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Assessment of seasonal variation in the mineral elemental composition of *Moringa oleifera* and *Murraya koenigii* leaves

Aju B Y[1], Rajalakshmi R[1], Mini S[2]
[1] Department of Botany, University of Kerala, Thiruvananthapuram, Kerala, India
[2] Department of Biochemistry, University of Kerala, Thiruvananthapuram, Kerala, India

Abstract
Moringa oleifera and *Murraya koenigii* are two medicinal plants used by the south Indians in their day today life. *Moringa oleifera* leaves are used as vegetable and *Murraya koenigii* leaves as spicy leaves to garnish dishes. There is a traditional belief that *Moringa oleifera* leaves are not recommended during the month of August (Karkkidaka). But there are no scientific data are available behind this belief. The present study is to explore the scientific reason behind the traditional belief. The objective of the present study is to determine the nutritional quality of leaves of *Moringa oleifera* and *Murraya koenigii* for emphasizing mineral element concentrations in different seasons. Soil analysis and weather data analysis was carried out to assess the mineral elemental variation in the leaves. The present study revealed that most of the mineral elements including heavy metals were found to be accumulated in the leaves of *Moringa oleifera* during the month of August. Soil and weather data substantiate the mineral elemental variation in these two plants. Most of the minerals were accumulated in the leaves of *Moringa oleifera* in the post monsoon season (August) compared to *Murraya koenigii*. So it is essentially required to check for contaminant load before processing it for further pharmaceutical purposes or for local human consumption.

Keywords: *Moringa oleifera*, *Murraya koenigii*, seasonal variation, minerals

Introduction

Quite a lot of studies have been reported the nutritional value of *Moringa oleifera* and *Murraya koenigii* leaves (Ferreira *et al*., 2008; Handral *et al*., 2012) [27, 29]. However the safety of the consumption of *Moringa oleifera* and *Murraya koenigii* leaves as dietary supplement is not studied intentionally. Since there is a common belief that eating of *Moringa oleifera* leaves in the month of Karkkidaka (August) may create gastrointestinal problems. Indeed, this belief is not groundless; poisonings associated with the presence of microbial contaminants, chlorinated pesticides, heavy metals and other chemical toxins in plants were reported regularly (Clemens and Ma, 2016) [16]. Among these, heavy metal toxicity is the major poisonings. Heavy metals are easily accumulated in leafy vegetables, as compared to grain or fruit crops (Mapanda *et al*., 2005) [38] depending on the soil condition and season. Availability of heavy metals in the environment due to anthropogenic activities is the major problem nowadays. However, in field conditions, the levels of toxic metals in plants (both terrestrial and aquatic) vary widely depending upon the influence of environmental factors and the type of plant itself (Wong, 1996) [55]. Many factors influence the uptake of metals in plants, include the growing environment, such as temperature, soil pH, soil aeration and fertilization, the availability of the elements in the soil, the type of leaves, soil moisture and plant energy supply to the roots and leaves (Yamamoto and Kozlowski, 1987) [59]. Besides the micronutrient estimation, quantification of heavy metal accumulation is also important in plants like *Moringa oleifera* which are expected to absorb heavy metals in varying environmental conditions. Therefore the present study aimed to evaluate the nutritional quality of *Moringa oleifera* and *Murraya koenigii* leaves under varying environmental condition along with that of soil analysis.

The following objectives have been achieved in this study

1. Analysis of nutritional quality of leaves of *Moringa oleifera* and *Murraya koenigii* for emphasizing mineral element concentrations in different seasons.
2. Analysis of soil samples in different seasons to evaluate the essential mineral and heavy metal accumulation in soil.
3. Analysis of weather data collected from Meteorological Department to find out the environmental changes in the study period.

Materials and methods

1. Material

Fresh leaves of *Moringa oleifera* and *Murraya koenigii* were collected from the plants grown in similar soil and environmental condition. That is, these two plants are grown within one meter limit. For mineral element analysis leaf samples were collected during April, August and December, 2016, representing summer, post monsoon and winter season respectively. Samples were collected randomly from all branches of the tree.

2. Analysis of Mineral elements using ICMS

The inductively coupled mass spectrometry system (Thermo Scientific ICAP Qc) was used for analysing the mineral elements. The external calibration solutions were prepared from standard certified multi element solution (MERCK).Thermo scientific BRANSTEAD Smart2pure water containing 1% suprapur grade Nitric acid (MERCK) was used to get a range of concentrations 25ppb, 50ppb, 100ppb, 250ppb, 500 ppb for all elements. Samples containing higher concentration of elements than this calibration range are diluted and analyzed by applying dilution factors. The ion optics was tuned using Thermo scientific Tune-B ICAP-Q solution in standard mode and

KED mode. Mass and detector calibration was conducted using Thermo scientific Setup solution ICAPQ.For sample preparation all the acids used for the ICP-MS analysis were of suprapur grade (Merck, USA) and high purity water from Milli-Q water purification system (Thermo Scientific, Barnstead, Smart 2 pure) was used for dilution and preparation of sample. All glass and plastic utensils were thoroughly acid cleaned and then rinsed with Milli-Q water before use. For the microwave digestion (Anton Paar, Multiwave 3000), 0.1gram of sample was mixed with HNO3 (7ml) and Hydrogen peroxide (1ml) and the vessel was immediately closed to avoid contamination. The samples were hold for 25min, zero ramp time at 400 watts and then retained for 30 min, 5 ramp at 500 watts. After cooling, the vessels were opened and the samples are diluted to 100ml with Milli Q water (Thermo Scientific, Barnstead, Smart 2 pure). The vessels were closed and shaken thoroughly to complete the dissolution. The details of the instrument are given below.

Operating Conditions

Peristaltic pump speed	40 rpm
Cool flow	14.00 L/min
Sampling depth	5
Plasma power	1550 watts
Auxiliary flow	0.80 L/min
Nebulizer flow	0.97
No. of main runs	5

3. Soil analysis

Soil samples were collected during April, August and December in the Year 2016, representing three seasons, summer, post monsoon and winter respectively, from the field where the two study materials *Moringa oleifera* and *Murraya koenigii* trees are grown. A composite soil sample from the main rooting zone was taken by digging soil up to 20 cm depth. Non soil particles were removed from the soil. Soil was dried and sieved through a 2 mm sieve and stored in the labelled polythene sampling bags. Soil analysis was conducted with this stored soil samples. Soil pH, electrical conductivity, organic carbon, porosity, water holding capacity, bulk density, soil particle density and cation exchange capacity were estimated using standard procedures (Richards, 1954., Jackson, 1973 [31, 45]., Anderson and Ingram, 1993., Daubenmire, 1974., Piper, 1966., Misra,1968.) [3, 23, 40, 44]. Soil minerals such as sodium, potassium, calcium, phosphorous, potassium (Jackson, 1973) [31], magnesium (Richards, 1954) [45], sulphur (Chesnin and Yien, 1950) [15], boron (Dible *et al*., 1954), iron, manganese, zinc, copper, lead, cadmium and chromium (Hesse, 1971) [30] were estimated using standard procedures.

4. Statistical analysis and calculations

4.1 Statistical analysis

Statistical analysis was done as described by Bennet and Franklin (1967). All analysis was performed using the statistical package SPSS. Data were analyzed by one-way analysis of variance (ANOVA). All the values are expressed as mean value ± SEM. 'p' values of 0.05 or less were considered significant.

4.1.1 Daily intake of heavy metals

An assessment of the health risk posed in human beings by the consumption of contaminated leaf powder was made by comparing the concentration of the contaminants recorded from *Moringa oleifera* and *Murraya koenigii* leaf powder with national and international safe limits. Daily intake of heavy metals through the consumption of the *Moringa oleifera* and *Murraya koenigii* leaf was calculated according to equation:

Daily intake of heavy metals (mg/day) = Daily recommended dose of leaf powder (g/day)× Heavy metal concentration(mg/g)in leaf powder.

The daily intake of metals was estimated according to the consumption for adults. A daily consumption rate of the heavy metals is compared with the values of provisionaltolerable daily intake (Joint FAO/WHO Expert Committee on Food Additives, 1999) [32].

4.1.2 Coefficient of Biological Absorption

Transfer Factor (TF) or Plant Concentration Factor (PCF) is a parameter used to describe the transfer of trace elements from soil to plant body and it is the function of both soil and plant properties. The transfer coefficient was calculated by dividing the concentration of heavy metals in plants by the total heavy metal concentration in the soil (Kachenko and Singh, 2006) [33]

$K_i^{bp} = C_i^r / C_i^p$

Where $\mathbf{C_i^r}$ is the content of the i^{th} heavy metal in a plant, mg/kg; $\mathbf{C_i^p}$is the content of the i^{th} heavy metal in soil, mg/kg.

Higher transfer coefficient represents relatively poor retention in soils or greater efficiency of plants to absorb metals. Low coefficient demonstrates the strong adsorption of metals to the soil colloids.

5. Analysis of weather condition in study period

In the present study three seasons such as summer (April), post monsoon (August) and winter (December) were selected for analysing the seasonal variation of mineral elements in *Moringa oleifera* and *Murraya koenigii* leaves. Weather data is collected from Meteorological Department, Thiruvananthapuram.

Results

1. Mineral elements in *Moringa oleifera* and *Murraya koenigii*

The variation of mineral elements in *Moringa oleifera* and *Murraya koenigii* leaves were analysed in three seasons within the same year at an interval of four months.*Moringa oleifera* and *Murraya koenigi* leaves showed rich amount of various micro nutrients such as Fe, Mn, Zn, Cu and Ni during the month of August. In *Moringa oleifera* Ni, Cu, Ba, Zn, Pb, Cr, Li and Cd accumulation was terrifically high on August compared to other two seasons. However Ga, Se, Ag, Cs, Tl and As were absent or negligibly low in the month of December. But these elements are appeared in August except Tl. But *Murraya koenigii* showed high amount of Tl in the samples collected on August. Ba accumulation was very high in *Murraya koenigii* during the month of December. The results are shown in table 1.

The results indicate that leaf samples of both plants contained Fe concentration between the range of 101.59±2.34 and 360.4 ± 8.94 mg/kg.The highest concentration of Fe was found in the *Moringa oleifera* post monsoon sample, whereas least amount was found in *Moringa oleifera* winter sample. Range of Cr concentration observed in April sample was1.87±0.02 (MO) and 1.33±

0.01mg/kg (MK), but a sudden elevation was observed in August in both the samples, *Murraya koenigii* was shown 12.48± 1.34mg/kg and *Moringa oleifera* 15.2 ± 0.09 mg/kg. The range of Zn concentration was 9.32±2.02 to 25±1.98 mg/kg. High concentration, that is, 24.8 ± 3.98 and 25±1.98 mg/kg was observed in the post monsoon season in *Moringa oleifera* and *Murraya koenigii* respectively. The range of Mn concentration observed was 17.15±2.23 to 32.24± 3.12 mg/kg. Compared to *Murraya koenigii* all the samples of *Moringa oleifera* have shown high concentration of Mn. Minimum and maximum concentration Ni was observed in *Moringa oleifera* samples 5.90 ± 1.10 and 5.11 ±0.23 mg/kg was observed in April and December but there is a sudden increase by 39.2 ± 1.2 mg/kg in August sample. *Murraya koenigii* showed gradual increase, in concentration. 6.12±1.2, 9.36± 0.23 and 13.9±1.2 mg/kg concentration was observed in April, December and August respectively. Lowest and highest amount of Cu was observed in *Moringa oleifera* samples (12.27± 0.45 and 43.1 ± 2.3 mg/kg). Here also a sudden raise was observed in post monsoon sample. But in *Murraya koenigii*27.6±2.34 mg/kg was observed in post monsoon sample. Maximum amount Pb (5.7 ± 0.34 mg/kg) was observed in *Moringa oleifera* post monsoon sample however, *Murraya koenigii* showed 0.4±0.01 mg/kg (least concentration) in post monsoon sample. All the samples except *Moringa oleifera* post monsoon sample showed below permissible limit of Cd concentration whereas *Moringa oleifera* post monsoon sample has shown high level (0.7± 0.01mg/kg) of Cd. The same trend was observed in As and Co. Concentration of Co was above permissible limit in *Moringa oleifera* post monsoon sample whereas, as above permissible limit was observed in *Murraya koenigii* post monsoon sample.

Table 1: Comparison of mineral elements in *Moringa oleifera* and *Murraya koenigii* in three seasons

	Mineral	Amount of elements in leaf sample (mg/kg)						Permissible limit (mg/kg) (Standard)	Range found in plants (mg/kg)
		Moringa oleifera			*Murraya koenigii*				
		April	August	December	April	August	December		
1	Li	0.43±0.01	1.6 ± 0.02	0.87 ± 0.01	0.47 ± 0.02	1.2 ± 0.01	0.95±0.03	-	-
2	Mg	2248.80± 12.32	3689.2 ±13.34	3504.29±12.40	2012.50± 12.78	2783±12.90	2107.76±13.67	-	-
3	Al	294.44 ± 5.64	929.3 ± 12.98	203.48 ± 12.92	199.78±3.98	734.9±8.90	923.97±12.90	-	-
4	Mn	32.24± 3.12	31.3 ± 3.34	31.86 ± 3.20	21.05± 2.34	27.6± 4.30	17.15±2.23	500b	-
5	Fe	253.15 ± 10.84	360.4 ± 8.94	101.59±2.34	251.72±6.67	348.8 ± 2.45	341.43±11.90	425b	400-500
6	Co	0.22 ± 0.03	1.2 ± 0.23	0.14± 0.01	0.12±0.01	0.30± 0.01	0.26±0.05	0.05-1.1f	-
7	Ni	5.90 ± 1.10	39.2 ± 1.2	5.11 ±0.23	6.12±1.2	13.9±1.2	9.36± 0.23	1.5 a	0.02-50
8	Cu	17.24 ± 2.87	43.1 ± 2.3	12.27± 0.45	14.94±3.78	27.6±2.34	25.25± 2.34	40d, 150	2.5
9	Zn	11.74 ± 1.45	24.8 ± 3.98	12.48± 0.34	9.32±2.02	25±1.98	16.05± 2.98	60e	20-100
10	Ga	0.07± 0.01	4.3 ± 1.2	0	0.06±0.01	10.2±2.78	0	-	-
11	Se	0	5.7 ± 1.34	0	0	0	0.38± 0.05	-	-
12	Ag	3.14 ±0.05	1.3 ±0.05	0	0.39± 0.01	0.5±0.01	3.20± 0.02	-	-
13	Cs	0.03 ± 0.01	0.1± 0.01	0.02 ± 0.01	0.01±0.00	0.1 ± 0.01	0.04±0.01	-	-
14	Ba	16.01 ± 1.23	23.4 ± 1.2	25.14 ±0.56	16.67± 1.2	59±3.98	56.60± 2.3	-	-
15	Tl	0.02 ± 0.01	0	0.04 ±0.01	0	3.9±0.12	0	-	-
16	Pb	0.99 ± 0.01	5.7 ± 0.34	1.08±0.01	0.67± 0.02	0.4±0.01	2.36± 1.2	0.3b	0.50-30
17	Cr	1.87 ± 0.02	15.2 ± 0.09	1.49±0.01	1.33± 0.01	8.9±0.02	12.48± 1.34	2.3b	-
18	As	0.05 ± 0.01	0.1 ± 0.0	0	0.05±0.01	0.2± 0.01	0	0.2e	-
19	Cd	0.08 ±0.01	0.7± 0.01	0.01±0.0	0.11±0.01	0.10±0.01	0.05±0.01	0.3c, 0.1b	2.4

A WHO/FAO (Codex Alimentarius Commission. Joint FAO/WHO, 2007) [19] and indian standard awashthi; bWHO (Codex Alimentarius Commission, Joint FAO/WHO, 2001 and codex alimentarius commission, 1994) [21]; cEuropean Union (EU), 2006 [25]; dWHO/FAO (FAO/ WHO,codex general standard for contamination and toxin in foods, 1996) [17]; eWHO (codex alimentarius commission, 1991) [20]; fAgency for toxic substance disease registry (ATSDR, 1994a) [6].

Table 2: Comparison of element concentration in recommended dose to tolerable dietary intake

No:	Mineral	Amount of elements in the daily recommended dose (µg)		Tolerable dietary intake (µg/day)	Remarks (based on an average of 60 days intake)
		Moringa oleifera August sample	*Murraya koenigii* August sample		
1.	Co	0.418	0.0972	-	-
2.	Ni	13.720	4.5036	1000	No health risk
3.	Cu	15.085	8.9424	2000	No health risk
4.	Ba	8.190	19.2132	-	-
5.	Pb	17.10	0.1296	214	risk
6.	Cr	5.320	2.8836	200	risk
7.	As	0.035	0.0648	7.00	No health risk
8.	Cd	0.245	0.0324	60	No health risk
9.	Li	0.560	0.3888	-	-

Heavy metal accumulation was high in August (post monsoon) sample. Therefore those samples were taken and calculated the daily intake of heavy metals, which is represented in the table 5.6. Concentration ofNi, Cu, Cd and

As was far below the tolerable intake value, whereas, Pb and Cr quantified with an average values of 60 days intake, was above their permissible limits.

2. Soil analysis

The results of soil analysis revealed the physical and chemical properties of the soil. In the present study, soil was slightly acidic with low electrical conductivity, organic carbon, bulk density, particle density and cation exchange capacity. The soil has considerable water holding capacity. The analysis of mineral content in soil revealed that most of the minerals P, K, Zn, Cd, Cr and Pb were accumulated during the summer season, when compared to other two seasons. However S, Fe, Mn, B and Cu were found to be higher at post monsoon season (August). At winter season (December) all the elements were present in a moderate level. The results are shown in tables 3 and 4. Exchangeable cat ions in soil sample were Ca, Mg Na and K in 49.61, 0.74, 0.28 and 2.5 CMol/kg respectively.

Table 3: Physical properties of soil sample

Sl.No	Character	Result
1	pH	6.2
2	Electrical conductivity (dS/cm)	0.15
3	Organic carbon (%)	1.43
4	Porosity (%)	47.59
5	Water holding capacity (%)	47.63
6	Bulk density (g/cm^3)	1.02
7	Particle density (g/cm^3)	1.57
8	Cationexchange capacity(CMol/kg)	0.5

Mineral analysis revealed that P, K and Zn accumulate more in summer season while concentration of S, B, Fe, Mn and Cu was high in post monsoon season. However toxic metals Cd, Cr and Pb concentration was high in summer season. But the concentration of all the detected metals were below the permissible limits recommended as standard and it is shown in the table 5.8.Transfer Factor of different heavy metal from soil to plant is presented in Table 5. Higher transfer factor, ≥ 5 was noticed for Mn, Fe and Cr. TF ≥ 10 of Cr was observed in *Moringa oleifera* and *Murraya koenigii* for August and December sample respectively. TF of Fe was high in all the samples. Variation was prominent in TF values depending upon the seasons.

Table 4: Comparison of soil mineral elements in three seasons

Sl.No	Elements	Amount of elements in soil sample (mg/kg)			Standard
		April (Summer)	August (Post monsoon)	December (Winter)	
1	P	40.03 ± 1.23	33.22±1.54	35.03±3.20	20 - 40
2	K	130.98±2.34	123.2±1.34	126.34±5.67	150-250
3	S	8.12±0.56	8.66±0.56	7.34±0.89	10-20
4	B	0.27±0.01	0.88±0.03	0.34±0.01	20
5	Fe	25.67±0.43	36.89±1.20	30.23±1.22	150[b]
6	Mn	4.2±0.03	6.4±0.98	5.12±0.03	437[b]
7	Zn	15.09±1.23	13.32±1.20	14.05±1.20	50-100[c]
8	Cu	15.43±1.01	17.48±1.63	14.76±1.34	6-60[a]
9	Cd	0.39±0.01	0.27±0.02	0.29±0.01	0.07-1.1[a]
10	Cr	1.45±0.02	1.12±0.01	1.01±0.03	65[b]
11	Pb	8.31±0.67	7.78±0.05	7.12±0.43	10-70[a]

Results are presented as mean ± SEM (n=6) aFAO/WHO, codex general standard for contaminants and toxins in foods, 1996; bWorld Health Organization, 2000; cWHO 1998.

Table 5: Transfer factor (TF) of mineral elements from soil to *Moringa oleifera* (MO) and *Murraya koenigii* (MK) in different seasons

Heavy metal	Transfer factor (TF)					
	April		August		December	
	MO	MK	MO	MK	MO	MK
Mn	7.68	5.01	4.89	4.31	6.22	3.34
Fe	9.86	9.81	9.76	9.45	3.36	11.29
Cu	1.12	0.97	2.46	1.57	0.83	1.71
Zn	0.78	0.62	1.86	1.87	0.88	1.14
Pb	0.12	0.81	0.73	0.05	0.15	0.33
Cr	1.29	0.92	13.57	7.95	1.48	12.35
Cd	0.21	0.28	2.59	0.37	0.03	0.17

Analysis of weather condition in study period

In the present study three seasons such as summer (April), post monsoon (August) and winter (December) were selected for analysing the seasonal variation of mineral elements in *Moringa oleifera* and *Murraya koenigii* leaves. Variation in the concentration of mineral elements in the soil at these three different seasons was also assessed. The data collected from meteorological department revealed that the maximum temperature was reported in the month of April (36.4°C) and the minimum temperature at the month of Februry (19.3°C) of 2016. Maximum rain fall was occurred during the months of May and June and minimum rain fall on January. Maximum temperature recorded daily in three seasons is given in the figure 1, 2 and 3. Daily rain fall in the summer season (May to July) is given in the figure 4. Monthly average temperature and rainfall in the year, 2016 is given in the table 6.

Table 6: Weather report showing monthly average temperature and rain fall in the year 2016

Month	Max. Temp.(°C)	Min.Tem.(°C)	Avge. Rain fall (cm)
January	33.40 ±0.69	23.50 ± 1.24	0
February	34.10± 0.90	24.30 ± 1.80	0.07 ± 0.34
March	34.90 ±0.74	25.90± 1.02	0.59 ±3.04
April	35.10 ± 0.63	27.30 ±0.63	1.7 ± 5.02
May	33.10 ±2.35	25.60± 1.42	13.6 ± 27.25
June	30.63 ±1.72	24.10 ± 0.89	13.8 ±17.01
July	31.10 ±0.98	24.10 ± 0.67	6.34±9.65
August	31.66 ±0.70	24.60± 0.48	1.78±3.82
September	31.65 ±0.70	24.30± 0.67	0.50±0.90
October	32.37 ± 1.10	24.21± 0.66	1.62±3.82
November	32.10 ± 1.50	24.00 ±0.60	1.40±3.81
December	33.10 ± 0.964	23.60±0.83	1.30±4.62

Data from Meteorological Department, Thiruvananthapuram

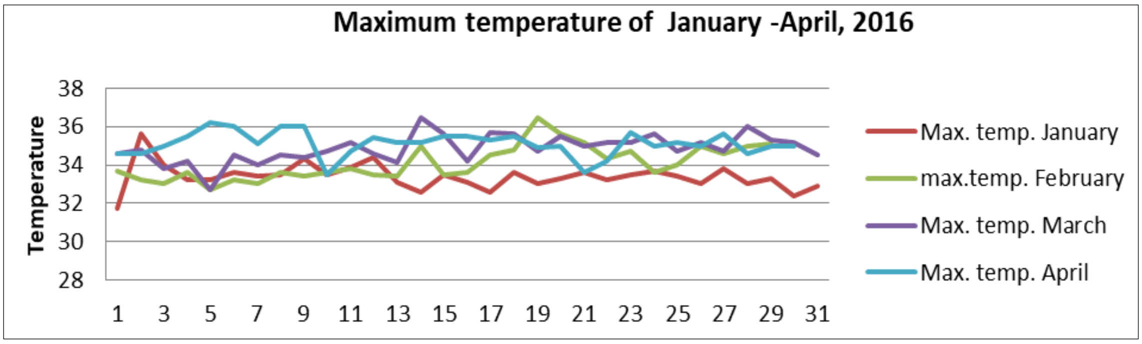

Fig 1: Maximum temperature recorded daily in the summer season (January-April), 2016

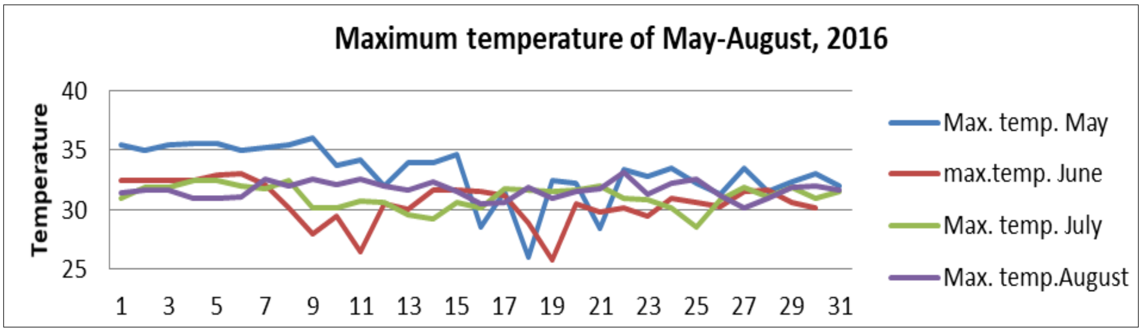

Fig 2: Maximum temperature recorded daily in the post monsoon season (May-August), 2016

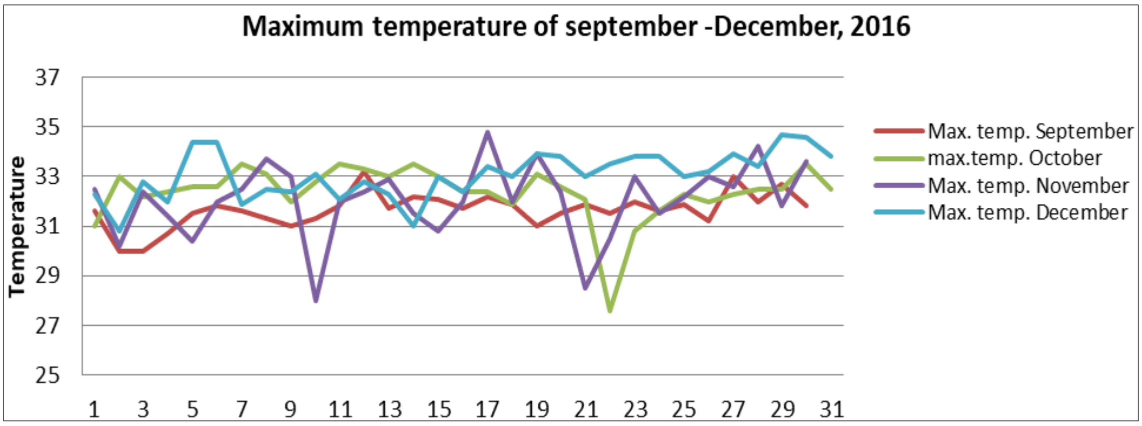

Fig 3: Maximum temperature recorded daily in the winter season (September- December), 2016

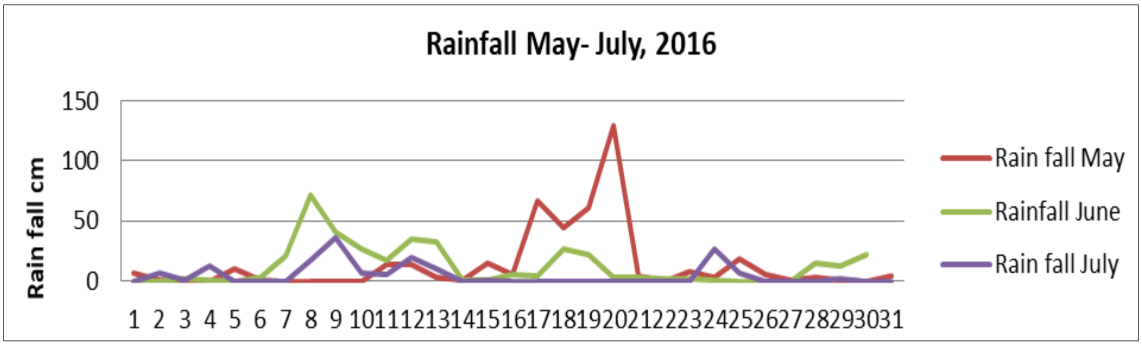

Fig 4: Rainfall recorded daily in the monsoon season (May- July), 2016

Discussion

People use different plants in many respects, as a food for nutritional purpose and medicine for treatment of diseases. The nutritious value as well as the toxicity of the plants is due to their chemical composition and these chemicals have significant role in the regulation of various body systems. Their excess or deficiency may disturb normal biochemical functions of the body (Kumar *et al*., 2012) [34]. Toxicity of medicinal herbs is of much greater concern today than ever before. In recent years, much emphasis is being laid on toxic element contents, as several western countries have banned Ayurvedic drugs.

In the present study seasonal variation was observed in the concentration of mineral content in *Moringa oleifera* and *Murraya koenigii* leaves. Li, Mg, Al, Mn, Fe, Co, Ni, Cu, Zn, Ga, Se, Ag, Cs, Ba, Tl, Pb, Cr, As, Cd were the minerals analysed. All of them were present in both the plants but seasonal distribution of mineral elements showed slight variation. Most of the mineral elements were accumulated during post monsoon season both in *Moringa oleifera* and *Murraya koenigii* leaves. The order of preferential accumulation of mineral elements during post monsoon in *Moringa oleifera* is Mg >Al > Fe > Cu > Ni > Mn > Zn > Ba > Cr > Se >Pb > Ga > Li >Ag > Co > Cd >As > Cs > Tl and in *Murraya koenigii* is Mg > Al > Fe > Ba > Mn> Cu > Zn > Ni > Ga > Cr > Tl> Li > AG > Pb > Co >As > Cs > Cd > Se.

In both *Moringa oleifera* and *Murraya koenigii,* Ni, Ba, Ag, Ga, Cr andPbwerenoticed in significant amounts than the permissible limit of WHO. However, Cu and Se contents were slightly higher than the permissible limits in *Moringa oleifera* post monsoon sample. Similarly, Tl, a toxic element, was detected in *Murraya koenigii* post monsoon sample and Ba concentration was very high in winter sample.

Metals such as zinc, iron, manganese and copper are essential elements since they play an important role in biological systems and only become harmful at high concentrations, whereas chromium, lead and cadmium are non-essential elements as they are toxic, even at low concentration levels (Monni *et al*. 2000; Blaylock and Huang 2000) [11, 42]. From this study it is clear that in both the plants, toxic metal accumulation was very high during the post monsoon season (August). In *Moringa oleifera* Cu, Ni, Ba, Pb, Cr, Li and Cd accumulation was terrifically high on August compared to other two seasons.

The daily intake of Zn was estimated as 15.73 mg, which represents 56 % for a 60 kg adult (WHO, 1993). So WHO's recommended limit of zinc in medicinal plant is 60 mg/kg. In the present study the range of Zn concentration was 9.32±2.02 to 25±1.98 mg/kg; it is in the recommended limit. Higher concentrations, ie, 24.8 ± 3.98 and 25±1.98 mg/kg was observed in the post monsoon season in *Moringa oleifera* and *Murraya koenigii* respectively.

The results of the present study indicate that Fe concentration range between 101.59±2.34 and 360.4 ± 8.94 mg/kg. The WHO recommended level of iron in medicinal plants is 425 mg/kg, while its dietary intake is 10 – 28 mg/day (WHO,1998), whereas the range of Mn concentration observed in the present study was 17.15±2.23 to 32.24± 3.12 mg/kg. Compared to *Murraya koenigii* all the samples of *Moringa oleifera* have shown high concentration of Mn. WHO's maximum permissible limit of Mn in medicinal plants is 200 mg/kg, while its daily intake is 11mg/day (WHO, 1998).

WHO's permissible limit of Cu in medicinal plants is 40mg/kg. Lowest and highest amount was observed in *Moringa oleifera* samples. A big hike (from 12.27± 0.45 to 43.1 ± 2.3 mg/kg) was observed in post monsoon sample. This value is above the permissible limit. Cu is an essential substance to human life, but in high doses it can cause anaemia, liver, kidney damage, stomach and intestinal irritation. Cu accumulates easily in the body; hence, chronic low level intakes of heavy metals have damaging effects on human beings and other animals (Bermudez *et al*., 2011) [10]. The daily intake of Cu was estimated as 10.75 mg, which represents 38 % for a 60 kg adult (WHO, 1993). However *Murraya koenigii* showed the value (27.6±2.34 mg/kg) below the permissible limit even in post monsoon sample.

Maximum amount lead (5.7 ± 0.34 mg/kg) was observed in *Moringa oleifera* post monsoon sample however, *Murraya koenigii* showed 0.4±0.01 mg/kg (least concentration) in post monsoon sample. According to China food hygiene standard in 1994 the standard limit of lead for vegetables and fruit is 0.2 mg/kg while it is 0.3 mg/kg for WHO (Codex Alimentarius Commission, Joint FAO/WHO). It is found that in all samples, lead concentration is more than permitted level. Lead is a toxic element that can be harmful to plants, although plants usually show ability to accumulate large amounts of lead without visible change in their appearance or yield. In many plants Pb accumulation can exceed several hundred times the threshold of maximum level permissible for human consumption. High Pb concentrations may be attributed to plants located near roads of heavy traffic. The toxic effects of Pb focus on several organs, such as liver, kidneys, spleen and lung, causing a variety of biochemical defects. The daily intake of Pb was reported in literature by Tripathi *et al*. (1997) is 0.521 mg per day. Although Pb concentrations were high in *Moringa oleifera* post monsoon sample, low consumption of leaves in this season is commended.

According to WHO, permissible limit of chromium in medicinal plant is 2.3 mg/kg, while its daily dietary intake is 0.2mg (WHO, 1998). Range of Cr concentration observed in this study was 8.9±0.02 to 15.2 ± 0.09 mg/kg. So all the samples have shown high amount of Cr than the permissible limit. The WHO recommended level of Cadmium in medicinal plants is 0.1 mg/kg (WHO, 2005). and European Union (EU), (2006) [25] recommended level is 0.3 mg/kg. All the samples except *Moringa oleifera* post monsoon sample exhibited below permissible limit of Cd concentration whereas *Moringa oleifera* post monsoon sample has shown high level (0.7± 0.01mg/kg) of Cd, that is, above the permissible limit. The same trend was observed in arsenic and cobalt. In *Moringa oleifera* post monsoon sample exhibited above permissible limit of Co concentration whereas, arsenic concentration was very high in *Murraya koenigii* post monsoon sample. Li is toxic to all plants, causing a chlorosis-like condition. Uptake and sensitivity to lithium are species dependent. In general, more lithium is taken up by plants from acidic than alkaline soils. The U.S. Environmental Protection Agency (EPA) in 1985 [52] estimated the daily Li intake of a 70 kg adult to range from 650 to 3100µg. In *Moringa oleifera* and *Murraya koenigii* post monsoon samples exhibited high amount of Li compared to other seasons. Minimum and maximum concentration of Ni was observed in *Moringa oleifera* samples. Minimum concentration (5.90 ± 1.10 and 5.11 ±0.23 mg/kg) was observed in April and December but there was anunexpected increase by 39.2 ± 1.2 mg/kg in August sample. *Murraya koenigii* showed gradual increase, in concentration that is, 6.12±1.2, 9.36± 0.23 and 13.9±1.2 mg/kg concentration was observed in April, December and August respectively. WHO's permissible limit of nickel in medicinal plant is 1.5 mg/kg, while its routine requirement for mankind is 1mg/ day (WHO, 2005). The result of the present study showed that Ni concentration is very high in all the samples.

The contamination of *Moringa oleifera* and *Murraya koenigii* leaves by heavy metals like Pb, Cr, Cd, As may pose a threat to humans because they are not biodegradable. With reference to the RDA limits and the present study regarding the occurrence of heavy metals in the leaves of *Moringa oleifera* and *Murraya koenigii*, there is a need for dose fixation study to give an indication of comparative safety and prevention of any possible cumulative toxicity of heavy metals during its prolonged use. The total arsenic, barium, cadmium, cobalt, copper, chromium, lead, lithium and nickel in *Moringa oleifera* and *Murraya koenigii* post monsoon leaf samples were found at high concentrations when compared to permissible limit. Previous studies reported that concentration of heavy metals in *Moringa oleifera* and *Murraya koenigii* leaves are not exceeding the recommended dietary allowance of RDA (Chary *et al*., 2008) [13]. However the present study recommends that *Moringa oleifera* leaves are not suitable for direct consumption as food supplement during the month of August. Though it is highly recommended for other seasons especially winter because winter samples showed permissible level of micro elements and most of the toxic elements are totally absent or within the acceptable limit. This may be the reason behind the traditional belief that *Moringa oleifera* leaf is not recommended during the month of Karkkidaka (August). So present study recommend that there is no health risk associated with the consumption of these plants in other seasons with respect to their metal contents. However, the content of Pb and Cr must be monitored frequently to avoid any possible cumulative toxicity of heavy metals during its prolonged use.

Annan *et al*. (2013) [4] states that soil conditions and locations determine the elemental contents in the leaves. The pH and electrical conductivity of soil provide useful information about the nutrient content and acidity of the soil (Smith and Doran, 1996) [50]. The results of the present study revealed the slight acidic (pH - 6.2) nature of the soil. The amount of moisture held by soil particles determines the electrical conductivity. The soil collected for the present study is silt with medium conductivity.

Soil organic carbon is the carbon present in the organic matter of soil. In the present study the amount of organic carbon present in the soil was very low. The information concerning the pore size of the soil is very useful in characterizing soil as a medium for plant growth and other uses (Danielson and Sutherland, 1986) [22]. Soil porosity gives an idea about the amount of water and air stored in the soil. In the present study the soil particles have an adequate space between them. Water holding capacity refers to the amount of water held between field capacity and wilting point. In the present study showed that soil has good water holding capacity. Bulk density is the ratio of the mass of dry solids to the bulk volume of the soil. In the present study the soil showed a higher bulk density indicating its low organic content.

Plant nutrients can exist either as cation or anion. Cation exchange capacity is an indication of negative charges on the soil. So it can absorb or release positively charged cations into the soil solution where plants can access these nutrients. Soil with good amount of organic matter has high cation exchange capacity (McMullen, 1995) [39]. In the present study the soil has a low cation exchange capacity (CEC) indicating low amount of organic matter in the soil. The soil with low cation exchange has not enough negatively charged particles to hold the positively charged cation, such as potassium.

Mineral analysis of soil revealed that macronutrients P, K and S accumulate more in summer season while micro elements B, Fe, Mn, Zn and Cu have shown high accumulation in post monsoon season. However, heavy metals concentration was high in summer season. The concentration of all the detected metals were below the permissible limits recommended and it is shown in the table 5.8. The maximum allowable limit of iron recommended by WHO in soil is 150 mg/ kg. The concentration of iron was too low in the study area compare with the maximum allowable limit of WHO. From the soil analysis it is concluded that the soil of the study area was not polluted by any metal. But *Moringa oleifera* and *Murraya koenigii* showed some amount of heavy metals in their leaves.

Heavy metals bioavailability to plants is strongly related to the concentration of the element in the soil solution. The accumulation of metals from soils to plants depends on many factors such as metal forms, plant species and plant parts and soil properties. The solubility of the trace metals in the soil and its uptake by plants vary considerably with pH and the redox potential within the soil or the root system. The associated anion and the particle size also have a great influence on growth and metal uptake by plants. The present study showed that the concentrations of metals in leaves were generally higher than that of the soil. In order to evaluate the accumulating capacity of heavy metals from soils to plant, a quantitative evaluation of the relationship between metals concentration in leaves and in soil was made by calculating the transfer factor for the soil/plant system.

Generally transfer factor varies from one plant to another plant, suggesting a selectivity of the plants in absorbing elements from soils. In the present study, the TF of different heavy metal from soil to leaves are presented in table 5.9. Higher transfer factors reflect relatively poor retention in soils or greater efficiency of plants to absorbs metals. Low transfer factor reflects the strong adsorption of metals to the soil colloids (Wierzbicka *et al*., 2007) [54]. The mobility of metals from soil to plants is a function of the physical and chemical properties of the soil and of plant species, and is altered by innumerable environmental and human factors (Alloway and Ayres, 1997) [2]. The highest TF value was found for Mn, Fe and Cr. These might be due to higher mobility of these heavy metals with a natural occurrence in soil (Alam *et al*., 2003) [1] and the low retention of them in the soil than other toxic cations (Zurera *et al*., 1987) [60].

Variation was prominent in TF values depending upon the seasons. Cr accumulation was very high in both plants and *Moringa oleifera* showed high TF value on August however *Murraya koenigii* showed highest value on December.Under a given environmental condition, the uptake of a metal by a plant can be estimated from the biological absorption co efficient (Fergusson, 1990) [26]. However, in field conditions, the relationship works best only when the concentration of the metal in the soil is not too high (Shaw and Panigrahi 1986) [49]. Because of the influence of environmental factors and the type of plant itself, the levels of heavy metals in plants vary widely (Wong, 1996) [55]. If the other facts are constants, the uptake of a metal by different plant species may be compared.As far as the growing environment is concerned the increase in pH, means the environment becoming more alkaline, and decrease in redox potential, i.e., the environment becoming more reducing, result in decrease in availability of heavy metals, or metals in general to plants (Misra and Mani 1991) [41].Generally, a fluctuation in pH values during different seasons of the year has a considerable influence on the availability of nutrients to crops. Low soil pH (pH between 1 and 4) which is a highly acidic condition tightly holds soil nutrients which are unavailable for plant uptake and most soil nutrients are available for plant uptake in the pH range of 5.5 - 6.5. (Ronen, 2016) [46]. The present study soil pH was 6.2 means increase in availability of metals in the soil. It must, however, be stated that, plants may absorb elements from the soil and environment, some of which may be toxic to humans.

Climate also determines the elemental contents in plants(Annan *et al*., 2013) [4].So weather report was analysed in this study to understand the mineral up take in *Moringa oleifera* and *Murraya koenigii* plants in varying environmental condition such as extreme temperature and rainfall. Temperature was very high in April (summer); average maximum and minimum temperature recorded was 35.10 ± 0.63°C and 27.30 ±0.63°C respectively. Precipitation rate was very less in summer, 2016.From January to April, maximum rain fall (1.7 ± 5.02 cm) was recorded in April. However temperature was maximum when compared to January to March. In January there was no rainfall and maximum temperature recorded was 33.4 ± 0.69°C.But in February and March low precipitation was recorded; 0.07 ± 0.34 cm and 0.59 ± 3.04 cm respectively. Summer season, 2016 was very hot and attained maximum temperature of 36.4°C intermittently. In this season both *Moringa oleifera* and *Murraya koenigii* plants experienced severe drought stress. Soil temperature and drought has marked effects on plant growth, morphology, longevity, respiration, and membrane fluidity, which influence plant nutrient acquisition efficiency. Earlier studies generally suggest that soil warming enhances nutrient uptake (Bassiri Rad, 2000).

Several studies have reported the effects of temperature on plants (Cheng *et al*., 2010; Li *et al*., 2012) [14, 35], however, there were very few studies that have dealt with the interactive effects of higher temperature and heavy metals on plants in polluted soils. In general soil warming can affect the nutrient and heavy metal pathway through altering release of soluble metal ions in to soil solution via decomposition of soil organic matter, and the destruction of soil aggregates, thereby changing metal bioavailability, its uptake and distribution in plant tissues (Rustad *et al*., 2001; Sardans *et al*., 2008) [47, 48]. It has been reported by several authors that an increase of soil organic matter decomposition due to warming can reduce cation-exchange capacity and thus the capacityof soil to retain soil nutrients and heavy metals (Sardans *et al*., 2008) [48]. For instance, Antoniadis and Alloway (2001) [5] reported that soil organic matter decomposition due to higher temperature could facilitate the increase in available fraction of heavy metals leading to greater amount of metal uptake by plants. It has also been reported that elevated temperature can increase the release of trace elements from organic to exchangeable complex through enhancing the soil enzymatic activity(Cao and Ma, 2004) [12] and thereby increasing plant metal uptake. It is known that the elevated temperature increase the active sites on the root surface and/or change the lipid composition of the plasma membrane (Lynch and Steponkus, 1987) [37] and thereby its fluidity, and so facilitate both passive and active metal flux through the membrane (Fritioff *et al*., 2005) [28]. Li *et al*. (2012) [35] found that the elevated temperature increased Cu, Zn and Fe leaf concentrations in *Solanum tuberosum*. The rise in Cu, Zn and Fe accumulation may have resulted from increased enzyme activity (Nilsen and Orcutt, 1996) [43] and protein synthesis (Nilsen and Orcutt, 1996) [43] in plants under increased temperature. So result of the present study also indicates that higher temperature greatly influences the element uptake or its accumulation in *Moringa oleifera* and *Murraya koenigii* plants through enhancing physiological processes and consequently the nutrient demand. It is clear from the high transfer factor of mineral elements from soil to plant in different seasons. The decrease in soil moisture content could probably be due to high soil moisture evaporation which generally occurs during a drought period. Under an evapo-transpiration rate, utilization of water by crops declines when 50% of the required soil moisture is lost. Water is the primary factor determining soil nutrient mineralization and uptake by crops. In general, the increased accumulation of heavy metals in plant tissues as a consequence of elevated temperature can reduce the growth by altering the photosynthesis, transpiration, and other metabolic activities (Li *et al*., 2011) [36]. Taken together these results suggest that plant metal uptake under warming condition may be differentially affected as a result of increased soil organic matter accumulation, decomposition, enzyme activity, mineralization, photosynthetic capacity and plant biomass production.

Conclusion
In the present study both the plants showed seasonal variation in the concentration of mineral elements. Most of the minerals were accumulated in the leaves of *Moringa oleifera* in the post monsoon season compared to *Murraya koenigii*. So it is essentially required to check for contaminant load before processing it for further pharmaceutical purposes or for local human consumption.

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