Elemental Analysis in the Indigenous Medicinal Plant *Moringa oleifera* and their Association with Ameliorative Activity

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Summary: "Trace elements aid numerous biochemical processes in the human body. The main cause of many health problems in man are either deficiency or excess of minerals. Hence, screening of the definite bio-active" constituents of plant source and evaluation of elemental composition of the extensively used medicinal plants is greatly vital. An effort was made to find out the elemental constituents of diverse parts of Moringa *oleifera* including fruit, leaves, bark, stem and flower at trace levels from Laki Marwat, Bannu and D.I. Khan. Trace elements were quantified applying atomic absorption spectrophotometer (AAS). The amount of Selenium (Se), Zinc (Zn), Copper (Cu) and Iron (Fe) in stems, flowers, barks, leaves and fruits of Moringa *oleifera* from Laki Marwat is relatively greater than those of Bannu and D.I. Khan. The results reveal that the amounts of trace elements in various aerial parts of M. *oleifera* are highly dependent on harvesting sites."Various aerial parts of M. *oleifera* were observed to have ample amounts of Zn, Se, Fe and Cu.

Keywords: *M. oleifera*; Trace elements; Atomic absorption spectrophotometer; D.I. Khan; Lakki Marwat.

Introduction

Medicinal plants have always been valued for curative purposes in folk cultures [1]. Several researchers described the significance of trace elements (minerals), which increased the alertness of minerals in such plants [2]. Trace elements are those elements which, although present in minute quantities in the body tissues, but stand essential. It is evident from recent studies"that an adequate amount of micronutrients / minerals is required in the regulation of metabolic pathways and functions of enzymes. Thus, scarce supply of these micronutrient may upshot in the shortage of enzymes synthesis and may lead to various disease including metabolic dysfunction"[3, 4]."The information on levels of trace elements is essential for shaping the usefulness of these plants in treatment of diverse diseases via their pharmacological actions and to expand effective conventional awareness on the therapeutic prospective of medicinal plants"[5]. Moringa *oleifera* also called tree of life. It is specie of monogeneric family, the Moringaceae, which is native to south Asia where it grows on the foot hills of the Himalayas, but is widely grown in the tropics and sub-tropics [6]."In Pakistan, it is"locally known"as Sohanjna. It is widely cultivated and grown in all provinces of Pakistan. It is a multipurpose herbal plant and"consumed as a vegetable to improve nutrition. It is utilized as a medicinal plant to treat diseases and improve health. It is refined for live fencing and used as scavenge for livestock [7, 8]. It has been recognized as a plant with several health benefits possessing medicinal and nutritional significances [9].

Various parts of the subject plant contain significant amount of diverse elements and used as mineral supplement formulation in both mankind and animal diets. Phytochemical investigations show that *M. oleifera* is a rich source of vitamins and minerals [10, 11]. Aerial parts of the subject plant are edible and consumed by human since long"[12]."Recent studies show its therapeutic and medicinal properties of the subject plant which comprise the treatment of various health conditions, such as glandular, catarrh, hysteria, cholera, infections, bronchitis, asthma, abnormal blood pressure, fever, headaches, swelling, chest congestion, joints pain, psoriasis, anemia, semen deficiency, pimples, anxiety, lactation, blackheads, diabetes, pregnancy, blood impurities, scurvy, respiratory disorders, sore throat, intestinal worms sprain and tuberculosis"[13]. Various parts of Moringa *oleifera* contain a profile of important minerals. The leaves, pods, seeds, gums, bark and flowers of Moringa are used in more than 80 countries including Pakistan to relieve mineral and vitamin deficiencies' [14].

Verification of minerals content of plant materials across diverse agro-ecologies is required in the selection and formulation of plant-based mineral supplement in animal and human nutrition. *Moringa oleifera* could be used as available source of minerals. It

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is therefore of greater importance to investigate the essential trace elements of *Moringa oleifera*."The undertaken study was conducted to assess and compare trace elements concentration of different aerial parts"of *Moringa oleifera* from different regions of Khyber"Pakhtunkhwa and promote awareness related to mineral composition of medicinally important edible plants.

Experimental

Sample collection

Samples of Moringa *oleifera* were collected from diverse parts of KPK, Pakistan and placed in the herbarium. Plant samples were identified by Plant taxonomists, Department of plant Sciences, Quaid-e-Azam university, Islamabad and labeled with voucher specimen No. ISL-1367). Various aerial parts of Moringa *oleifera* were cleaned with distilled water three times to remove possible contaminants and dried in an oven at 65 °C for 24 hours or as long as required until constant weight was obtained. They were then milled in a stainless steel mill and finally screened into a mesh of 70 micron for powder formation.

Reagents

Analytical grade chemical and reagents obtained from BDH and sigma-Aldrich were used for sample preparation and experiments. Hydrochloric acid (HCL 25%), Nitric acid (HNO_3 65%) Perchloric acid ($HClO_4$ 65%) were used foe acid digestion process. Standard solution of (Se Zn, Cu and Fe 1000mg/l)"were used."For dilution and sample preparation processes pure water having resistivity of"18 MΏ cm"was used, which was acquired from Milli-Q water purification system. Acid washed glassware's were used in routine analyses.

Digestion

The process of digestion was carried out with slight modification in already optimized method [15]. "Approximately 1 g of each aerial part of *Moringa oleifera* was weighed into a 50 ml beaker and 7 ml of a mixture of concentrated nitric acid and perchloric acid were added in a ratio of 5:1. They were capped with clock glass and heated slowly in the oven to complete the digestion of the sample, carefully removing the acid vapors in the extraction hood. The beaker containing the digested sample was cooled, quantitatively transferred into a volumetric flask and produced up to 25 ml by adding deionized water.

Instrumentation

The samples were analyzed in triplicate for selenium using AAS equipped with hydride generation system, Analytic Jena (Vario VI) HS-55 Batch system and atomic Absorption Spectrophotometer (Contra 700, Analytic Jena) was used for zinc, iron and copper analysis of the samples.

Validation of Analytical Methods

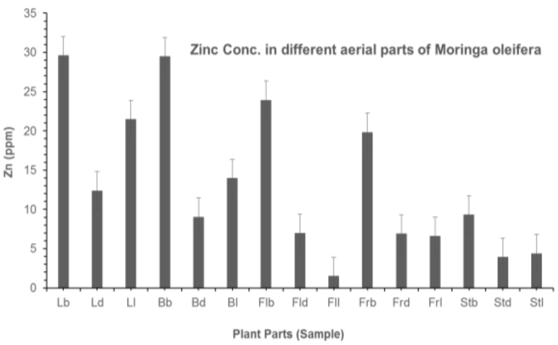
For the purposes of validation, the laboratorial comparison (ILC) was used for quality control of plant samples. In this regard, a composite sample was prepared from 5 different aerial parts of the plant. The composite sample was centrifuged well. The same composite sample was also analyzed by AAS with the hydride generation system. Both results were compared"and shown in Table-1.

"*Statistical analysis"*

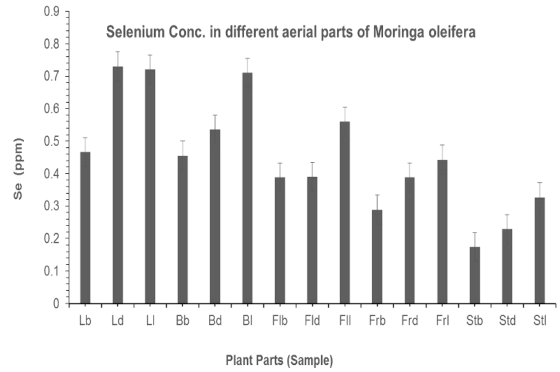
Triplicate analyses were performed. Mean and standard deviation were calculated. The data was processed through variance analysis (ANOVA) in connection to find out the significance of differences in the elemental composition of Moringa *oleifera* samples. For identification of homogenous subsets of means, the Duncan test was carried out at"$P \leq 0.05$.

Results and Discussion

The amount of mineral elements in Moringa leaves, barks, flowers, fruits, stems and variations by localities are presented in Table-1, and summarized in Fig. 1. The highest Se contents (0.730ppm) were found in the leaf of M. oleifera collected from D.I. Khan. This was considerably higher ($P \leq 0.05$) than other plant samples from Bannu and Lakki Marwat and the least Se content (0.176 ppm) was found in the stem samples from Bannu (Table-2 Fig. 1). Similarly highest Zn content (27.20 ppm) was found in the leaf samples of M. oleifera from Lakki Marwat (Table-2 Fig. 1), which was considerably higher ($P \leq 0.05$) than other plant samples from Bannu and Lakki Marwat. Least Zn content (3.00 ppm) was found in the stem samples from Bannu (Table-2 fig. 1). The highest Cu content (16.05 ppm) was found in the leaf samples of M. *oleifera* from Lakki Marwat. This was considerably higher ($P \leq 0.05$) than other plant samples from Bannu and Lakki Marwat and the least Cu content (3.18 ppm) was found in the stem samples from Bannu (Table-2 Fig. 1). The highest Fe content (459.073 ppm) was found in the leaf samples of M. oleifera from Lakki Marwat. This was considerably higher ($P \leq 0.05$) than other plant samples from Bannu and D.I.Khan and the least Fe content (120.22 ppm) was found in the stem samples from Bannu (Table-2 Fig. 1).



(A)



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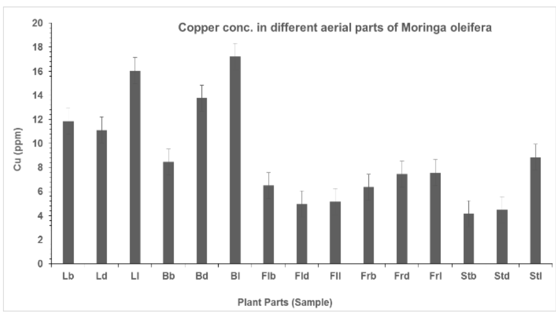
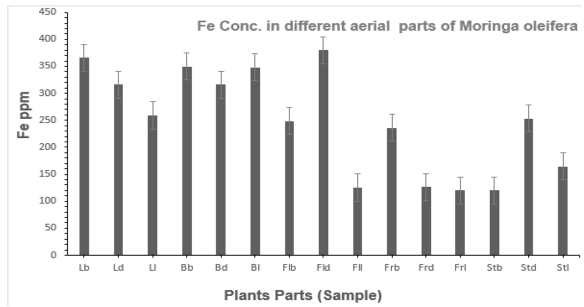


(C)



(D)

Fig. 1: Concentration of Selenium (A) ,Zinc(B),Copper(C) and Iron (D) in different aerial Parts of *Moringa oleifera*.

Lb: Leaf of M.O from Bannu
Ld: Leaf of M.O from D.I.khan
Ll: Leaf of M.O from L.Marwat
Bb: Bark of M.O from Bannu
Bd: Bark of M.O from D.I.khan
Bl: Bark of M.O from L.Marwat
Flb: Flower of M.O from Bannu
Fld: Flowere of M.O from D.I.khan
FlL: Flower of M.O from L.Marwat
Frb: Fruit of M.O from Bannu
Frd: Fuit of M.O from D.I.khan
Frl : Fruit of M.O from L.Marwat
Stb: Stem of M.O from Bannu
Std: Stem of M.O from D.I.khan
Stl: Stem of M.O from L.Marwat

Table-1: Inter-Lab comparison of the composite plant samples for Se, Zn, Cu and Fe.

Laboratories	Concentration (ppm)			
	Se	Zn	Cu	Fe
Chemical Metrology Division, NPSL (Contra 700, Analytic Jena	0.795±0.166	28.05 ±0.64	10.87±0.60	370±0.15
Chemical Metrology Division, NPSL (Contra 700, Analytic Jena)	0.695±0.155	25.08±0.96	9.50 ±0.56	369±0.16
Abdul Wali Khan University, KPK (AA Analyst 100, Perkin Elmer, with Zeeman Background Correction)	0.546±0.152	23.055±0.67	8.47±0.56	350±0.50

Values are the mean of three replicates ± SD

Table-1"showed that the concentration level of Se falls between mean values of 0.79 plus minus one standard deviation of 0.166. Similarly, the values of others elements are shown in Table-2, which indicate descriptive statistics of samples of different concentrations at different levels of plant-parts and locations. The sample has five different parts, such as leaf, bark, fruit, flowers, and stems each at three locations (Bannu, Lakki Marwat and D.I. Khan)."The mean and standard deviations (SD) of plant parts at Bannu, Lakki, and D.I. Khan were shown in third and fourth column of the Table-2. Leaf has highest mean value with a standard deviation of 0.002, however, stem has minimum mean value of 0.179 with a standard deviation of 0.002, at District Bannu for the selenium concentration. For district Lakki Marwat, flower has larger mean value with a zero SD; however, for D.I. Khan, the leaf has the largest mean value with no variations."The amount of Selenium in all aerial parts of Moringa *oleifera* from diverse regions was minor than the limits recommended in MPL (Maximum Permissible Level). While, samples of stem, fruit and flower possess very low conc. of selenium. Our results regarding Se content in Moringa leaves are consistent with the results of scientists [16, 17] but differ from [18]. In literature [19], most vegetables and fruits cultivated in non seleniferous soils contain selenium 0.01 µg g -1."The low concentration of Se in vegetables and fruits is considered safe level in many places [20].

Table-2: Descriptive Statistical Analysis among dependent Variables. Mineral Concentration (ppm) in different aerial parts of *Moringa oliefera*

Region	Sample	Selenium (Se)		Zinc (Zn)		Copper (Cu)		Iron (Fe)	
		Mean	StDev	Mean	StDev	Mean	StDev	Mean	StDev
Bannu	Leaf	0.465	0.00	20.70	0.10	11.06	0.05	365.77	0.02
	Bark	0.455	0.00	16.57	0.05	8.47	0.00	260.51	0.01
	Flower	0.390	0.00	6.37	0.02	7.19	0.00	208.57	0.02
	Fruit	0.290	0.00	4.57	0.05	4.37	0.00	175.77	0.01
	Stem	0.176	0.00	3.10	0.05	3.18	0.01	120.23	0.01
Total		0.407	0.18	10.91	6.69	6.85	2.93	226.17	86.28
L. Marwat	Leaf	0.457	0.00	27.20	0.01	16.05	0.00	459.07	0.064
	Bark	0.719	0.00	19.53	0.05	10.24	0.02	281.07	0.06
	Flower	0.560	0.00	8.75	0.01	8.30	0.00	225.23	0.02
	Fruit	0.443	0.00	7.35	0.00	4.57	0.00	199.06	0.05
	Stem	0.329	0.00	4.57	0.05	4.47	0.02	145.15	0.00
Total		0.436	0.07	3.00	0.05	8.72	4.42	261.91	111.64
D.I. Khan	Leaf	0.730	0.00	24.60	0.20	11.85	.005	376.44	0.04
	Bark	0.537	0.00	16.56	0.05	9.78	.010	276.43	0.02
	Flower	0.410	0.02	7.56	0.05	8.07	0.06	229.69	0.02
	Fruit	0.390	0.00	5.36	0.05	4.45	0.01	186.56	0.01
	Stem	0.231	0.00	4.00	0.00	4.11	0.01	133.16	0.01
Total		0.460	0.17	11.62	8.10	7.65	3.11	240.46	85.75

The transport of selenium from soil and water to humans is due to livestock and agricultural harvests. In mankind, the transfer may diverge from 10-5000 μg/day in areas with low selenium deficient soil. According to the recommendations of US Food and Nutrition Board in 1980, there should be 60-75 mg/day intake of selenium"[21]. Selenium conc. in aerial parts of Moringa *oleifera* obtained from diverse regions of KPK is relatively lower than the recommended limits by MPL/FAO/WHO (1984) and reflect tolerable"[22]."Selenium is a vital element in both human and animal nutrition. Moreover, the anti-tumorigenic impacts of Selenium complexes have been explained in a various animal models and described that supplemental dose of Se may reduce the risk of cancer in human [23].

The highest Zinc concentration (27.20 ppm) was observed in the leaf of Moringa *oleifera* from Lakki Marwat Table-(2 figure 1). This value was considerably higher ($P \leq 0.01$) than other aerial parts of M. *oleifera.* The least Zinc concentration (3ppm) was found in the stem of Moringa oleifera from Bannu region. This was drastically lower ($P \leq 0.01$) than in leaf. The Zn concentration in the sample of Bark from Lakki Marwat was found to be (19.53 ppm) and was usually higher ($P \leq 0.01$) as compared to the Zinc concentration from other regions. The Zn concentration in the flower of M. *oleifera* from Lakki Marwat was considerably ($P \leq 0.01$) higher than the flower samples from other regions i.e. Bannu and D.I. Khan. Similarly high Zinc concentration (7.35ppm) was observed in fruit sample from Lakki Marwat. It was observed that aerial parts of Moringa *oleifera* samples collected from Lakki Marwat possess higher conc. of Zinc as compared to other two regions such as D.I. Khan and Bannu (Table-2 and fig. 1). Various aerial parts of Moringa *oleifera* possess amount of Zn in the following order; leaves > bark > seed > flower > fruit > stem. The results are in close agreement with the findings described in literature"[7, 24, 25].

"However, "our results regarding zinc concentration in different parts of Moringa illustrate inconsistent variation with the results obtained in other studies"[26]. It is evident from the results that Zn conc. in all samples of Moringa *oleifera* is within the permissible limits (27.4 ppm) as described by FAO/WHO"[27]. Though, for medicinal plants the limits of zinc concentration by WHO is not yet to be documented [28]. The range of Zn in agriculture foodstuffs should be between 15-200 ppm."Zinc is one of the major indispensable micronutrients in the human corpse and mostly found in the cytosol as an intracellular ion. Zinc plays a vital role in wounds healing and functions as an antioxidant. Moringa *oleifera* extract showed great wound healing action against excision, restored incision and deadspace wounds. This effect could be due to Moringa *oleifera* high zinc content"[29]. The concentration of Copper (16.05ppm) in the leaf samples of Moringa *oleifera* from Lakki Marwat was found to be significantly higher ($P \leq 0.01$) than other leaf samples from Bannu and D.I. Khan.

The samples of leaves of Moringa *oleifera* from D.I. Khan and Lakki Marwat were generally higher in their Cu content. The content of Cu in the samples of Moringa *oleifera* barks from Bannu and D.I. Khan was found to be (8.47 ppm) and (9.78 ppm) respectively. Whereas, the Cu concentration in the samples of barks native to Lakki Marwat was found to be (10.22 ppm) and was generally higher as compared to other regions. The samples of fruit of Moringa *oleifera* from Bannu, D.I. Khan and Lakki Marwat were found to be (4.37 ppm), (4.44 ppm and 4.57 ppm) .The content of Cu in the samples of Stem from Bannu and D.I. Khan was found to be (93.17 ppm and 4.10 ppm) respectively. Whereas, the Cu concentration in the samples of Stem native to Lakki Marwat was found to be (4.45 ppm) and was generally higher as compared to other regions.

The aerial parts of Moringa oleifera from Lakki Marwat contained higher concentration of Cu as compared to other two regions i.e. Bannu and D.I. Khan"(Table-2 and Fig. 1). Various aerial parts of Moringa *oleifera* from diverse regions of KPK contain high amount of copper. The sequence of Cu concentration found as; Leaves > bark > flower > fruit > stem. The samples of flower, fruit and stem have not showed any significant concentration of copper. Similarly in contrast to our study some researchers [7] described a reasonably low conc. of Cu in Moringa leaves and pods in diverse areas of Pnnjab, Pakistan. In some studies [31-33] a tendency of squat Cu contents is reported in Nigerian plant dietetic intakes. The undertaken study is consistent with the findings of [33-34]. Similar studies [34-35] have been carried out on Spinach, C"nidoscolus species and green leafy vegetable cultivated on soil fortified with different chemical fertilizer.

It is noted from the present study that all the samples analyzed for copper concentrations has exceeded the established limit i.e. 3 ppm according to JECFA, FAO and WHO Expert Committee on Food Additives [28]. However, for medicinal plants the WHO [29] limits for copper has not yet been established. Although in medicinal plants, permissible limits for copper set by China and Singapore are in the range 20 ppm -150 ppm respectively. Some studies [14] reveal that the sort of copper conc. in 50 medicinally significant leafy plants cultivated in India is 17.6-57.3 ppm."Copper is the 3rd most plentiful element in the body. It is concerned with haemoglobin formation and acts as a significant catalyst for iron absorption. Copper deficiency may be a threat aspect for cardiovascular diseases, osteoporosis, and anaemia [4].

The concentration of Iron (Fe) in the M. *oleifera* leaves from D.I. Khan and Bannu was found to be (376.44 ppm) and (365.77 ppm) respectively. While, the Fe conc. in the sample leaves inhabitant to Lakki Marwat was observed to be (459 ppm). The samples of Moringa *oleifera* leaves from D.I. Khan and Lakki Marwat possess quite comparable higher conc. of Fe. The contents of Fe in the barks from Bannu and D.I. Khan were observed to be (260.50 ppm) and (276.40 ppm) respectively."Wheile, Fe conc. in the samples of barks inhabitant to Lakki"Marwat was 281 ppm and is usually elevated as compared to other regions. The level of Fe in the M. oleifera flower from Bannu, D.I. Khan and Lakki Marwat is 208.55 ppm, 229.67 ppm and 225.2 ppm respectively. The Fe contents in Moringa oleifera fruit collected from Bannu, D.I. Khan and Lakki Marwat were estimated 175.77 ppm, 186.55 ppm and 199.0 ppm respectively. The contents of Fe in Moringa *oleifera* stem from Bannu and D.I. Khan is 120.22 ppm and 135.15 ppm respectively. While, the Fe conc. in Moringa *oleifera* stem inhabitant to Lakki Marwat is 145.15 ppm and was usually greater as compared to other regions. The aerial parts of Moringa *oleifera* from Lakki Marwat contain higher concentration of Fe as compared to other two regions such as Bannu and D.I. Khan"(Table-2 & Fig. 1).

Different aerial parts of M. oleifera from various regions of KPK revealed higher concentration of Fe in a series as; Leaves > bark > flower > fruit > stem. The samples of flower, fruit and stem showed low concentration of Iron. It is noted that all individual concentrations and average concentrations exceed the limit established for iron according to JECFA - Joint FAO / WHO Expert Committee on Food Additives, which is 15 ppm for leafy vegetables.

Iron concentration was highest in the leaves (459 ppm) and was lowest in the stem (120.22ppm). Leaves of Moringa *oleifera* are the pottential source of iron most consumed, therefore act as a potential source of Fe in human diet. Iron plays a vital role in the production of haemoglobin and myglobin which are transporter of oxygen in the blood vessels. Iron deficiency may result in anemia. In comparison to the undertaken studies some researchers [36, 37] have studied similar nutritional potential of M. *oleifera.* The level observed in this study is very much comparable to the levels in the present study. The high concentration of iron in the leaf of Moringa *oleifera* is a good sign because it is needed by both plants and human. On average, Moringa leaves contained a higher concentration of mineral nutrients than locally sourced vegetables. Moringa leaves also contained substantial amounts of flavonols, justifying the use of Moringa as a natural antioxidant.

In the current study, plant parts were used as an independent variable, which has five different levels, at three different locations such as Bannu, Lakki and D.I. Khan. In this research, one of the aims was to test the interaction effect between plant parts and locations and also to check the contribution of indepdent variable. Plant parts and locations explain variations in dependent variables such as Se, Fe, Cu and Zn. So, for such kind of analysis, Analysis of Variance was the appropriate statistical technique rather than multiple t-tests. Multiple t-test in such cases can increase type-I error, thus rejecting true null hypothesis. Furthermore, the application of multiple t-test fails to check for the interaction effect.

The influence of main effect such as plant parts and locations on Se and other dependent variables such as Zinc, Copper and Iron can only be tested through ANOVA. The results of the ANOVA, Duncan's Multiple Comparison Test (DMRT) were shown in the below Table 4. ANOVA results showed overall model fitting and statistical significance of the individual variables however, it does not convey any information pair-wise comparison. So, to compare different groups means and to examine the groups which show statistical significance, Duncan;s multiple comparison test was used.

Table-3 shows comparison of the trace elements in the aerial parts of subject plant, where statistical analysis confirms that the mean difference is significant at the 0.05 level. Table 4 reveals results of ANOVA, which consists of six columns and six rows. Column-1 shows source of variations, coulm-2 and 3 sums of squares and degrees of freedom. However, column 4, 5 and 6 representing mean square, F-ratios and p-values. In Table 4 locations, plant parts and interaction effect were found statistically significant both at 0.05 and 0.01 level of significance with F-Values 249.976, 5176.21 and 1786.889 respectively. The results suggesting overall model fitting.

The quantity of edible parts of Moringa to be eaten according to the literature should be more than 100 g to achieve the quantity needed of the minerals per day [38]. The superiority of Moringa *oleifera* over other vegetables lies in the nutritional value of its leaves, pods and seeds."According to some researchers [39] 100 g of Moringa *oleifera* leaves possess 10 times greater amount of vitamin A as compared to carrots, 17 times greater calcium contents as compared to milk, 15 times higher potassium contents as compared to bananas, 25 times greater iron contents as compared to spinach, 9 times greater protein contents as compared to yogurt and much more vitamin C than in oranges. Hence, Moringa *oleifera* is a better sink of minerals and vitamins and can be measured as a high-quality substitute to be applied to improve micronutrients deficiencies. The bark and stem of *Moringa oleifera* contain mineral elements that are of high nutritional value. But the leaves are richer in mineral elements than the bark, fruit, flower and stem. Hence, elemental composition may not be appropriate to assess absolutely the nutritional values of the non edible bark and stem as source of minerals for human. Further research is required to find out the accessibility of nutrients and impacts of processing on chemical and nutritive values of the plant."It is also related to estimate the contents of anti-nutrient factors that are abundantly present in the plant.

Conclusion

All the aerial parts of plant analyzed in this study were found to contain the selected essential elements but in varying concentrations. The topographical distribution also exhibited great variation in light metal concentrations."There was a significant variation in micro elements level in Moringa leaf, bark, fruit, flower and stem from different regions in Bannu, D.I. Khan and Lakki Marwat. Among different elements the highest light metal concentration was reported for iron followed by Zinc and Copper, while the lowest was reported for Se. "The geographical distribution might be attributed to the variable uptake of minerals by the plant materials. Finally some organs of Moringa *oleifera* are good source of important minerals and this plant might be explored as a reasonable supplement and ready source of dietary minerals in animal and human food.

Table-3: Comparative trace elements concentration in different aerial parts of *Moringa oleifera.*

Plant Parts(I)	Plant Parts(J)	Mean Differences(I-J)	Std Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
Leaf	Bark	0.002	0.003	1.00	0.006	0.009
	Flower	0.097*	0.003	0.00	0.089	0.105
	Fruit	0.176*	0.003	0.00	0.168	0.184
	Stem	0.305*	0.003	0.00	0.298	0.313
Bark	Leaf	0.002*	0.003	1.00	0.009	0.006
	Flower	0.095*	0.003	0.00	0.008	0.103
	Fruit	0.174*	0.003	0.00	0.167	0.182
	Stem	0.304*	0.003	0.00	0.296	0.311
Flower	Leaf	0.097*	0.003	0.00	0.105	0.089
	Bark	0.095*	0.003	0.00	0.103	0.088
	Fruit	0.079*	0.003	0.00	0.071	0.087
	Stem	0.208*	0.003	0.00	0.201	0.216
Fruit	Leaf	0.176*	0.003	0.00	0.184	0.168
	Bark	0.174*	0.003	0.00	0.182	0.167
	Flower	0.079*	0.003	0.00	0.087	0.071
	Stem	0.129*	0.003	0.00	0.122	0.137
Stem	Leaf	0.305*	0.003	0.00	0.313	0.298
	Bark	0.304*	0.003	0.00	0.311	0.296
	Flower	0.208*	0.003	0.00	0.216	0.201
	Stem	0.129*	0.003	0.00	0.137	0.122

Based on estimated marginal means*, The mean difference is significant at the 0.05 level, Adjustment for multiple comparisons: Bonferroni [b].

Table-4: Variation of trace elements within the region, plant parts and and region versus plants.

Sources	Selenium (Se)					Zinc (Zn)				
	S.S	d.f	M.S	F	PV	S.S	d.f	M.S	F	PV
Intercept	8.50	1	8.50	294772.41	0.00	4863.87	1	4863.87	940181.55	0.00
Region	0.02	2	0.01	000349.98	0.00	0071.99	2	0035.99	006957.94	0.00
Parts	0.60	4	0.15	005176.21	0.00	1301.01	4	0325.25	062871.09	0.00
Region * Parts	0.41	8	0.05	001786.89	0.00	0750.53	8	0093.82	018134.50	0.00
	Copper (Cu)					Iron (Fe)				
Intercept	27.79	1	270.79	4638774.98	0.00	2653930.37	1	2653930.37	2374291583.05	0.00
Region	26.43	2	13.22	0022704.16	0.00	009712.513	2	0004856.26	0004344563.58	0.00
Parts	50.71	4	125.93	0216287.45	0.00	0372562.39	4	0093140.59	0083326577.95	0.00
Region * Parts	26.49	8	3.311	0005686.89	0.00	009122.92	8	0001140.37	0001020207.41	0.00

S.S = Sum of Squares, d.f = Degree of Freedom, M.S = Mean Square, F = F. Value, PV = P. Value

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Conflict of Interest Statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

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