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Estimation of Heavy Metals, Essential Trace Elements and Anti-Nutritional Factors in Leaves and Stems from *Moringa oleifera*

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Abstract: This study was conducted to estimate the concentrations of 8 trace elements including (Mn, Fe, Cu, Zn, Ni, Ar, Cr, and Pb) and 4 anti-nutritional factors including (saponins, oxalates, phytate, and cyanogenic glycosides) in leaves and stems from *Moringa oleifera*. The concentrations in samples analyzed were found to be in the range of 0.88-1.88 mg/kg for Mn, 11.95-25.25 mg/kg for Fe, 0.35-1.22 mg/kg for Cu, 6.92-17.96 mg/kg for Zn, 0.03-0.07 mg/kg for Ni, 0.955-1.45 mg/kg for Cr and 0.564-0.85 mg/kg for Pb. However, Arsenic was not detected in all the samples analyzed. As for the anti-nutritional factors, the concentrations in samples analyzed were found to be in the range of 111.35-123.42 mg/kg for saponins, 69.5-509.4 mg/kg for oxalates, and 0.38-0.156 mg/kg for phytate and 316.95-325.27 mg/kg for cyanogenic glycosides. The values of all these elements were found significantly below the recommended maximum tolerable guidelines level proposed by WHO/FAO except for lead (Pb), Pb was found slightly higher than the recommended limit as described. Our findings of this study reveal that most of the trace elements found in *M. oleifera* are below the recommended maximum tolerable limits; therefore it is safe for both human and animal consumption as well.

Keywords: *Moringa oleifera*, Heavy Metals, Trace Elements and Anti-nutritional Factors

1. Introduction

Heavy metals are considered to be the most hazardous form of pollutants when consumed in a considerable amount through contaminated foods and received much attention in recent years because of their non-biodegradability, elevated toxicity, persistence and bioaccumulation [1-2]. In the recent years, human exposure to heavy metals has been increased enormously which can be engendered by the continuous use of hazardous metals as starting materials in different industrial processes [3]. The main route of heavy metals intake by humans is the consumption of foodstuffs from contaminated areas through contaminated foods [4]. Heavy metals are classified as essential (if they have important role in human biochemical processes such as Iron (Fe), Copper (Cu), Zinc (Zn) and

Manganese (Mn), and as toxic (no fundamental role in human biochemical processes like Mercury (Hg), nickel (Ni), Cadmium (Cd), Lead (Pb), Tin (Sn), Chromium (Cr), or Arsenic (As)) [5]. Despite their role in human biochemical processes, most of these heavy metals are highly toxic when the concentration is exceeding the safe permissible limits and accumulate in important human organs like the lungs, kidney, heart, or liver; thereby invoking in serious health problems [6-7]. Plants and the ecosystem are at serious threat due to the exposure of heavy metals. An obvious example of the outbreak of heavy metals would be the wastewater from industrial processing plant due to their constitution of high concentrated heavy metals leading to pollution of soils, natural water resources and the entire environment [8-9]. Leaves and root of some vegetable species such as spinach, cabbage, watercress and drumstick uptake some of these heavy metals when they are cultivated with heavy metal contained water and soil [10]. The consumption of heavy metal contaminated vegetables thus possess risks to humans when consumed leading to the accumulation of the metals in the human systems.

Moringa oleifera, commonly known as horseradish tree or drumstick tree by locals of different parts of the world is a cruciferous plant belonging to the Moringaceae family. *M. oleifera*has resulted with higher quantities of proteins, fats, vitamins, minerals, phenolic acids and polyphenols, flavonoids, alkaloids and carotinoids [11]. *M. oleifera* is popular for its edible leaves and flowers and also for the potential to be used as food, medicine or cosmetic oil [12-13]. Several studies, both *in vivo* and *in vitro* have demonstrated the beneficial effects of the *M. oleifera* in humans [14]. Several studies have revealed the medicinal properties of *M. oleifera* leaves and also being used for medicinal purposes as well as nutraceuticals due to their high antioxidant constituents and other nutrients, which are commonly deficient in people living in under-developed countries [15]. Leaves have already been used for the cure of diseases such as malaria, typhoid fever and also against hypertension and diabetes [16-17]. The roots, bark, fruits, flower, seed and seed oil of *M. oleifera* have also reported of having a number of biological functions that includes protection against gastric ulcer, anti-diabetic and hypotensive effects [18]. Since *M. oleifera* is enriched with the essential nutrients, estimation of the concentration of toxic heavy metals in *M. olefera* is an ultimate necessity to facilitate the safe consumption.

2. Materials and Methods

2.1. M. oleifera Sample Processing

The whole plants of *M. oleifera* were collected from the local of Jashore, Bangladesh. Roots, stems and leaves were processed and stems and leaves were then separated for this study. Leaves and stems then went through cleaning and the samples were then rinsed manually after collection with normal tap water followed by distilled water to remove adhering dirt.

2.2. Determination of Heavy Metal and Essential Trace Elements Compositions

The samples were then first introduced to sun drying and then dried using air drying at room temperature. Samples were then chopped, sieved and stored airtight at room temperature for analysis of trace elements, heavy metals. Solution was prepared according to the study [19] with slight modification. Aqua Regia and distilled water was primarily used to prepare the solution. Dried powdered sample of *M. oleifera* leaves and stems were separately weighted and 1g of each sample was placed in a beaker with 10 cm^3 of aqua regia, stirred continuously. Then 5 cm^3 of distilled water was added to the mixture, stirred again. The resulting solution was filtered into a volumetric flask and made up to the mark using distilled water. The samples were analyzed for heavy metal using atomic absorption spectrophotometer.

2.3. Determination of Anti-nutritional Factors

For the estimation of anti-nutritional factors different standard methods were used. Oxalates and phytates were determined as described [20-21] respectively while saponins and cyanogenic glycosides were determined using the guidelines as described [22] (AOAC, 1999).

2.4. Statistical Analysis

Data obtained were analyzed using Microsoft Excel 2007 and graphpad prism version (8.1.0) software. The data were expressed in terms of descriptive statistics while the figures were presented with Mean values as (Mean ± SD) and a p-value less than 0.05 were considered as significant.

3. Result and Discussion

In the present work, a comparative estimation were carried out in order to assess the concentrations of trace elements (Mn, Fe, Cu, Zn, Ni, Ar, Cr and Pb) and anti-nutritional factors (saponins, oxalates, phytate and cyanogenic glycosides) in leaves and stems from *M. oleifera*. A total of 8 trace elements and 4 anti-nutritional factors were analyzed. The average heavy metal concentrations (mg/kg) are in the following order for *M. oleifera* leaves: Cr>Pb>Ni>As; *M. oleifera* stems: Cr>Pb>Ni>As (table 1). The average essential trace elements concentrations (mg/kg) are in the following order for *M. oleifera* leaves: Fe>Zn>Mn>Cu (figure 1); *M. oleifera* stems: Fe>Zn>Cu>Mn (figure 1). Likewise, for the anti-nutritional factors the average concentrations (mg/kg) are in the following order for *M. oleifera* leaves: cyanogenic glycosides>saponins>Oxalates>phytates; *M. oleifera*stems: Oxalates>cyanogenic glycosides>saponins>Phytates (table 2). The concentration of nickel in the leaves and stem of *M. oleifera*were found 0.06±0.007 mg/kg and 0.03±.005mg/kg respectively, concentration of chromium in the leaves and stem of *M. oleifera* were found 1.26±0.24 mg/kg and

1.008±0.09 mg/kg respectively, concentration of lead in the leaves and stem of *M. oleifera* were found 0.78±0.05 mg/kg and 0.53±0.06 mg/kg respectively.

Table 1. *The (mean ± standard deviation) concentration of heavy metals in the leaves and stems of M. oleifera.*

Samples	Heavy Metals analysis			
	Ni (mg/kg)	**As (mg/kg)**	**Cr (mg/kg)**	**Pb (mg/kg)**
*M. Oleifera*Leaves	0.06±0.007	ND	1.26±0.24	0.78±0.05
*M. Oleifera*Stems	0.03±0.005	ND	1.008±0.09	0.53±0.06

However, arsenic was not detected in any sample analyzed. All of the samples investigated were found below the recommended maximum tolerable guideline level designed by WHO/FAO except for lead (Pb) content in both leaves and stems. According WHO/FAO guidelines [23] the permissible limit for nickel, arsenic, chromium and lead is 0.2 mg/kg, 0.43 mg/kg, 2.30 mg/kg and 0.3 mg/kg respectively. Since the arsenic was not detected, our inference is that this area is free from arsenic pollution and safe for vegetable species such *M. oleifera* cultivation. However, the lead content in both leaves and stems were found slightly exceeding the limit as described in WHO/FAO [23].

Essential trace elements studied are presented in figure 1. The concentration of manganese in the leaves and stem of *M. oleifera* were found 1.76±0.1 mg/kg and 1.03±0.2 mg/kg respectively [figure 1(a)], concentration of iron in the leaves and stem of *M. oleifera* were found 25.103±0.21 mg/kg and 12.34±0.53 mg/kg respectively [figure 1(b)], concentration of copper in the leaves and stem of *M. oleifera* were found 0.39±0.04 mg/kg and 1.10±0.11 mg/kg respectively [figure 1(c)] and concentration of zinc in the leaves and stem of *M. oleifera* were found 17.28±0.60 mg/kg and 7.46±0.52 mg/kg respectively [figure 1(d)].

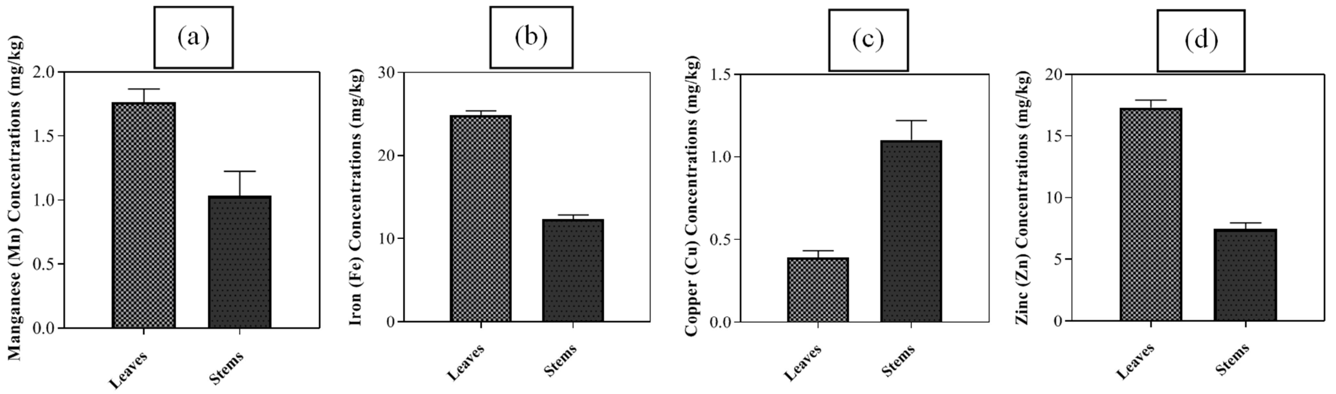

Figure 1. *Essential trace element concentrations analysis in the leaves and stems from from M. oleifera. (a) Manganese Concentrations in leaves and stems; (b) Iron Concentrations in leaves and stems; (c) Copper Concentrations in leaves and stems; (d) Zinc Concentrations in leaves and stems.*

All of the samples investigated were found below the recommended maximum tolerable guideline level designed by WHO/FAO. According WHO/FAO guidelines [23] the permissible limit for manganese, iron, copper and zinc is 6.61 mg/kg, 48 mg/kg, 40 mg/kg and 60 mg/kg respectively [23]. The significant amount of essential trace elements not exceeding the permissible in the leaves and stems of *M. oleifera* indicate the *M. oleifera* plant parts possess robust minerals contents which is responsible for many human biochemical process [24]. Our investigation is supported by some other studies such as [19] and Opaluwa *et al*. 2012 [25]. Their finings regarding the trace elements analysis were found nearly identical to our findings. Moreover, the concentration of the metals analyzed ranges was not varied significantly [25]. The concentrations of anti-nutritional factors in the leaves and stems of *M. oleifera* are presented in table 2. The concentration of saponins in the leaves and stem of *M. oleifera* were found 112.1±.65 mg/kg and 123.1±0.52 mg/kg respectively, concentration of oxalates in the leaves and stem of *M. oleifera* were found 70.19±0.60 mg/kg and 509.03±0.47 mg/kg respectively, concentration of phytate in the leaves and stem of *M. oleifera* were found 0.15±0.00 mg/kg and 0.46±0.09 mg/kg respectively and concentration of Cyanogenic Glycosides in the leaves and stem of *M. oleifera* were found 325.10±0.50 mg/kg and 317.15±0.30 mg/kg respectively.

Table 2. *The (mean ± standard deviation) concentrations of anti-nutritional factors in the leaves and stems of M. oleifera.*

Samples	Anti-nutritional factors analysis			
	Saponins (mg/kg)	**Oxalates (mg/kg)**	**Phytates (mg/kg)**	**Cyanogenic Glycosides (mg/kg)**
*M. Oleifera*Leaves	112.1±.65	70.19±.60	0.15±.00	325.10±.50
*M. Oleifera*Stems	123.1±.52	509.03±.47	0.46±.09	317.15±.30

The concentrations found in our investigation for anti-nutritional factors are also supported by other study [25]. The results described in the study [25] have found mostly similar with the concentrations assessed for saponins, oxalates, phytates and cyanogenic glycosides. Another study Makkar and Becker (1997) reported that tannins, saponins, and cyanogenic glycosides were found in the stem of *Moringa oleifera* but noticeably the concentrations were merely negligible [29]. Anti-nutritional factors such as tannins, phytates and Cyanogenic Glycosides have been found responsible for the complications associated with indigestion of food and flatulence, lowering nutrient bio-availability through antagonistic competition with nutrient [26] However, these anti-nutrients can easily be removed by soaking, boiling and frying during the processing of the particular vegetables for consumption [27-28]. The low content of these anti-nutrients in the leaves and stems of *M. oleifera* plant is a good forecast for the utilization of those plant parts as dietary components to facilitate nutrients consumption. In addition, these Anti-nutritional factors are important since they can also be used medically and pharmacologically. Tannic acid, as an astringent and has been known to be used in the treatment of bedsores and minor ulceration [30]. Other anti-nutrients saponins have been used in the manufacture of shampoos, insecticides and various drug preparations [31].

4. Conclusion

In summary, this study reveals that the concentration of heavy metals such as nickel, chromium, and arsenic in stems and leaves of *Moringa oleifera* were below the maximum tolerable level suggested by the Joint FAO/WHO expert except for the lead content in both leaves and stems which was found slightly exceeding the limit as recommended. However, if *Moringa oleifera* is cultivated applying efficient cultivation techniques, the burden of heavy metals can be reduced to a greater extent. Arsenic was not found in the samples indicating that the area is probably safe from arsenic pollution. The concentrations of anti-nutritional factors such saponins, oxalates, phytates and cyanogenic glycosides were found low. In addition, the anti-nutritional contents can easily be removed by some methods such as soaking, boiling and frying during the processing of *Moringa oleifera*. *Moringa oleifera* also significantly contained some essential trace elements such as manganese, iron, copper and zinc which are important for many physiological functions in the body. Therefore, different plant part of *M. oleifera* can be a promising source of essential micro-nutrients and would be efficacious while applying different parts of *M. oleifera* as food ingredients to Infants formula, food supplements and food product development.

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Conflicts of Interest

The authors declare that they have no competing interests.

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