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Inductively Coupled Plasma Mass Spectrometric Determination of Heavy Metals in *Moringa oleifera* Lam. Leaves

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ABSTRACT

Leaves of *Moringa oleifera* Lam. collected from urban and rural gardens in Thailand were determined for 11 heavy metals (Al, As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, and Zn) by using inductively coupled plasma - mass spectrometry (ICP-MS). At urban and rural gardens, the concentrations of Al, As, Cd, Cr, Hg, Mn, Ni, and Pb in *M. oleifera* leaves were found within permissible limits and normal ranges. In contrast, Cu and Zn concentrations in some samples of *M. oleifera* leaves from both gardens were higher than permissible limits. Fe concentrations in all samples from both gardens also exceeded the permissible limit. Therefore, consumers should be aware of the risk for ingestion of *M. oleifera* leaves due to the excess concentrations of Cu, Fe, and Zn. However, heavy metal concentrations in *M. oleifera* leaves from urban gardens were higher than those from rural gardens. It is concluded that the environmental pollution in urban areas played a significant role in increasing the concentrations of heavy metals which might pose a threat to the quality of *M. oleifera* leaves with consequences for consumer health.
Keywords: *Moringa oleifera*, Heavy Metals, Leaves, ICP-MS

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INTRODUCTION

Moringa oleifera Lam. (Moringaceae) is distributed in many countries such as Thailand, Cambodia, India, and Pakistan [1]. This plant is used as a vegetable and medicinal herb [2]. *M. oleifera* leaves are a good source of *β*-carotenes, amino acids, phenolic compounds, vitamins, and minerals, especially calcium and iron [3]. Therefore, the leaves are used as antitumor and antirheumatic drugs as well as cardiac and circulatory stimulants in folk medicine [2]. Furthermore, various parts of *M. oleifera* such as leaves, roots, seeds, barks, fruits, flowers and immature pods act as antipyretic, antiepileptic, anti-inflammatory, antiulcer, antispasmodic, diuretic, antihypertensive, cholesterol lowering, antioxidant, antidiabetic, hepatoprotective, antibacterial and antifungal activities [4].

Aluminium (Al), arsenic (As), cadmium(Cd), chromium (Cr), copper (Cu), iron (Fe), mercury (Hg), manganese (Mn), nickel (Ni), lead (Pb), and zinc (Zn) are heavy metals found in common edible plants. And some of them especially As, Cd, Hg, and Pb are environmental pollutants and toxic to human health. They enter the human body through food, air, and water. As is used as pesticides and semiconductors while Cd and Pb are used in various types of industries, especially batteries and paints [5]. Exposure to Hg occurs through the use of disinfectants, antifungal agents, and organometallics [6]. As a consequence of increasing population, automobile exhausts, and industrial factories, the environment is affected by various heavy metals. Plants grown in heavy metal contaminated soil and water can translocate and accumulate those metals into their tissues [7]. Human exposure to heavy metals by consuming heavy metal contaminated plants makes it necessary to monitor these metals in edible plants.

The concentrations of 11 heavy metals such as Al, As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, and Zn have not been reported in *M. oleifera* leaves grown in Nakhon Pathom province, Thailand. The purpose of this pilot study was to determine the concentrations of those 11 heavy metals in *M. oleifera* leaves that cultivated in urban and rural gardens in Nakhon Pathom province, a growing city in Thailand, by using inductively coupled plasma - mass spectrometry (ICP-MS). Data obtained are needed to evaluate the risk and quality of *M. oleifera* leaves.

MATERAILS AND METHODS

Materials

The ultrapure water ASTM type I, 18.2 MΩ x cm used for determination of 11 heavy metals was generated by a TKA Gen Pure ultra pure water machine (TKA Was seraufbereitungs systeme GmbH, Germany). Nitric acid, an analytical reagent grade (lot K40352656 935), used for digestion was purchased from Merck, Darmstat, Germany. An ICP multi element standard solution XIII (Lot HC813513, Agilent, USA) was diluted with 5% v/v nitric acid in ultrapure water. All laboratory glassware containers used for metal analysis were immersed in 20% v/v nitric acid solution overnight and rinsed several times with ultrapure water to eradicate metal contamination.

Plant Material

M. oleifera plants were cultivated in organic gardens using natural fertilizers and pesticides. Leaves of *M. oleifera* were harvested from urban and rural gardens in 7 districts (amphoes) including Mueang Nakhon Pathom (MNP), Kamphaeng Saen (KS), Nakhon Chai Si (NCS), Don Tum (DT), Bang Len (BL), Phutthamonthon (P), and Sam Phran (SP) in Nakhon Pathom province, Thailand, during the period September 2011 to December 2011. The immature leaves were collected at approximately 2 meters of the tree's height. The leaf samples were placed in plastic bags, labeled, sealed, and sent to the laboratory immediately. The voucher specimens were deposited in the Faculty of Pharmacy, Silpakorn University, Thailand.

Plant Treatment

The fresh leaves of *M. oleifera* were washed 3 times in distilled water and rinsed 3 times with ultrapure water. All samples were completely dried in hot air oven at 50°C. The dried leaves were then grounded separately with an IKA MF-10 Microfine Grinding Mill (Werke GmbH & Co. KG, Germany), equipped with a 0.5 mm pore size sieve and high-grade stainless steel grinders. The powdered samples were stored in dried plastic bags inside a desiccator to protect them from moisture before nitric acid digestion.

Sample Digestion

The digestion method was modified from that of Zheljazkov and Warman [8]. Each powdered sample (1 g) was digested with 60% v/v nitric acid solution in a 100 ml Pyrex beaker covered with watch glass on a hot plate at 120-130°C. The clear digested solution was allowed to cool down to room temperature and then diluted by adding ultra pure water to the final volume before analysis by using ICP-MS. Each powdered sample was digested in triplicate.

Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)

The concentrations of 11 heavy metals (Al, As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, and Zn) in diluted solutions were determined by an ICP-MS spectrometer (Model 7500 ce, Agilent). Collision/reaction cells were adopted for removing spectral interferences in ICP-MS. The operating conditions employed for determination were an ICP RF power of 1500 W, an argon nebulizer gas flow rate of 0.9 l/min, and a nebulizer pump speed of 0.1 rps. The data are presented as the mean of triplicate determinations and the results are representative of three independent experiments.

Validation Method

For the quantitative ICP-MS analysis of 11 heavy metals in *M. oleifera* samples, the calibration was most commonly achieved by external standardization. The signal intensities of all isotopes were measured in a blank as well as in 6 different concentrations of standard solutions which covered a concentration range of interest. Standard solutions were prepared in 5% v/v nitric acid solution by diluting an ICP multi element standard solution XIII.

The parameters of method validation including range of linearity, limits of detection (LODs), limits of quantification (LOQs), specificity, precision under repeatability conditions and within-laboratory reproducibility were evaluated. The procedure and calculation were modified according to the European Standard for the analyses of heavy metals [9,10].

RESULTS AND DISCUSSION

Data Validation

For validation of an analytical method, there were no certified reference materials available for estimating 11 heavy metals (Al, As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, and Zn). Therefore, recovery studies of these heavy metals were done in order to validate the method. The recoveries of 11 heavy metals were between 97.98% and 105.21%. For analytical parameters, all the measurements were accomplished using the quantitative mode analysis. The correlation coefficients (r^2) for all calibration curves were at least 0.9989, exhibiting good linear relationships over the concentration ranges. The LODs and LOQs were calculated as three and ten times of the standard deviation (SD) of ten measurements, respectively. The blanks were prepared by the same procedure as used for samples. The LODs for all the studied metals were 2.52 µg Al/L, 4.09 µg As/L, 1.58 µg Cd/L, 0.95 µg Cr/L, 2.38 µg Cu/L, 4.32 µg Fe/L, 3.56 µg Hg/L, 1.25 µg Mn/L, 1.58 µg Ni/L, 4.89 µg Pb/L, and 2.18 µg Zn/L. The LOQs were 7.36 µg Al/L, 12.35 µg As/L, 4.80 µg Cd/L, 3.15 µg Cr/L, 7.19 µg Cu/L, 17.15 µg Fe/L, 11.05 µg Hg/L, 4.12 µg Mn/L, 4.59 µg Ni/L, 16.85 µg Pb/L, and 6.49 µg Zn/L. The precision was expressed by the relative standard deviations (RSD) for n=10. The intraday repeatability (RSD, 0.235 to 1.347%) and interday reproducibility (RSD, 0.351 to 1.430%) exhibited the good precision. This method is suitable for determination of 11 heavy metals in *M. oleifera* leaves.

Heavy Metal Concentrations in *M. oleifera* Leaves

Heavy metals are considered potential carcinogens and are associated with various diseases, such as cardiovascular disease, bone disease, kidney disease, gastrointestinal diseases, reduced general intellectual capacity, and cancer [11]. Serious health problems can occur as a result of excessive accumulation of heavy metals from eating contaminated herbal plants. Due to the toxicity of heavy metals in herbal plants, permissible limits of various heavy metals have been set. In Thai Herbal Pharmacopoeia 2000, the permissible limits of heavy metals in herbal plants have been fixed for As (4 mg/Kg), Cd (0.3 mg/kg), and Pb (10 mg/Kg) based on acceptable daily intake (ADI) [12]. Under the regulation of the prevention of food adulteration act, 1986 in Thailand, the permissible limits for Cu, Fe, Hg, and Zn in herbal plants have been set at 5, 15, 0.5, and 5 mg/Kg, respectively [13]. The data about heavy metal concentrations in *M. oleifera* leaves used as traditional medicines and food is very important for evaluation the risk to human health. Therefore, the concentrations of heavy metals in *M.*

oleifera leaves were determined in the present work. Leaf samples of *M. oleifera* were harvested from urban gardens and rural gardens in 7 districts of Nakhon Pathom province, Thailand, such as Mueang Nakhon Pathom (MNP), Kamphaeng Saen (KS), Nakhon Chai Si (NCS), Don Tum (DT), Bang Len (BL), Phutthamonthon (P), and Sam Phran (SP). Urban gardens were located near busy roads while rural gardens were located far from busy roads. The leaf samples were digested with nitric acid and then were analyzed by ICP-MS. Heavy metal concentrations from the analysis, as well as permissible limits from Thai Herbal Pharmacopoeia and the prevention of food adulteration and normal ranges from the previous report, are shown in Table 1.

As shown in Table 1, all leaf samples from urban and rural gardens in 7 districts were safe from As, Cd, Hg, and Pb since the concentrations of those heavy metals were less than permissible limits (4, 0.3, 0.5, and 10 mg/Kg, respectively). However, the concentrations of 11 heavy metals (Al, As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, and Zn) in almost all *M. oleifera* leaf samples from urban gardens were higher than those from rural gardens. These results implied that the concentrations of heavy metals were increased when gardens exposed to environmental pollution, especially vehicular pollution. A previous report showed that heavy metals accumulated in plant leaves originated from environmental pollution, especially heavy metals spewed by high traffic [7]. The present study attempts to reveal that air pollution in urban areas should reflect the concentrations of heavy metals accumulated in plant leaves. Therefore, the consumption of *M. oleifera* leaves cultivated in urban gardens close to the traffic streets is an important factor considered by consumers.

Although Cu, Fe, and Zn are essential elements, their excessive concentrations in herbal plants can cause toxicity in consumers [14]. The concentrations of Cu, Fe, and Zn are shown in Table 1. The concentrations of Cu in leaf samples from urban and rural gardens in MNP, KS, NCS, DT, and BL were lower than the permissible limit (5 mg/Kg), whereas those concentrations in P (urban 6.459, rural 5.506 mg/Kg) and SP (urban 8.462, rural 6.625 mg/Kg) were higher than Cu permissible limit. The concentrations of Fe in all leaf samples from urban and rural gardens in 7 districts were between 22.130 and 65.356 mg/Kg that were higher than the permissible limit (15 mg/Kg). The concentrations of Zn in leaf samples from urban and rural gardens in MNP, DT, and BL were lower than permissible limit (5 mg/Kg), whereas those concentrations in KS (urban 6.302, rural 5.868 mg/Kg), NCS (urban 5.618, rural 5.126 mg/Kg), P (urban 14.329, rural 9.861 mg/Kg), and SP (urban 14.235, rural 14.213 mg/Kg were higher than permissible limit (5 mg/Kg). However, the highest concentrations of Cu (8.462 mg/Kg), Fe (65.356 mg/Kg), and Zn (14.235 mg/Kg) were found in the leaf samples from an urban garden in SP. From the comparison of Cu, Fe, and Zn concentration ranges in *M. oleifera* leaves between the previous report and the present study, the results indicate that those concentration ranges (Cu 0.792-8.462, Fe 22.130-65.356, and Zn 1.100-14.235 mg/Kg) of most samples in the present study were lower than those concentration ranges (Cu 7-11, Fe 180-280, and Zn 15-30 mg/kg) in the previous report [15]. In conclusion, although Cu, Fe, and Zn concentrations in all leaf samples were lower than those concentrations in the previous report, all leaf samples showed Fe concentrations higher than the permissible limit (15 mg/Kg) and some leaf samples showed Cu and Zn concentrations higher than the permissible limits (Cu 5, Zn 5 mg/Kg) as shown in Table 1. It was suggested that the samples of *M. oleifera* leaves might be unsafe from Cu, Fe, and Zn.

Table 1. The concentrations (mg/Kg) of 11 heavy metals in *M. oleifera* leaves

Districts and gardens	The concentrations (mg/Kg) of 11 heavy metals										
	As	Cd	Hg	Pb	Cu	Fe	Zn	Al	Cr	Mn	Ni
MNP*											
Urban garden	n.d.	n.d.	n.d.	n.d.	1.325	41.437	3.120	39.347	0.128	41.031	1.421
Rural garden	n.d.	n.d.	n.d.	n.d.	0.792	22.328	1.979	35.208	0.085	33.249	1.065
KS*											
Urban garden	0.039	n.d.	0.025	n.d.	1.985	49.256	6.302	45.392	0.135	43.089	1.689
Rural garden	0.003	n.d.	n.d.	n.d.	0.806	35.489	5.868	38.961	0.096	34.685	1.230
NCS*											
Urban garden	0.031	0.004.	0.112	2.561	2.355	53.289	5.618	53.610	0.385	49.107	2.178
Rural garden	n.d.	n.d.	n.d.	0.012	0.929	46.231	5.126	42.639	0.260	43.728	1.579
DT*											
Urban garden	n.d.	n.d.	n.d.	n.d.	1.239	40.312	3.597	36.985	0.371	43.126	1.053
Rural garden	n.d.	n.d.	n.d.	n.d.	0.896	22.130	1.100	31.220	0.210	32.120	0.990
BL*											
Urban garden	n.d.	n.d.	0.002	1.236	4.356	43.156	3.347	45.128	0.237	43.029	1.436
Rural garden	n.d.	n.d.	0.001	0.963	3.456	31.179	2.208	30.065	0.028	36.251	1.236
P*											
Urban garden	0.005.	0.006	0.472	2.450	6.459	59.963	14.329	53.267	0.878	48.081	2.126
Rural garden	n.d.	n.d.	0.124	1.239	5.506	38.965	9.861	40.131	0.376	34.698	1.691
SP*											
Urban garden	1.014	0.007	0.152	3.459	8.462	65.356	14.235	65.230	0.285	49.157	3.168
Rural garden	0.821	0.002	0.004	2.105	6.625	49.230	14.213	56.639	0.456	48.728	2.563
Permissible limits	4#	0.3#	0.5▽	10#	5▽	15▽	5▽	-	-	-	-
Normal ranges□	-	-	-	-	-	-	-	1,000	0.03-14	50-90	0.02-5

n.d.: not determined; %SD: 0.005-0.625

*Mueang Nakhon Pathom (MNP), Kamphaeng Saen (KS), Nakhon Chai Si (NCS), Don Tum (DT), Bang Len (BL), Phutthamonthon (P), and Sam Phran (SP)

#Permissible limits set by Thai Herbal Pharmacopoeia 2000 [12]

▽Permissible limits set by the prevention of food adulteration act, Thailand, 1986 [13]

□Normal ranges set by the previous reports [15-17]

There is no information about permissible limits of Cr, Ni, Mn, and Al in herbal plants set by Thai Herbal Pharmacopoeia [12] and the regulation of the prevention of food adulteration act [13]. The results obtained for the concentrations of Cr, Ni, Mn, and Al in *M. oleifera* leaf samples are shown in Table 1. The concentration ranges of Cr, Ni, Mn, and Al were 0.028-0.878, 0.990-3.168, 32.120-49.157, and 30.065-65.230 mg/Kg, respectively. According to average value, the heavy metal concentrations in the leaf samples were in the order of Al (43.844 mg/Kg) > Mn (41.434 mg/Kg) > Ni (1.673 mg/Kg) > Cr (0.281 mg/Kg). The highest concentrations of Ni (3.168 mg/Kg), Mn (49.157 mg/Kg), and Al (65.230 mg/Kg) were found in the leaf sample from an urban garden in SP while the highest concentration of Cr (0.878 mg/Kg) was detected in the leaf sample from an urban garden in P. The concentration range of Mn in *M. oleifera* leaves in the present study (32.120-49.157 mg/Kg) was lower than that concentration range in the previous report (50-90 mg/Kg) [15]. There is no previous report of Cr, Ni, and Al concentrations in *M. oleifera* leaves. However, the concentration ranges of Cr (0.028-0.878 mg/Kg) and Ni (0.990-3.168 mg/Kg) in *M. oleifera* leaf samples were in the normal ranges of Cr (0.03-14 mg/Kg) and Ni (0.02-5 mg/Kg) in general plant leaves [16]. Furthermore, the concentration range of Al in *M. oleifera* leaves in the present study (30.065-65.230 mg/Kg) was lower than the concentration (1,000 mg/Kg) in approximately 25 plant families accumulating Al in their leaves [17]. The results suggested that the samples of *M. oleifera* leaves might be safe from Cr, Ni, Mn, and Al.

The above results showed that except for Cu, Fe, and Zn, all other concentrations of heavy metals including As, Cd, Hg, Pb, Cr, Ni, Mn, and Al in *M. oleifera* leaf samples were found within permissible limits and normal ranges. It was determined that *M. oleifera* leaves in the present study were probably unsafe for human consumption because of Cu, Fe, and Zn concentrations. However, some heavy metals especially for As, Cd, Hg, and Pb are toxic heavy metals and their presence even at very low concentration causes toxic effects to human health [18]. Therefore, people should be aware of the daily consumption of heavy metals accumulated in *M. oleifera* leaves.

CONCLUSION

In the present study, *M. oleifera* leaves showed high concentrations of Al, Fe, and Mn and low concentrations of As, Cd, Hg, Pb, Cu, Zn, Cr, and Ni. However, the concentrations of As, Cd, Hg, Pb, Cr, Ni, Mn, and Al were found within permissible limits and normal ranges while those of Cu, Fe, and Zn were higher than permissible limits. Therefore, people should be aware of the potential risks of heavy metals associated with the consumption of *M. oleifera* leaves containing Cu, Fe, and Zn in excess of permissible limits. In Thailand, the quality standard for heavy metals in *M. oleifera* leaves is still unclear. The results of the present study show the need for a systematic control of heavy metals in *M. oleifera* leaves used as herbal medicines.

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