Comparison of Eleven Heavy Metals in *Moringa Oleifera* Lam. Products

C. LIMMATVAPIRAT*, S. LIMMATVAPIRAT, J. CHAROENTEERABOON, C. WESSAPAN, A. KUMSUM, S. JENWITHAYAAMORNWECH AND P. LUANGTHUWAPRANIT
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Silpakorn University, Nakhon Pathom-73000, Thailand

Eleven heavy metals in various products of *Moringa oleifera* were analyzed to determine eleven heavy metals (Al, As, Cd, Cr, Cu, Fe, Pb, Mn, Hg, Ni, and Zn) using Inductively Coupled Plasma-Mass Spectrometry. The products of *M. oleifera* were purchased in Nakhon Pathom, Thailand. All products were digested with nitric acid solution before determining the concentrations of heavy metals. The recoveries of all heavy metals were found to be in the range of 99.89-103.05%. Several criteria such as linearity, limits of detection, limits of quantification, specificity, precision under repeatability conditions and intermediate precision reproducibility were evaluated. Results indicate that this method could be used in the laboratory for determination of eleven heavy metals in *M. oleifera* products with acceptable analytical performance. The results of analysis showed that the highest concentrations of As, Cr, Hg, and Mn were found in tea leaves while the highest concentrations of Al, Cd, Cu, Fe, Ni, Pb, and Zn were found in leaf capsules. Continuous monitoring of heavy metals in *M. oleifera* products is crucial for consumer health.

Key words: *Moringa oleifera,* Heavy Metals, Products, Leaves, ICP-MS

Moringa oleifera Lam. (family, *Moringaceae* and genus, *Moringa*) is a useful Thai plant. The leaf extracts have hypocholesterolemic[1] and hypolipidemic effects[2]. Additionally, the leaves have been reported to have antioxidant[3], hypoglycemic[4], and antiatherosclerotic activities[5]. The seed extracts showed antispasmodic, antiinflammatory and diuretic activities[6,7]. Furthermore, the seed extract has been shown to have ameliorative effect on liver fibrosis in rats[8]. A recent study of the nutritional value of *M. oleifera* leaves found that the dried leaves are composed of amino acids such as alanine, threonine, tyrosine, methionine, valine, phenylalanine, isoleucine, leucine, histadine, lysine, tryptophan, and cystine, fatty acids such as α-linolenic acid, heneicosanoic acid, γ-linolenic acid, palmitic acid, and capric acid, and several minerals such as calcium, phoshorus, magnesium, potassium, sodium, sulphur, zinc, copper, manganese, iron and selenium. Moreover, the leaves also reported to consist, vitamin A, vitamin E, β-carotene, fiber, condensed tannins, and polyphenols[9]. The phytochemical investigation for bioactive compounds of *M. oleifera* seeds showed the presence of glycosides such as 4-alpha-L-rhamnosyloxy-benzylglucosinolate, niazimicin and niazirin, beta-sitosterol and moringa oil[8]. In summary, the nutritional characterization of leaves and seeds from *M. oleifera* indicated that they are rich in nutrients and possess some medicinal properties. Therefore, leaves and seeds of *M. oleifera* were prepared for herbal medicines, dietary supplements, and functional drinks.

The popular uses of leaves and seeds from *M. oleifera* raise the question about safety and health of their products, especially due to the heavy metal concentrations. Daily exposure to heavy metals above the permissible limits has been associated with mental retardation, cancer, neuropathy, hepatic dysfunction and renal failure[10]. Our previous studies concerning the simultaneous analysis of heavy metals have

***Address for correspondence**
E-mail: chutima@su.ac.th

Accepted 22 July 2015
Revised 20 January 2015
Received 01 January 2014
Indian J Pharm Sci 2015;77(4):485-490

determined the concentrations of eleven heavy metals including aluminum (Al), arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), lead (Pb), manganese (Mn), mercury (Hg), nickel (Ni), and zinc (Zn) in raw leaves and leaf capsules of *Moringa oleifera* Lam. by using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)[11,12]. The results indicate that the concentrations of Al, As, Cd, Cr, Hg, Mn, Ni, and Pb in all samples of raw leaves as well as those of Al, As, Cr, and Hg in all samples of leaf capsules were within permissible limits and normal ranges. In contrast, the concentrations of Fe in all samples of raw leaves as well as those of Cu, Fe, and Zn in all samples of leaf capsules were higher than permissible limits. Furthermore, the concentrations of Cu and Zn in some samples of raw leaves as well as those of Cd, Mn, Ni, and Pb in some samples of leaf capsules were also higher than permissible limits and normal ranges. The overall conclusion is that samples of leaf capsules showed higher concentrations of Cd, Cu, Fe, Mn, Ni, Pb, and Zn compared to those in samples of raw leaves. Therefore, the present study was aimed at investigating the concentrations of those heavy metals in several commercial products made from leaves and seeds of *M. oleifera*. Finally, the results are compared with our previous reports.

The ultrapure water ASTM type I, 18.3 MΩ.cm resistivity, obtained by purifying distilled water with the TKA GenPure UltraPure Water Machine (TKA Wasseraufbereitungssysteme GmbH, Germany) was used for preparing all solutions. Nitric acid, an analytical reagent grade (lot K40352656 935), used for digestion was purchased from Merck, Darmstadt, Germany. An ICP multi-element standard solution XIII (Lot HC813513, Agilent, USA) was diluted with 5% v/v nitric acid solution. Working standard solutions of heavy metals were freshly prepared. All glassware and plastic bottles were cleaned by soaking with 20% v/v nitric acid solution for at least 24 h and rinsed several times with 5% v/v nitric acid solution to eliminate surface contamination.

Samples of *M. oleifera* products (n=35), tea leaves, dried seeds, leaf capsules, leaf powders, and functional drinks were purchased from several selected occasional markets and grocery stores in Amphoe Mueang, Nakhon Pathom province, Thailand, during the period Sep 2011 to Mar 2012. All samples were kept in the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Silpakorn University, Nakhon Pathom, Thailand. The dried samples were stored in a desiccator while the samples of functional drinks were stored in dry condition at room temperature in well-closed containers.

The samples of tea leaves and dried seeds were grounded separately with an IKA MF-10 Microfine Grinding Mill (Werke GmbH and Co. KG, Germany), equipped with a 0.5 mm pore size sieve and high-grade stainless steel grinders. All powder samples were stored in a desiccator to protect them from moisture before digestion. The sample digestion procedure was performed according to previous reports[11,12]. Approximately 1 g of each sample was digested by adding 10.0 ml of 60% v/v nitric acid solution in a 100 ml Pyrex beaker covered with watch glass on a hot plate at 120-130°. This mixture was heated until the solution was fully digested and clear. The clear digested solution was allowed to cool and then filtered. The digested filtrate was diluted to appropriate concentration with ultrapure water. The resulting digestate was analyzed using ICP-MS. All samples were digested in triplicate. The resulting concentrations for all samples were calculated by determining the average of the triplicate measurements.

The concentrations of eleven heavy metals including Al, As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, and Zn in resulting digestates were analyzed by an ICP-MS spectrometer (Model 7500 ce, Agilent). The operating conditions and acquisition parameters are given in Table 1. Prior to analysis, instrument optimization was performed using standard solutions. The calibration curves were prepared from an ICP multi-element standard solution XIII by diluting with 5% v/v nitric acid solution and storing as stock solutions. Working standard solutions were prepared daily in 5% v/v nitric acid solution.

TABLE 1: ICP-MS OPERATING CONDITIONS AND ACQUISITION PARAMETERS

Parameter	Value
Auxiliary gas flow	0.89 L/min
Plasma gas flow	15 L/min
RF power	1500 W
Mass range	7-208 amu
Total acquisition time	260 s
MS analytical settings	20 sweeps/reading 1 reading/3 replicate 3 replicates

ICP-MS: Inductively coupled plasma-mass spectrometry, RF: radio frequency

Heavy metal results from the ICP-MS were quantified against standard curves generated from one blank (0 μg/l) and six different concentrations of standard reference solutions run separately. Quality control was assessed by running a laboratory reagent blank once after every five samples. The concentrations of eleven heavy metals were expressed in mg/kg of samples. The validation parameters such as linearity, limits of detection (LODs), limits of quantification (LOQs), specificity, precision under repeatability conditions and within-laboratory reproducibility were examined. The procedures were performed under the European Standard for the analyses of heavy metals[13,14].

The validation of an analytical method was carried out to ensure reliability of the results. The results of regression analysis on calibration curves are presented in Table 2. Linearity was determined from the regression plots by the least squares method. The correlation coefficients (r^2) of all calibration curves were between 0.9990 and 0.9998, showing good linear relationships over the ranges of heavy metal concentrations. Reagent blank determinations were used to correct the instrument readings.

As shown in Table 3, the precision was expressed by the relative standard deviations (RSD) for n=10. The intraday repeatability (RSD, 0.126 to 0.843%) and interday reproducibility (RSD, 0.351 to 1.204%) exhibited the good precision. The LODs, LOQs, and recoveries of all heavy metals are shown in Table 4. The recovery and reproducibility of the method was estimated by spiking several already analyzed samples with varied concentrations of standard solutions of heavy metals and processed as previously described. The recoveries of all heavy metals were between 99.89 and 103.05%. The recoveries and RSDs listed were acceptable. This method is a useful tool for rapid determination of eleven heavy metals in *M. oleifera* products.

The concentrations of eleven heavy metals (Al, As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, and Zn) in *M. oleifera* products from this study are shown in Tables 5 and 6. The concentrations of As, Cd, Hg, Pb, Cu, Fe, and Zn in *M. oleifera* products are shown in Table 5. The ranking of toxic heavy metal concentrations in *M. oleifera* products was Pb>As>Cd>Hg while that of essential element concentrations was Fe>Zn>Cu. As shown in Table 6, the concentrations of Al (53.833-564.168 mg/kg) and Cr (0.093-2.631 mg/kg) from all analyzed samples were within the normal ranges (Al 1,000 and Cr 0.03-14 mg/kg) prescribed by the previous reports[15,16], except for those of Ni (11.360-115.417 mg/kg) from most of analyzed samples that showed the concentrations more than two times higher than the maximum level (5 mg/kg)[15,16]. Furthermore, the

TABLE 2: RESULTS OF REGRESSION ANALYSIS ON CALIBRATION CURVES

Heavy metals	Regression equations	Correlation coefficients (r^2)
As	y=0.2947x+0.9358	0.9997
Cd	y=0.3568x+0.1588	0.9995
Hg	y=0.2547x+0.9581	0.9998
Pb	y=0.3414x+0.8756	0.9998
Cu	y=0.0987x+0.0016	0.9990
Fe	y=0.0983x+0.0123	0.9992
Zn	y=0.9578x+0.0369	0.9997
Al	y=0.2458x+0.5692	0.9996
Cr	y=0.3458x+0.0456	0.9996
Mn	y=0.4597x+0.0165	0.9998
Ni	y=0.6215x+0.3264	0.9996

TABLE 3: RSD OF THE MEASUREMENTS

Heavy metals	RSD, %	
	Intraday precision	Interday precision
As	0.79	1.12
Cd	0.83	1.14
Hg	0.84	1.20
Pb	0.75	1.07
Cu	0.84	1.17
Fe	0.83	1.19
Zn	0.50	1.16
Al	0.83	1.20
Cr	0.13	0.35
Mn	0.57	0.78
Ni	0.45	0.56

RSD: Relative standard deviations and *n*=10

TABLE 4: LOD, LIMITS OF QUANTIFICATION AND RECOVERIES OF ALL HEAVY METALS

Heavy metals	LOD (μg/l)	LOQ (μg/l)	Recovery (%)
As	3.36	9.93	101.56
Cd	1.22	3.56	103.05
Hg	3.39	10.06	102.69
Pb	4.45	13.13	101.05
Cu	1.56	4.57	99.89
Fe	3.55	9.96	100.06
Zn	2.08	6.12	100.23
Al	2.47	7.18	101.69
Cr	0.87	2.24	102.41
Mn	1.05	3.05	103.01
Ni	1.70	4.95	102.35

LOD: Limit of detection, LOQ is limit of quantification and *n*=10

TABLE 5: CONCENTRATIONS OF As, Cd, Hg, Pb, Cu, Fe, AND Zn IN *MORINGA OLEIFERA* PRODUCTS

Products	The concentrations (mg/kg) of heavy metals						
	As	Cd	Hg	Pb	Cu	Fe	Zn
Tea leaves							
Sample number 1	0.386	0.115	0.128	2.175	21.365	250.067	139.679
Sample number 2	0.186	0.075	0.057	2.156	7.442	201.823	81.866
Sample number 3	0.735	0.155	0.087	3.155	7.798	201.587	105.574
Sample number 4	1.574	0.182	0.054	3.285	6.915	324.286	113.406
Sample number 5	0.130	0.160	0.052	1.214	5.879	174.723	49.287
Sample number 6	0.333	0.080	0.366	2.328	10.680	372.398	59.093
Sample number 7	0.568	0.100	0.091	2.227	11.245	469.085	86.784
Sample number 8	0.123	0.068	0.050	1.175	3.015	115.352	41.326
Sample number 9	0.456	0.120	0.236	2.567	12.402	388.025	59.332
Sample number 10	0.598	0.135	0.298	2.893	12.691	396.230	61.028
Dried seeds							
Sample number 11	0.225	0.075	0.055	1.705	7.542	92.655	114.879
Sample number 12	0.380	0.141	0.065	1.235	4.234	223.130	55.831
Sample number 13	0.761	0.078	0.084	1.121	6.478	86.753	82.016
Sample number 14	0.402	0.152	0.068	1.212	4.325	220.120	56.320
Sample number 15	0.653	0.069	0.082	0.012	6.120	85.326	81.306
Leaf capsules							
Sample number 16	0.459	0.087	0.088	2.344	13.714	197.927	103.604
Sample number 17	0.180	0.102	0.171	1.548	28.294	168.549	87.442
Sample number 18	0.030	0.355	0.055	24.032	16.165	922.311	156.518
Sample number 19	0.028	0.277	0.056	8.860	13.747	569.412	133.592
Sample number 20	0.541	0.069	0.072	1.650	11.569	256.217	72.714
Sample number 21	0.646	0.126	0.060	1.902	6.529	317.988	74.354
Sample number 22	0.445	0.074	0.070	1.522	7.277	652.592	59.852
Sample number 23	0.523	0.068	0.069	1.563	10.986	154.236	71.265
Sample number 24	0.631	0.123	0.063	1.963	6.452	312.580	75.230
Sample number 25	0.493	0.598	0.068	1.426	7.023	593.951	55.639
Leaf powders							
Sample number 26	0.025	0.072	0.038	2.751	2.692	87.876	107.929
Sample number 27	0.189	0.069	0.051	1.591	3.126	37.831	57.010
Sample number 28	0.135	0.089	0.065	1.689	10.235	98.769	105.369
Sample number 29	0.129	0.102	0.087	1.987	12.369	102.360	104.256
Sample number 30	0.235	0.112	0.097	1.872	11.362	84.239	89.325
Functional drinks							
Sample number 31	0.012	0.056	0.023	0.501	3.201	102.359	42.356
Sample number 32	0.032	0.012	0.010	0.043	1.235	112.365	39.254
Sample number 33	n.d.	n.d.	n.d.	0.001	0.892	89.235	33.361
Sample number 34	0.056	0.023	0.018	0.005	2.012	98.236	47.012
Sample number 35	0.021	0.015	0.011	0.013	1.892	105.692	39.235

%SD: 0.012-1.066, n.d.: not determined, SD: standard deviation

concentrations of Mn (92.301-216.675 mg/kg) from some of analyzed samples (Table 6) were higher than the maximum level (90 mg/kg)[15,16].

Overview of Tables 5 and 6, the concentrations of As (<1.574 mg/kg), Cd (<0.182 mg/kg), Hg (0.010-0.366 mg/kg), Pb (0.001-3.285 mg/kg), Al (53.833-439.106 mg/kg), and Cr (0.093-2.631 mg/kg) from all analyzed samples of tea leaves, dried seeds, leaf powders, and functional drinks were fairly low and comparable to previous records[15,16]. Whereas the concentrations of Cu (6.452-28.294 mg/kg), Fe (154.236-922.311 mg/kg), and Zn (55.639-156.518 mg/kg) from all analyzed samples of leaf capsules were higher than those found previously in 2010[15,16]. The concentrations of Fe (37.831-469.085 mg/kg), Zn (33.361-139.679 mg/kg), and Ni (8.361-91.391 mg/kg) from all analyzed samples of tea leaves, leaf powders, and functional drinks were also higher than those of 2010's data[15,16]. Moreover, the concentrations of Fe (86.753-223.130 mg/kg) and Zn

TABLE 6: THE CONCENTRATIONS OF Al, Cr, Mn, AND Ni IN *MORINGA OLEIFERA* PRODUCTS

Products	The concentrations (mg/kg) of heavy metals			
	Al	Cr	Mn	Ni
Tea leaves				
Sample number 1	256.221	1.604	62.086	42.833
Sample number 2	206.662	1.347	216.675	31.616
Sample number 3	182.419	0.893	77.047	13.992
Sample number 4	266.378	1.911	55.624	22.339
Sample number 5	248.715	0.505	63.803	12.612
Sample number 6	404.342	2.417	78.929	91.391
Sample number 7	409.831	1.429	104.526	32.427
Sample number 8	131.653	0.732	29.567	15.766
Sample number 9	425.362	2.498	89.362	86.325
Sample number 10	439.106	2.631	92.301	87.012
Dried seeds				
Sample number 11	53.833	0.566	19.854	9.631
Sample number 12	195.869	0.736	17.075	2.310
Sample number 13	61.424	0.282	11.970	5.968
Sample number 14	187.926	0.802	16.985	2.430
Sample number 15	60.897	0.236	10.981	5.813
Leaf capsules				
Sample number 16	246.548	1.043	45.613	2.973
Sample number 17	209.902	1.128	57.180	9.229
Sample number 18	290.017	1.291	143.133	56.829
Sample number 19	512.629	1.199	207.015	115.417
Sample number 20	263.584	1.204	124.272	12.175
Sample number 21	336.070	1.582	55.405	4.915
Sample number 22	564.168	1.766	148.692	3.937
Sample number 23	259.987	1.123	120.398	12.036
Sample number 24	326.281	1.495	54.398	4.890
Sample number 25	534.321	1.689	139.254	3.601
Leaf powders				
Sample number 26	286.208	2.425	53.332	8.361
Sample number 27	242.933	1.665	64.338	16.068
Sample number 28	277.365	0.754	78.356	33.564
Sample number 29	298.361	0.897	96.326	41.230
Sample number 30	302.560	1.235	79.235	56.234
Functional drinks				
Sample number 31	102.356	0.561	35.693	12.036
Sample number 32	99.325	0.689	29.365	14.910
Sample number 33	79.210	0.093	19.354	11.360
Sample number 34	89.356	0.108	30.290	23.026
Sample number 35	98.320	0.198	31.291	19.031
Normal ranges*	1000	0.03-14	50-90	0.02-5

*Normal ranges prescribed by the previous reports. %SD: 0.001-0.956, SD: Standard deviation

(55.831-114.879 mg/kg) from all analyzed samples of dried seeds were higher than those of 2010's data[15,16].

To achieve the aim of this study, the method was developed for rapid determination of eleven heavy metals in *M. oleifera* products. For various products from *M. oleifera* the average concentrations of heavy metals decrease in the order: Al (255.718 mg/kg)>Fe (247.608 mg/kg)>Zn (78.373 mg/kg)>Mn (73.135 mg/kg)>Ni (26.408 mg/kg)>Cu (8.54 mg/kg)>Pb (2.449 mg/kg)>Cr (1.164 mg/kg)>As (0.362 mg/kg)>Cd (0.122 mg/kg)>Hg (0.087 mg/kg). Among *M. oleifera* products, tea leaves had the highest average concentrations of As (0.509 mg/kg), Hg (0.142 mg/kg), Cr (1.597 mg/kg), and Ni (43.6313 mg/kg) while leaf capsules had the highest average concentrations of Cd (0.188 mg/kg), Pb (4.681 mg/kg), Cu (12.176 mg/kg), Fe (414.576 mg/kg), Al (354.351 mg/kg), and Mn (109.536 mg/kg). Moreover, leaf powders had the highest average concentration of Zn (92.778 mg/kg). However, the concentrations of As (<1.574 mg/kg), Hg (<0.366 mg/kg), Al (53.833-564.168 mg/kg), and Cr (0.093-2.631 mg/kg) in all *M. oleifera* products were lower than those found previously in 2010[15,16].

In addition, Tables 5 and 6 showed that the concentrations of heavy metals in *M. oleifera* products were higher than those in raw leaves when compared to our previous study[11]. The concentrations of heavy metals in leaf capsules as shown in Tables 5 and 6 were similar to those found in our previous report[12]. Prevalence of heavy metals in *M. oleifera* products especially elevated levels of As, Cd, Hg, and Pb, pose severe health risks to the consumers. Continuous monitoring of heavy metals in *M. oleifera* products is important for quality control of *M. oleifera* products.

Acknowledgements:
This research work was supported by the Office of the National Research Council of Thailand (NRCT) and the Faculty of Pharmacy, Silpakorn University, Nakhon Pathom, Thailand.

Financial support and sponsorship:
This research work was supported by the Office of the National Research Council of Thailand and the Faculty of Pharmacy, Silpakorn University, Nakhon Pathom, Thailand.

Conflicts of interest:
There are no conflicts of interest.

REFERENCES

1. Ghasi S, Nwobodo E, Ofili JO. Hypocholesterolemic effects of crude extract of leaf of *Moringa oleifera* Lam in high-fat diet fed wistar rats. J Ethnopharmacol 2000;69:21-5.
2. Rajanandh M, Satishkumar M, Elango K, Suresh B. *Moringa oleifera*

Lam. a herbal medicine for hyperlipidemia: A pre–clinical report. Asian Pac J Trop Dis 2012;2:S790-5.
3. Vongsak B, Sithisarn P, Mangmool S, Thongpraditchote S, Wongkrajang Y, Gritsanapan W. Maximizing total phenolics, total flavonoids contents and antioxidant activity of *Moringa oleifera* leaf extract by the appropriate extraction method. Indian Crop Prod 2013;44:566-71.
4. Jaiswal D, Kumar Rai P, Kumar A, Mehta S, Watal G. Effect of *Moringa oleifera* Lam. leaves aqueous extract therapy on hyperglycemic rats. J Ethnopharmacol 2009;123:392-6.
5. Chumark P, Khunawat P, Sanvarinda Y, Phornchirasilp S, Morales NP, Phivthong-Ngam L, *et al*. The *in vitro* and *ex vivo* antioxidant properties, hypolipidaemic and antiatherosclerotic activities of water extract of *Moringa oleifera* Lam. leaves. J Ethnopharmacol 2008;116:439-46.
6. Cáceres A, Saravia A, Rizzo S, Zabala L, De Leon E, Nave F. Pharmacologic properties of *Moringa oleifera* 2: Screening for antispasmodic, antiinflammatory and diuretic activity. J Ethnopharmacol 1992;36:233-7.
7. Usman M, Barhate S. Phytochemical investigation and study of antiinflammatory activity of *Moringa oleifera* Lam. Int J Pharm Res Dev 2011;3:114-9.
8. Hamza AA. Ameliorative effects of *Moringa oleifera* Lam seed extract on liver fibrosis in rats. Food Chem Toxicol 2010;48:345-55.
9. Moyo B, Masika P, Hugo A, Muchenje V. Nutritional characterization of Moringa (*Moringa oleifera* Lam.) leaves. Afr J Biotechnol 2011;10:12925-33.
10. Obi E, Akunyili DN, Ekpo B, Orisakwe OE. Heavy metal hazards of Nigerian herbal remedies. Sci Total Environ 2006;369:35-41.
11. Limmatvapirat C, Limmatvapirat S, Charoenteeraboon J, Phaechamud T. Inductively coupled plasma mass spectrometric determination of heavy metals in *Moringa oleifera* Lam. leaves. Res J Pharm Biol Chem Sci 2013;4:161-8.
12. Limmatvapirat C, Limmatvapirat S, Charoenteeraboon J, Phaechamud T. Simultaneous analysis of eleven heavy metals in *Moringa oleifera* Lam. leaf capsules by ICP-MS, in: Proceedings of the International Conference on Life Science and Biological Engineering (LSBE), Tokyo, Japan; October, 2013.
13. Commission Decision 2002/657/EC, Council Directive 96/23/EC, The Performance of Analytical Methods and the Interpretation of Results; August, 2002.
14. Commission Regulation (EC) No. 333/2007, The Methods of Sampling and Analysis for the Official Control of the levels of Lead, Cadmium, Mercury, Inorganic tin, 3-MCPD and Benzo (a) Pyrene in Foodstuffs; March, 2007.
15. Sauveur A, Broin M. Growing and Processing *Moringa* leaves. *Moringa* news, *Moringa* Association of Ghana, France; 2010. p. 55-63.
16. Agyarko K, Darteh E, Berlinger B. Metal levels in some refuse dump soils and plants in Ghana. Plant Soil Environ 2010;56:244-51.