

Mineral composition and assessment of human ingestion risk of twelve accessions of *Moringa oleifera* Lam

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Abstract

Increasing consumption of moringa leaf powder in Ghana is boosted by popular claims that the plant contains a rich array of minerals, vitamins and other protective substances. However, the safe doses of this product among gender classes and vulnerable groups are unknown. This knowledge gap demands an analysis of the mineral composition of the product and estimation of the risk (hazard) potential associated with the ingestion of dried leaf samples of the moringa plant. Mineral composition (Na, Mg, P, K, Ca, Mn, Cr, Fe, Cu and Zn) of dried leaf samples of twelve accessions of *Moringa oleifera* Lam. was determined using the Atomic Absorption Spectrometry aided by a fast sequential Atomic Absorption Spectrometer. Results of the study indicate that the samples showed variation with respect to macro and micro minerals content and that dried leaf powder of moringa can serve as an excellent source of minerals. Additionally, an estimation of hazard exposure calculated as hazard index presented overall indices of 0.0866 and 0.0443 for males and females respectively. Even though the values (0.076-0.097; 0.038-0.051) obtained from the samples are within safe limits, it is essential that consumption of moringa leaf powder is done on sound advice from a nutritionist since crucial factors such as gender, age, health status and exposure time contribute to individual dose needs and influence mineral toxicity.

Keywords: moringa leaf, mineral composition, risk assessment, toxicity potential, hazard indices

INTRODUCTION

Mineral toxicity (hazard or risk) is a condition where the concentration of any one of the minerals is abnormally high in the body, and where there is an adverse effect on health [1]. It occurs when there is an accidental ingestion of too much of any mineral, as with drinking ocean water (sodium toxicity) or with over-exposure to industrial pollutants, household chemicals, or certain drugs. Mineral toxicity may also apply to toxicity that occurs as a result of certain diseases or injuries. For example, haemochromatosis leads to iron toxicity, Wilson's disease results in copper toxicity while hyperkalemia resulting from severe trauma leads to potassium toxicity [1-4].

The pathways for intake of minerals are ingestion, inhalation and dermal contact. Evidence has shown that the ingestion pathway of intake is the most significant contribution to human health risk assessment of particulates, industrial and mining hazards, waste products and food toxics [5-6].

Moringa oleifera Lamarck is well known as one of the world's most useful plants found within Moringaceae family [7-8]. Growing popularity of the crop is attributable to its high nutritive value which includes the acclaimed edible oil content [9]. Dried leaf powder of

moringa contains an array of minerals including sodium (Na), magnesium (Mg), phosphorus (P), potassium (K), calcium (Ca), manganese (Mn), chromium (Cr), iron (Fe), copper (Cu) and zinc (Zn). Although various parts of the plant including leaves, flowers, pods, seeds, bark and roots are consumed, the dried leaf powder is the most popular among consumers in Ghana and other parts of the world. It is consumed as a beverage but may also be added as condiment to porridges, soups and sauces [10].

The moringa plant is reported to be a 'super excellent' source of dietary minerals but there are variations among accessions for content of these minerals [11]. There is no information on local accessions regarding levels of mineral composition thereby presenting a challenge to users of moringa leaf powder in the country. Although consumption of vegetables and herbs is generally claimed to be safe to human health, the effective use of dried leaf powder of moringa as a supplementary source of dietary nutrients must follow recommended dose, taking into consideration gender, age, health status and vulnerability of the consumers. This study, therefore, investigated mineral composition as well as the corresponding toxicity potential in leaf powder of twelve Ghanaian accessions of *Moringa oleifera* Lam. (dried weight basis). The specific objectives were to

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- (i) assess variation in macro and micro mineral composition of twelve accessions of *Moringa oleifera* Lam. collected from five geographical regions of Ghana and one sourced from India;
- (ii) evaluate the toxicity (hazard) potential of these accessions among adult male and female users and;
- (iii) evaluate the contribution of metallic minerals to the male and female hazard indices.

MATERIALS AND METHODS

Chemicals used

All chemicals including metallic ions used as reference standards and ethanol (EtOH) were of analytical grade.

Plant materials

Twelve accessions of *Moringa oleifera* Lam. were collected from five geographical regions of Ghana and it included an accession sourced from India, as seeds, nursed and hardened, and grown on the Research Farm of the Biotechnology and Nuclear Agriculture Research Institute (BNARI) of the Ghana Atomic Energy Commission (GAEC), Kwabinya, in the Greater Accra Region of

Ghana. The BNARI research farm is located at latitude 05°40' N and longitude 0° 13' W, and elevated at 76 m above sea level within the Coastal Savannah agro-ecological zone. Kwabinya has an annual average temperature of 28°C and the study area receives an annual rainfall less than 1000 mm [12]. The soil at the site is the Nyigbenya-Haatso series, which is a typically well-drained savannah ochrosol (Ferric Acrisol) derived from quartzite schist.

Each accession was represented by a single line planting of ten (10) plants at a spacing distance of 3 × 3 m. Matured leaves were harvested from eight-month old plants growing in the field and within height of 2 m from the ground level. Some phytochemical properties of the twelve accessions of the moringa (water extract basis) are shown in Table 1 below.

Table 1. Phytochemical properties of twelve accessions of *Moringa oleifera* Lam. Accession

Accession	Total Phenolics Content (mg/g/GAE)±SE	Total Antioxidant Activity (% DPPH. Scavenging Activity)±SE
BNR2	67.83±0.61	25.15±0.08
BNR4	101.20±1.95	24.30±0.15
BNR5	96.53±1.64	18.93±0.15
BNR6	87.89±0.32	19.40±0.06
BNR8	83.78±2.08	19.93±0.03
BNR9	55.84±1.14	13.46±0.22
BNR10	96.13±1.21	21.57±0.23
BNR11	85.21±0.62	18.59±0.03
BNR12	109.70±1.82	39.36±0.02
BNR13	104.54±1.23	24.00±0.00
BNR14	112.15±0.61	30.29±0.04
BNR15	99.46±0.57	19.59±0.07

Note: Data was adapted from Owusu-Ansah, 2010.

Preparation of plant samples

A kilogram each of freshly harvested leaves of *Moringa oleifera* Lam. from all the twelve accessions (namely BNR2, BNR4, BNR5, BNR6, BNR8, BNR9, BNR10, BNR11, BNR12, BNR13, BNR14 and BNR15) was kept at ambient temperature (approximately 29±4°C) for five days to dry, followed by oven drying at a temperature of 45°C overnight to obtain crisp leaf samples. Dried samples were ground to fine powder using an electronic laboratory blender (38BL 40 Waring blender). The pulverised leaf samples were secured in ziploc bags, well labelled and stored at 4°C until needed.

Determination of micro and macro minerals in leaf samples of twelve accessions of *Moringa oleifera* Lam.

Metallic micro mineral (Mn, Cr, Fe, Cu and Zn ions) composition was determined by acid digestion of the dried and pulverized samples of moringa leaves using Milestone laboratory protocol [13]. 5 mL of NHCl as well as 1mL of H₂O₂ were added to 0.50 g moringa sample. The mixture was kept in a programmed microwave oven which operated in a 5-step manner at an average temperature of 400°C and a pressure of 100 psi for 20 minutes (with vent duration of additional 5 minutes) to achieve the desired digestion, after which the digestate was allowed to cool followed by transfer into a 25 mL test tube where digested sample volume was made up to the 20 mL mark with distilled water. The metallic micro mineral composition was measured using the fast sequential Atomic Absorption Spectrometer, AAS, technique (Varian AA240FS). Determination of the concentrations of the other mineral ions (Na⁺,

Mg²⁺, PO₄³⁻, K⁺ and Ca²⁺) involved wet preparation of 5.0 g of moringa sample in dilutions followed by leaching, filtration and centrifugation processes [14]. Atomic absorption spectrometry analyses of Ca²⁺, Mg²⁺, Na⁺ and K⁺ were carried out using the flame Sherwood aspirator at 440 nm while the vis-uv spectrophotometer (Shimadzu Corp., Tokyo, Japan) was employed for the determination of PO₄³⁻ at 780 nm. The final concentrations of all analysed minerals were calculated using the following formula:

$$\text{Concentration} = \frac{(\text{AAS reading} \times \text{Nominal volume})}{\text{Initial sample weight}}$$

The final phosphorus composition however was estimated from a standard calibration curve followed by the multiplication of the phosphate concentration by a common factor. Thus, P = [PO₄³⁻] × 0.3261. The analysis of each mineral for each of the twelve accessions was carried out in triplicate.

Estimation of hazard quotient and hazard index in leaf samples of twelve accessions of *Moringa oleifera* Lam.

Estimation of moringa leaf powder daily intake (MDI) was based on the assumptions that: (i) one tablespoonful of moringa leaf powder is approximately 0.004 kg, thus presenting prescribed thrice daily dosage of 0.012 kg and (ii) the main pathway of intake of moringa by consumers is ingestion. The minerals, whose compositions were determined previously, were subjected to MDI estimation, followed by hazard quotient (HQ) and hazard index (HI) calculations using the exposure formula [15-16].

That is, **Hazard Quotient (HQ)** = $\frac{MDI}{RDI}$

But, **MDI** = $\frac{C \times CR \times EF \times ED \times 10^{-3}}{BW \times AT}$

where, MDI = moringa leaf powder daily intake (0.012 kg by mineral content, mg/kg/day);

RDI= recommended daily intake (mg/kg/day);

C = concentration of individual mineral in 3 table spoonfuls (0.012 kg) of moringa leaf (mg/kg),

CR = contact rate, adopted from spinach exposure (4.068 mg/day);

EF = exposure frequency (365 days/year);

ED = exposure duration (1 year);

BW = body weight (70 kg) and;

AT = averaging time (70 years) and

10⁻³ = unit conversion factor.

Finally, Hazard Index (HI) = HQ_{Na} + HQ_{Mg} + HQ_P + HQ_K + HQ_{Ca} + HQ_{Mn} + HQ_{Fe} + HQ_{Cu} + HQ_{Zn} [14-15].

Statistical analysis

Using SPSS version 16.0.2, (SPSS Inc., USA) significant differences among concentrations of macro and micro minerals were determined by analysis of variance (ANOVA) with statistical significance defined as P ≤ 0.05 [17].

RESULTS

Variation in mineral composition among twelve accessions of *M. oleifera* Lam.

There were significant variations in macro mineral composition including sodium (Na), manganese (Mg), phosphorus (P), potassium (K) and calcium (Ca) in dried leaf samples of the twelve accessions of *Moringa oleifera* Lam (Table 2).

Table 2. Variation in macro and micro minerals composition of twelve Ghanaian accessions of *M. oleifera* Lam.

Accession	Macro Mineral Composition ± SE (mg/kg)				
	Na	Mg	P	K	Ca
BNR2	220.00 ±4.47 ^b	18.20 ±1.08 ^a	0.57±0.00 ^b	1860.00 ±4.02 ^d	51.30 ±1.69 ^b
BNR4	347.00 ±4.49 ^e	28.60 ±0.46 ^{cd}	0.53±0.00 ^a	2140.00 ±3.16 ^e	49.50 ±1.21 ^b
BNR5	287.00 ±5.01 ^c	17.80 ±0.71 ^a	0.57±0.00 ^b	1870.00 ± 5.53 ^d	74.50 ±0.71 ^c
BNR6	300.00 ±3.16 ^{cd}	23.60 ±0.68 ^b	0.52±0.00 ^a	2970.00 ±8.37 ^f	95.80 ±1.15 ^e
BNR8	240.00 ±0.00 ^b	30.80 ±0.86 ^{de}	0.59±0.01 ^c	1840.00 ±2.40 ^d	92.70 ±1.41 ^{de}
BNR9	200.00 ±0.00 ^{cd}	32.40 ±1.24 ^{ef}	0.58±0.00 ^b	1070.00 ±3.91 ^a	86.90 ±1.80 ^d
BNR10	387.00 ±4.81 ^f	24.10 ±0.24 ^b	0.60±0.00 ^d	2070.00 ±4.81 ^e	43.30 ±0.34 ^a
BNR11	430.00 ±0.00 ^g	26.50 ±0.99 ^c	0.65±0.00 ^e	2980.00 ±2.40 ^f	71.90 ±1.60 ^c
BNR12	42.00 ±1.32 ^a	34.30 ±0.63 ^f	0.57±0.00 ^b	987.00 ±1.75 ^a	90.60 ±1.21 ^{de}
BNR13	330.00 ±0.00 ^{de}	27.00 ±0.99 ^c	0.64±0.00 ^e	1610.00 ±3.40 ^b	72.40 ±1.50 ^c
BNR14	333.00 ±2.40 ^{de}	28.70 ±0.80 ^{cd}	0.69± 0.00 ^f	1920.00 ±0.00 ^d	77.20 ±1.29 ^c
BNR15	567.00 ±2.40 ^g	27.50 ±0.94 ^c	0.57±0.00 ^a	1710.00 ±7.46 ^c	74.60 ±1.38 ^c

Accession	Micro Mineral Composition ± SE (mg/kg)				
	Mn	Cr*	Fe	Cu	Zn
BNR2	19.76±0.43 ^f	< 0.006	121.00±0.93 ^d	6.41±0.29 ^b	3.61±0.15 ^{bc}
BNR4	24.91±0.49 ^g	-do-	105.00±1.79 ^{bc}	7.23±0.84 ^{bc}	3.36±0.20 ^b
BNR5	15.24±0.75 ^{cd}	-do-	104.00±1.18 ^{bc}	7.21±0.74 ^{bc}	3.54±0.37 ^{bc}
BNR6	17.95±0.87 ^e	-do-	103.00±0.88 ^{bc}	6.33±0.86 ^b	2.99±0.15 ^a
BNR8	17.95±0.57 ^e	-do-	131.00±2.16 ^e	1.79±0.40 ^a	2.92±0.42 ^a
BNR9	13.12±0.62 ^a	-do-	118.00±1.03 ^d	7.16±0.47 ^{bc}	3.69±0.30 ^{cd}
BNR10	16.49±0.80 ^d	-do-	103.00±1.58 ^{bc}	2.11±0.59 ^a	2.92±0.20 ^a
BNR11	15.05±0.43 ^{bc}	-do-	92.36±1.37 ^a	7.16±0.42 ^{bc}	4.05±0.25 ^{ef}
BNR12	15.00±0.56 ^{bc}	-do-	92.99±1.51 ^a	6.40±0.28 ^b	3.88±0.33 ^{de}
BNR13	15.93±0.29 ^{cd}	-do-	98.64±1.30 ^{ab}	8.53±0.25 ^d	4.85±0.15 ^g
BNR14	12.99±0.36 ^a	-do-	109.00±1.11 ^c	7.07±0.32 ^{bc}	4.24±0.26 ^f
BNR15	13.84±0.79 ^{ab}	-do-	100.00±1.310 ^b	8.05±0.35 ^{cd}	4.73±0.29 ^g

Note: Means with different letters in a column are significantly different (P≤ 0.05) according to the Tukey's test. SE = standard error of the means. * Traces of Cr could not be detected by the Instrumentation and method.

Content of P in all the accessions was comparatively low in relation to the other macro minerals. The P content was lowest for BNR6 (P = 0.52 mg/kg) and highest for BNR14 (P = 0.69 mg/kg). Again from Table 3, the levels Of the micro minerals namely manganese (Mn), chromium (Cr), copper (Cu), zinc (Zn) and iron (Fe) in dried leaf samples of the twelve accessions of *M. oleifera* Lam. were also significantly different. The observed concentration of Cr was low in all accessions of moringa evaluated. It was below 0.006 mg/kg and detection was not possible by AAS equipment.

Variation in hazard index of twelve accessions of *Moringa oleifera* Lam. amongst males and females

A comparative moringa leaf powder daily intake (MDI) of *Moringa oleifera* Lam. in relation to recommended daily intake (RDI) in estimating hazard index (HI) is as shown (Table 3). For leaf powder of the twelve accessions of moringa, phosphorus presented the least mean MDI of 0.000003 mg/kg/day while potassium recorded the highest of 0.006976 mg/kg/day. The results suggest that BNR10 had the least hazard indices for males (0.076) and females (0.038).

Table 3. Mean mineral content values per daily intake of moringa as compared to recommended daily intake

Acc.	Moringa Mineral Daily Intake (mg/kg/day) ^{10⁻³}										Hazard Index (unitless)	
	Na	Mg	P	K	Ca	Mn	Cr	Fe	Cu	Zn	Male	Female
BNR2	0.800	0.066	0.002	6.764	0.1865	0.072	—	0.440	0.023	0.013	0.096	0.049
BNR4	1.262	0.104	0.002	7.782	0.1800	0.091	—	0.382	0.026	0.012	0.097	0.051
BNR5	1.044	0.065	0.002	6.800	0.2709	0.055	—	0.378	0.026	0.013	0.085	0.043
BNR6	1.091	0.086	0.002	10.800	0.3484	0.065	—	0.375	0.023	0.011	0.088	0.045
BNR8	0.873	0.112	0.002	6.691	0.3371	0.065	—	0.476	0.007	0.011	0.090	0.044
BNR9	0.727	0.118	0.002	3.891	0.3160	0.048	—	0.429	0.026	0.013	0.087	0.044
BNR10	1.407	0.088	0.002	7.527	0.1575	0.060	—	0.375	0.008	0.011	0.076	0.038
BNR11	1.564	0.096	0.002	10.836	0.2614	0.055	—	0.336	0.026	0.015	0.081	0.042
BNR12	0.153	0.124	0.002	3.589	0.3294	0.055	—	0.338	0.023	0.014	0.077	0.040
BNR13	1.200	0.098	0.002	5.854	0.2633	0.058	—	0.359	0.031	0.018	0.085	0.045
BNR14	1.211	0.104	0.003	6.982	0.2807	0.047	—	0.396	0.026	0.015	0.084	0.043
BNR15	2.068	0.100	0.002	6.218	0.2713	0.050	—	0.364	0.029	0.017	0.083	0.043
Overall HI											0.087	0.044

Note: The hazard quotient calculation from which hazard indices of moringa were estimated made use of United States Department of Agriculture, USDA, RDI values for males and females [25]. Hazard index for Na was not estimated since its RDI value is not available.

On the contrarily, BNR4 showed the highest hazard indices for males (0.097) and females (0.051) which exceeded the estimated overall hazard indices of 0.087 and 0.044 for males and females respectively (Table 3).

DISCUSSION

Mineral composition is relevant because macro minerals, especially, are important for making edible parts (dry matter) of moringa palatable as food or feed [18]. Values obtained for the minerals Ca, Mg and P in this study were lower when compared to estimates documented in a review which made use of secondary data from at least nine reference sources [11]. But the lower P content for instance, is supported by report of 0.0031 mg/kg for moringa from Sudan [19]. On the contrarily, results obtained for other minerals (K, Mn Fe, Cu and Zn) are higher than those obtained in an earlier review [11]. The popular spinach which bears some similarity to moringa has the following composition on mg/kg basis: Ca (12.50), Cu (trace amount), iron (0.12), Mg (4.80), Mn (trace amount), P (1.50), K (40.00), Na (2.50) and Zn (trace amount) [2]. Differences between these values and those obtained in the current study may be explained by the differences in age of the leaf, the geographical location, methods of preparation and conservation, and methods of analyses considered by the primary data consulted [11]. Again, the secondary data used for the evaluation involved the inclusion of data on fresh leaves of moringa.

The nutrient composition revealed by the results indicates that, moringa leaf could be compared to other leafy vegetables [20-21]. However, the contrasts do not support the claim that moringa leaf is a superior nutrient source since phosphorus content of cassava leaf and amaranth leaf exceed that of moringa leaf [22-23].

The results show that the three least potentially hazardous accessions are BNR10 (0.076, 0.038), BNR12 (0.077, 0.040) and BNR11 (0.081, 0.042). These accessions were sourced from the Upper East Region of Ghana, suggesting a possible close genetic relationship.

In spite of the promising nutritive virtue of moringa [24], the mineral content of the accessions used in this study had concentrations below the RDI. The requirement of macro minerals in the body is in larger quantities where as the need of micro minerals is in trace amounts according to RDI specification. This demands that powder must be consumed under the guidance of a nutritionist.

The additive effects of the mineral ions which were calculated as hazard index (HI) from HQ values revealed 0.087 and 0.044 for males and females respectively. These values were less than unity which is an indication that, the mineral intake over a 70-year period is safe. Also, the male HI was about twice (i.e. 95.36%) that of the female which conforms to increased life expectancy risk of males [25]. However, the heavy metallic ions (Mn, Fe, Cu and Zinc) contributed over 94.0% to the index, which is undesirable. Thus, concentrations of heavy metals in leaf samples of moringa higher than the recommended dosage during an individual's life span may pose a non-carcinogenic health risk for humans. This is because minerals are sourced from other foodstuffs, mineral-fortified water and atmospheric air.

The roles of metal ions in ageing have predominated owing to their contributions to oxidative stress, neurological disorders and radiation poisoning [15]. It is even possible that, the potential risk may be aggravating for vulnerable groups including, children, nursing mothers and the aged. Unfortunately, the RDI for such categories is rarely available for conclusive evaluation. Also, Ghana lacks large scale national data such as household surveys, national statistics reports and pilot studies which would serve as the relevant biomarkers for characterising the risk from foodstuffs.

CONCLUSION

Dried leaf powder samples of the twelve accessions of *Moringa oleifera* Lam used in this study exhibited variable composition in the minerals sodium, magnesium, phosphorus, calcium, manganese, iron, copper and zinc. The hazard indices estimated from the mineral composition indicated no toxicity potential among males or females but the contribution of metallic minerals alone amounting to over

94.0% of hazard (toxicity) index suggests the need to strictly adhere to use of recommended dose.

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