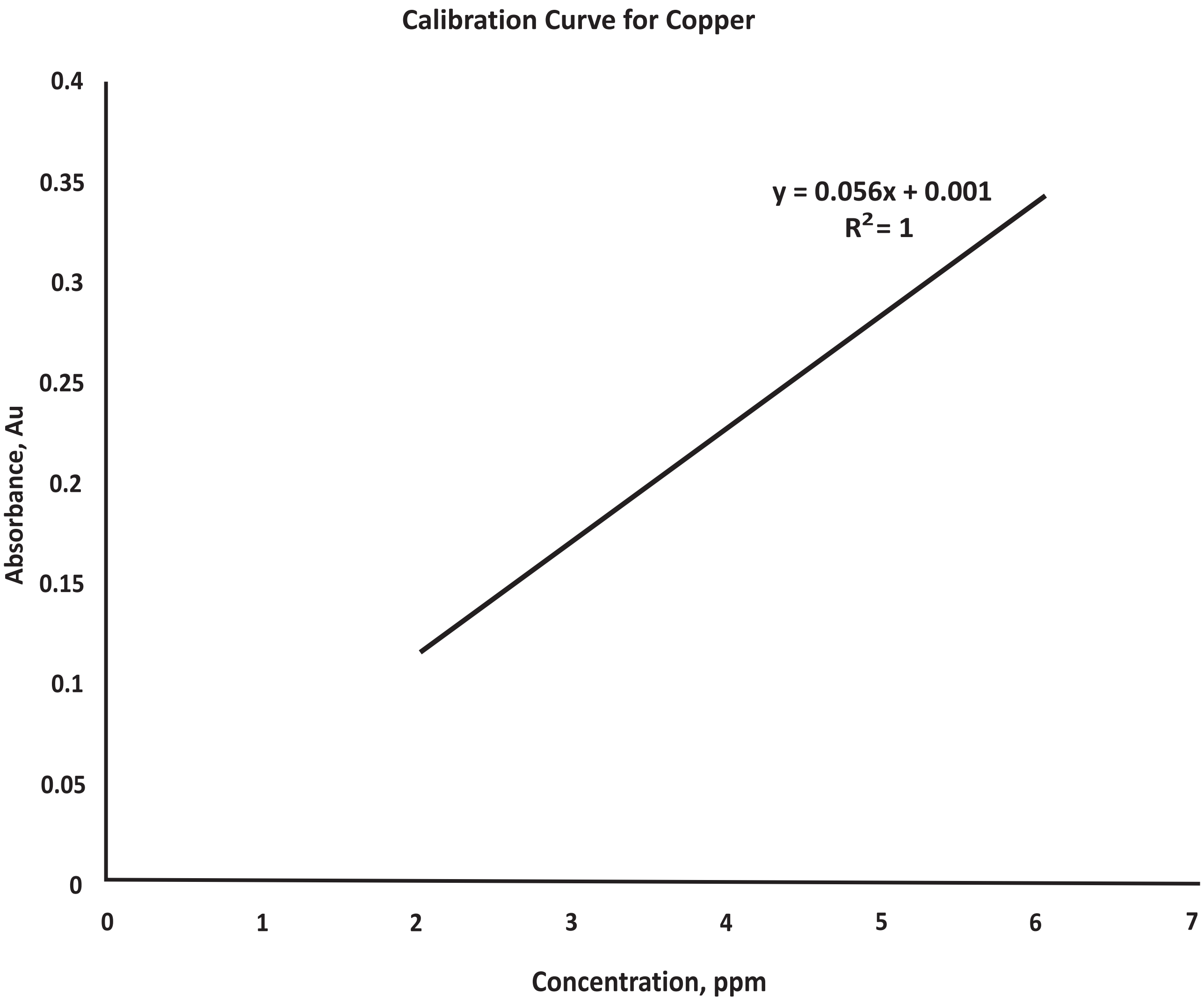

Figure 1: Calibration curve for copper

Table 2 enumerates the results of regression analysis from various calibration curves and the elemental concentrations of selected elements present in thirteen (13) *Moringa oleifera* products comprising tea leaves, seed powder and leaf powder marketed in southwest Nigeria are presented in Table 3. Heavy metals determined in the *Moringa* products include Cadmium, Lead and Copper while Calcium and Sodium are essential elements which were present in varying concentrations.

The concentration of cadmium ranged from 0.0011 to 0.0030 mg/kg with the lowest found in TMSP (0.0011mg/kg), while the highest was seen in AJMLP, (0.0030 mg/kg). SBMT, NIMT, and SBMSP had a cadmium concentration of 0.0014 mg/kg each. Other samples had cadmium concentrations of the order DFMLP (0.0015 mg/kg) < DFMSP=AJMT (0.0018 mg/kg) < TMT =LPMLP = LPMSP (0.0020mg/kg) <SBMP (0.0024 mg/kg) <BMMT (0.0029 mg/kg).

Table 2: Results of regression analysis from calibration curves

Heavy metal	Metal category	Regression equations	Correlation coefficients (*r*2)
Cd	Trace/Carcinogen	y=0.391x- 0.016	0.999
Pb	Trace/Carcinogen	y=0.011x-0.000	0.999
Cu	Trace/Beneficial	y=0.056x+0.001	1.000
Ca	Beneficial	y=0.006x+0.000	0.999
Na	Beneficial	y=0.184x+0.028	0.995

Table 3: Elemental concentrations of selected metals in mg/kg

	Elements				
	Cadmium	Lead	Calcium	Copper	Sodium
	WHO/NAFDAC Acceptable Limit (mg/kg) *				
	0.003	0.01	800	1.00	1.50 g
Sample Code Name	**Concentration (mg/kg)**				
SBMT	0.0014	0.0000	553.3000	0.3500	105.9000
SBMLP	0.0024	0.0050	320.6000	23.4000	607.3000
SBMSP	0.0014	0.0080	957.2000	13.0000	577.1000
TMSP	0.0011	0.0050	145.7000	11.2000	881.7000
TMT	0.0020	0.0040	570.2000	17.8000	174.4000
AJMT	0.0018	0.0110	647.3000	29.0000	777.0000
AJMLP	0.0030	0.0010	937.5000	18.0000	647.0000
NIMT	0.0014	0.0010	669.3000	29.1000	875.5000
DFMLP	0.0015	0.0020	771.4000	21.6000	947.6000
DFMSP	0.0018	0.0020	872.9000	36.7000	540.6000
BMMT	0.0029	0.0060	771.4000	21.6000	947.6000
MLP	0.0020	0.0030	823.0000	38.9000	200.1000
MSP	0.0020	0.0080	257.3000	28.0000	219.2000

Table 4: Comparison of Current study with Previous Results of Heavy Metal/Metal content for *Moringa oleifera* products

Heavy metal (mg/kg)	Tea leaves (range)	Dried seeds	Leaf capsules	Leaf Powder	Reference
Cd					
(PS)	0.068-0.182	0.069-0.152	0.068-0.598	0.069-0.112	Limmatvapirat *et al.* [37]
	0.0014-0029	0.0011-0.002	NS	0.0015-0.003	This study
Pb					
(PS)	1.175-3.285	0.012-1.705	1.426-24.032	1.591-2.751	Limmatvapirat *et al.* [37]
	0.0010-0.011	0.0020-0.008	NS	0.0010-0.005	This study
Cu					
(PS)	3.015-21.365	4.234-7.542	6.452-28.294	2.692-12.369	Limmatvapirat *et al.* [37]
	0.350-29.100	11.200-36.700	NS	18.000-38.900	This study
Ca					
(PS)	ND	ND	ND	ND	Limmatvapirat *et al.* [37]
	553.30-771.40	145.70-957.20	NS	320.60-937.50	This study
Na					
(PS)	ND	ND	ND	ND	Limmatvapirat *et al.* [37]
	105.90-947.60	219.2-881.70	NS	200.10-947.60	This study

PS=previous study; ND=not determined; NS=not sampled

DISCUSSION

The WHO gives the permissible levels of cadmium in tea as 0.003 mg/kg while the Chinese Cadmium Limit is 1.0 mg/kg[40] for tea hence, our samples would be suitable for human consumption since they all have values lower than the permissible levels for cadmium. Comparison of this result with limits given by the European Commission Regulation (ECR)[41] (Cd=0.20 mg/kg and Pb=0.1 mg/kg wet weight for leafy vegetables/herbs); the United States Food and Drug Agency (USFDA)[42] and United States Environmental Protection Agency (USEPA),[43] revealed all samples (n=13, 100%) had cadmium levels below the maximum permissible limit (MPL) of 50 µg/kg in consumable products. Table 2 presents the calibration data on regression equations and correlation coefficients.

High intake of cadmium may result in the irritation of the gastro-intestinal epithelium characterised by symptoms such as nausea, vomiting and abdominal pain. Additionally, the major toxic effects of long cadmium exposure include renal injury, obstructive pulmonary disease, osteoporosis, cardiovascular diseases;[44] as well as cancers of the lung, prostate, and kidneys.[45] The WHO being served by the International Agency for Research on Cancer (IARC), as source of information on cancer, has classified cadmium and its compounds as a Class I carcinogen;[32] hence, the reason for monitoring the levels of cadmium, lead, and other heavy metals in this study.

The concentration of Lead was least (0.0010 mg/kg) in the

AJMLP product, and the highest concentration of 0.0110 mg/kg was found in the AJMT product. These values were within the permissible levels of 0.01 mg/kg stipulated by the WHO for food products except for sample AJMT that had a value of 0.0110 mg/kg. All samples fell within permissible limits for lead by U.S. EPA,[43] for drinking water (0.015 mg/L) and 5.0 mg/kg for teas by Chinese regulatory authorities.[46] Our results agreed with that of Narin *et al*[47] who reported a range of 0.11-1.93 μg/kg for lead in *Moringa oleifera* in a previous study. Lead is a highly toxic metal that can cause deleterious and physiologically damaging effects including mental impairment, growth retardation, spasticity, skeletal abnormalities, and severe kidney damage even at low concentrations. Previous studies have linked growth disturbances in foetuses and children to lead while some plant parts toxicities have also been attributed to lead.[48,49] However, in this study, all the samples could be said to be free from lead contamination.

Calcium concentration in this study was lowest (145.7 mg/kg) in the TMSP and highest (957.2 mg/kg) in SBMSP. Previous studies conducted for calcium concentration in Nigeria reported 24.86 mg/g in medicinal plants and 286.07 ppm in *M. oleifera*;[50, 51] whilst that conducted in India in *M. oleifera* roots is 286.07 ppm.[52] An Ethiopian study on reported 2.6±0.13 to 5.64±0.25 mg/kg for *Moringa stenopetala* root.[53] High concentration of calcium is important because of Calcium also functions in some exocytosis, especially neurotransmitter release, and in muscle contraction and relaxation, normal heartbeat, stimulation of hormone secretion and activation of enzyme reactions in addition to its role in supporting growth and maintenance and reproduction of the human body.[54]

The concentration of Copper in the *Moringa* products ranged between 0.35 mg/kg in SBMT to 38.9 mg/kg in the LPMLP. Values ranging from 0.278± 0.021 mg/kg to 0.433±0.021 mg/kg have been reported for copper in *Moringa oleifera* leaves at some locations within Ibadan, south-west Nigeria in a previous study[55] and this agrees with our findings. The reference daily intake for copper for male and female is 0.9 mg/day.[56] Copper is an essential element for normal human growth but becomes a toxin at high levels. Hence, copper deprivation affects the function of copper enzymes, and this may lead to metabolic defects and pathologic changes including skeletal and vascular defects.[57] In view of the foregoing, the physiological importance of copper cannot be overemphasized.

The lowest value of Sodium concentration of 105.9mg/kg was found in the SBMT, and the highest concentration value of 947.6mg/kg was found in the DFMLP product. Sodium is a major electrolyte of blood and extracellular fluid and is required for maintenance of pH and osmotic balance in the body

Previous studies on the determination of the concentrations of heavy metals in raw leaves and leaf capsules of *Moringa oleifera* Lam. using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)[58] indicated Al, As, Cd, Cr, Hg, Mn, Ni, and Pb concentrations in eleven (100%) samples of raw leaves as well as those of Al, As, Cr, and Hg in all leaf capsules samples fell within permissible limits and normal ranges. They concluded that samples of leaf capsules showed higher concentrations of Cd, Cu, Fe, Mn, Ni, Pb, and Zn compared to those in samples of raw leaves.[58,37] However, results from our study gave lower levels of Cadmium, Lead and Copper than for tea leaves, dried seeds, leaf capsules and leaf powders used in Limmatvapirat *et al*[37] study. Table 4 gives comparison of our results with that of Limmatvapirat *et al*[37] summary. Although, heavy metal contaminations have earlier been reported in foodstuffs,[59,60] and other plant products,[61] only very few studies have been done on *Moringa* products.

Ensuring that authentic medicinal herb starting materials are free from impurities like heavy metal contamination is a major step in achieving quality end products (Govindaraghavan and Sucher, 2015). Also, they reported that the herbal medicines industry worldwide tests for heavy metals particularly Pb, Hg, Ass and Cd as contaminants. However, in this study, we tested for Pb, Cd, Cu, Ca, and Na. Though the latter trio are beneficial elements; it is still mandatory to set their limits since exceeding their safety thresholds could be deleterious to health.

CONCLUSION

This study has succeeded in determining the elemental concentrations of selected elements present in thirteen (13) *Moringa oleifera* products (seed, leaf powder and tea) marketed in southwest Nigeria. Results obtained in the present work compared well with previous reports. Based on present safety and permissible standards, the *Moringa* products selected in the present study were found to be safe for human consumption.

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