Original Research Paper

Volume - 7 | Issue - 8 | August - 2017 | ISSN - 2249-555X | IF : 4.894 | IC Value : 79.96

Food Science

HEAVY METAL CONTAMINATION IN SOIL AND PLANT TISSUES OF *Andrographis paniculata* (Burm.f.) Wall. ex Nees HARVESTED FROM DIFFERENT PLANTING LOCATIONS.

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ABSTRACT As one of the valuable herbs, *Andrographis paniculata* contains many bioactive compounds for medicinal purposes. However, it can also contain some undesirable components if not properly handled that can be harmful such as heavy metals. Therefore, the objective of this study was to determine heavy metals contaminations in soils and tissues of harvested plants from three different planting locations, University Agriculture Park (TPU), Universiti Putra Malaysia (UPM); Department of Agriculture, Serdang; and Bukit Pulau village, Melaka. Results showed that Al, As, Zn, Cr, Cu, Hg, Ni and Pb were detected in soil but only Al, Cr, Cu, Ni and Zn were detected in tissues. Contents of Al, Zn, Cr and Ni in soil were found significantly higher in Bukit Pulau village, but, TPU soil displayed higher concentrations of As, Cu and Pb. Samples collected from Department of Agriculture, Serdang had lower concentrations of Al, Zn, Cr and Cu than two other locations. Fortunately, all detected elements in soils or plant tissues were generally below safe levels as issued by local and international authorities.

KEYWORDS : *Andrographis paniculata*, heavy metals, soil, plant tissue.

1. Introduction

Andrographis paniculata or *hempedu bumi* generally are used in many cultures around the world for centuries to treat fever and infectious diseases. According to Chinese medicine theory, *A. paniculata* 'cools' and relieves internal heat, inflammation and pain and is used for detoxication (Mandal et al., 2001; Huang and Wu, 2002; Chao et al., 2009). Andrographolide is considered to be the most active and valuable constituent which is responsible for the therapeutics of the plant (Parichatikanond et al., 2010). However, this biomarker will be affected if raw materials are contaminated with heavy metals.

Many studies have shown that medicinal plants are able to accumulate heavy metals from contaminated soils and there is no exception with *A. paniculata.* These elements may be a necessity for the growth of plants or human consumption, but some of micronutrients (eg, Cu, Cr and Ni) may be toxic at high concentrations (Mclaughlin et al., 1999; Rahman et al., 2014). Atmospheric sedimentation of heavy metals accrued from mining, smelting and industrial activities had become a major source of soil contamination (Islam et al., 2015) nevertheless, the use of fertilizers, pesticides, sewage sludge, organic fertilizer and compost in agriculture also contributes to this contamination (Cui et al., 2005; Zheng et al., 2007; Islam et al., 2015,). Heavy metals uptake by *A. paniculata* is through metal transfer from sediments and water to food web (Mythili et al., 2011). All heavy metals can cause toxic effects to plants and subsequently, to humans if consumed in high concentrations. There are three main considerations in relation to heavy metal contaminations in herbal medicinal products; contamination during cultivation, cross-contamination during processing and/or intentional introduction of heavy metals as a therapy (Rahimi et al., 2012).

From the aspect of contamination during cultivation, it must be closely related to the agronomic practices. Usually, agricultural lands in many places of the world are polluted with heavy metal such as Cd, Cu, Zn, Ni, Co, Cr, Pb, and As. Several studies had proven that significant accumulation of metals in the soil in some areas are caused by agricultural, industrial or urban developments in China (Naveedullah et al., 2013, Bloemen et al., 1995, Gaw et al., 2006). However, under Malaysian scenario, contamination studies are still not exploited to the fullest. There are still gaps need to fill with study like this. Therefore, the objective of this study was to determine heavy metal concentrations in soils and tissues of A. paniculata harvested from different farms in various locations. This is to ascertain whether heavy metal contaminations of commercially grown *A. paniculata* are still within the safety limit or otherwise.

2. Materials and methods

2.1 Sampling Locations

The materials of study consisted of soil and plant tissue samples which were collected randomly from three different locations i.e. Vegetables Unit, University Agriculture Park (TPU), Universiti Putra Malaysia, Serdang, Selangor with an area (24 m × 27 m) planted with *A. paniculata.* The second location was in Bukit Pulau village, Melaka where the plants were grown scattered in between other herbs in an area of approximately 300 m^2. The final sampling location was in Agriculture Department, Serdang, Selangor where the plants were planted in an area of 20 m^2 with planting distance at 30 cm × 30 cm in 10 rows. Samplings were carried out in the morning to ensure that the plant samples were at optimum freshness and reached the laboratory in garden-fresh state.

2.2 Plant materials and soil sampling

Heavy metals determined were Al, As, Cd, Cr, Cu, Hg, Ni, Pb and Zn with four replications from each location. One hundred grams of leaves samples were collected randomly. Meanwhile, soil samples were dug using an auger at approximately 0 - 20 cm from soil surface (Cabrera et al., 1999; Cebula and Ciba, 2005). Four soil samples, representing four replications, were collected, and for every hole dug, approximately 500 grams was taken. In total, 2 kg of soil was collected from each location. Then, all the samples (soil and plant) were brought to the Postharvest Laboratory, Faculty of Agriculture, Universiti Putra Malaysia. Plant samples were washed thoroughly with running tap water to eliminate dust, dirt and possible parasites. Meanwhile, all other materials, like dead leaves and herbages, were removed from the soil samples.

2.3. Total heavy metal concentrations in soil

Four replications of soil sample for each location were dried at 105 °C for at least 16 hours until the weights became constant. Then, the dried soil samples were crushed by using a pestle and mortar to a size that can passed through a 63 µm stainless steel sieve under vigorous shaking to produce a homogeneous sample. Two grams of the prepared soil sample was digested with 15.0 ml nitric acid, 20.0 mL perchloric acid and 15.0 mL hydrofluoric acid and placed on a digestion block for three hours. After that, samples were left to cool for 1 min, then, transferred quantitatively with water added to a final volume of 100 mL. Final solutions of digested samples were stored at 4 °C prior to analysis. Extracted heavy metals in the soil samples were determined using the method of Opaluwa et al. (2012) and analysed using ICP-OES (Optima 8300, Pelkin Elmer, USA) equipment at Instrumentation Laboratory, Department of Land Management, UPM. Calibration standards, verification standards, and reagent blanks were included in the sample sequence. The standard solutions of elements Al, As, Cr, Cd, Cu, Hg, Ni, Pb and Zn were prepared in five different concentrations to obtain calibration curve for each element.

2.4. Total heavy metals concentrations in plant tissues

Leaf samples of *A. paniculata* (100 g) were dried in a convection oven at 60 °C for 24 hours until constant weights were obtained (Arık and Yaldız, 2010). Twenty-five milligram of sample was accurately weighed and added into a digestion flask. Digestion method was performed by heating at 100 °C with 8 mL of 51% sulphuric acid in a block digester, followed by evaporation almost to dryness. When the sample was slightly concentrated, 5 mL of hydrogen peroxide was added to the sample. Digestion continued until the colour of the sample solution turned clear. Samples were then filtered through a Whatman No.1 filter paper in a funnel. Then, filtered solution was made up to 100 mL for an accurate dilution with deionized water. Similar to soil sample, plant tissue samples were determined for heavy metal contents using the above ICP-OES machine (mentioned earlier) at the Instrumentation Laboratory, Department of Land Management, UPM. Calibration standards, verification standards, and reagent blanks were also included in the sample sequence.

2.5 Statistical analysis

The experimental design was a completely randomized design (CRD) with four replications. The differences of heavy metal concentrations in soil and tissue samples were analysed by one-way ANOVA at 95% confidence level using SAS version 9.4. The comparisons of treatment means were separated by Tukey's Studentized Range (HSD) test.

3. Results and discussion

The mean concentrations of heavy metals in soil (Table 1) revealed that most of the studied metals were present except for Cd and Hg which was not found in any of the locations. Among these metals, Al was found to be the highest for all locations (Table 1). Bukit Pulau village had significantly higher concentration ($p \leq 0.05$) of Al in the soil compared to other locations. Aluminum present in the soil in various forms and bound to soil constituents and the solubility of aluminum in the soil will increase as soil pH falls. Previous studies reported approximately 500,000 hectares of farm land has acidic soil and possibly high Al content (Haynes and Williams, 1993; Moir et al., 2000; Moir and Moot, 2010). These conditions extended with low available phosphorus and sulfur would limit the establishment and maintenance of legumes as well as affect the yield.

Table 1. Concentrations (mg kg^{-1}) of heavy metals (Al, As, Zn, Cd, Cr, Cu, Hg, Ni, and Pb) in soils collected from three planting locations (Taman Pertanian Universiti (TPU), UPM, Kg. Bukit Pulau, Melaka and Jabatan Pertanian, Serdang, Selangor).

Heavy metal concentration (mg kg^{-1})	Location		
	TPU	Kg Bukit Pulau	Jabatan Pertanian
Al	54.574±1.679b	87.711±5.879a	51.190±1.861b
As	0.212±0.002a	0.057±0.002b	0.063±0.004b
Zn	1.593±0.048a	1.508±0.053a	1.160±0.019b
Cd	ND	ND	ND
Cr	0.169±0.003b	0.847±0.021a	0.121±0.001b
Cu	0.044±0.002a	0.025±0.001b	0.051±0.002a
Hg	ND	ND	ND
Ni	0.025±0.001b	0.040±0.001a	0.011±0.001c
Pb	0.020±0.0005a	0.012±0.0008b	0.003±0.0004c

Means within a row followed by different alphabets are significantly different according to Tukey (HSD) at . SE = standard error, n = 12. ND = Not detected.

The other heavy metals, As, Zn, Cr, Cu, Ni and Pb, had lower concentrations (<2 mg kg^{-1}) in the soil (Table 1) when compared to Al. The lowest concentration of Zn was found in the soil and plant samples from Jabatan Pertanian compared to those from TPU and Kg. Bukit Pulau. Meanwhile, the soil in TPU was discovered to contain a higher concentration of As, about 0.155 mg kg^{-1} and 0.148 mg kg^{-1} more than Kg. Bukit Pulau and Jabatan Pertanian, respectively. Cr was found higher in Kg. Bukit Pulau than TPU and Jabatan Pertanian, 0.847 ± 0.021 mg kg^{-1}. The nickel content in the soil was found to be significantly different between planting locations where the mean concentration of nickel for each location was according the following descending order: Kg. Bukit Bunga TPU Jabatan Pertanian. The concentrations of Pb in soil were highly significantly different between locations. The highest concentration of Pb was detected in the TPU (0.020±0.0005 mg kg^{-1}) while the lowest was recorded in Jabatan Pertanian (0.003±0.0004 mg kg^{-1}). Nevertheless, all determined metals were within typical range of natural occurring metal concentration in Malaysia as mentioned in Table 2.

Table 2. Range of heavy metal (Al, Cd, Cr,Cu, Ni, Pb, Hg, As and Zn) concentrations (mg kg^{-1}) in Malaysian soils.

Heavy metal	Normal range in Malaysian soils (mg kg^{-1})
Al	33500 - 53900
Cd	0.09 - 11.90
Cr	0.02 - 14.40
Cu	4.0 - 19.8
Ni	0.70 - 28.90
Pb	0.18 - 36.00
Hg	0.02 - 0.42
As	1.1 - 43.0
Zn	6.9 - 54.3

Source: Ministry of Natural Resourses and Environment (2009).

Heavy metals may happens naturally from normal geological phenomenon (formation, weathering of rocks, and leaching) as well as the activities of exploration and exploitation of natural resources, industrial activities, agricultural practices, increasing population or urbanization (Al-Oud et al., 2011). Indeed, heavy metals in the environment can accumulate to toxic levels without any indication that can be seen clearly. However, we need to make sure contamination does not happen more rampant. Monitoring the heavy metals contamination of the soil had its own significance due to their influence on the ground and surface water, plants, animal and subsequently human health(Al-Oud, 2008; Suciu et al., 2008; Al-Oud et al., 2011).

The concentrations of Al, Zn, Cr, Cu and Ni (mg kg^{-1}) were determined in *A. paniculata* tissues planted in the same location where the soil samples were taken (TPU, Kg. Bukit Bunga and Jabatan Pertanian) and presented in Figure 1. Al was detected in plant tissues with no significant difference between Kg. Bukit Bunga and TPU but had substantial differences around 3.1 to 3.2 mg kg^{-1} higher, respectively, than Jabatan Pertanian.

A similar trend was observed on Cu content in the plant tissue. The lowest concentration of Cu was found in the Jabatan Pertanian, Serdang which is about 44 to 57% less than Kg. Bukit Bunga and TPU. However, Zn and Cr concentration were found to be significantly different between planting locations, the mean concentration of Zn in the plant tissues followed the descending order of planting locations: TPU Kg. Bukit Bunga Jabatan Pertanian. *A. paniculata* collected from TPU contained significantly (($p \leq 0.05$) higher Ni content (0.014±0.001 mg kg^{-1}) compared to Kg. Bukit Pulau and Jabatan Pertanian. Usually, some of the metals, like Al, Zn, Cu and Ni, are micronutrient elements for most biological systems in the plant and also human at appropriate concentrations, but if the dose is too high they could become toxic.

In general, this study showed that heavy metal concentrations in soils and plant tissues depended on planting locations. Generally, most of heavy metals in the plant were higher in TPU than other locations. This area has been actively used for planting activities for teaching or research purposes. Agriculture or planting activities are commonly involved a large amount agrochemical like fertilizer, pesticides, insecticides etc. (Naveedullah et al., 2013), thus, this condition could explain the presence of heavy metals in plant tissue of *A. paniculata.* Compositions of heavy metals in plants were strongly influenced by the level of heavy metals in soil, plant species and contaminants (Tahar and Keltoum, 2011). Cr is a non-essential compound that is extremely toxic to plants which could be detrimental to their growth and development, and it may be possible that the plants did not feature any specific mechanism of Cr transportation (Shanker et al., 2005; Ibnu Hajar et al., 2014). Toxic elements, such as As, Hg, Cd and Pb, in plants might accumulate to a toxic concentration, subsequently may raise potential health risks. The risks associated with metal contamination in herbal medicines are of great concern, a particularly element of As, Cd, Pb and Hg. Thus, the present of these elements in the soil should be given the utmost attention. It was very fortunate that all three locations had no detection of Cd and Hg, whereas these circumstances will lead to a lack of opportunities for Cd and Hg absorbed by *A. paniculata.* A high concentration of Cd in herbal medicine is one of the elements that triggered concerns over human health.

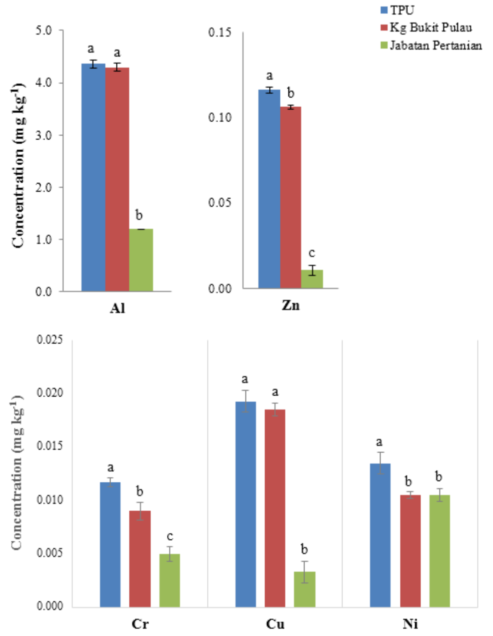

Figure 1. Concentration of Al, Zn, Cr, Cu and Ni in *A. paniculata* tissue collected from three planting locations, Taman Pertanian Universiti, UPM, Kg Bukit Pulau, Melaka and Jabatan Pertanian, Serdang, Selangor.

Means within a bar followed by different alphabets are significantly different according to Tukey (HSD) at $p \leq 0.05$. n = 12.

Furthermore, various herbal medicines, including ginseng and other Chinese herbal medicine purchased from Italy, United States, Europe and Asia, have already been detected with high concentrations of Cd, reaching 0.75 mg.L^{-1} (De Pasquale et al., 1993; Khan et al., 2001; Ting et al., 2013). For instance, the elevated concentrations of As and Pb in foods could probably be due to the ground water irrigation containing As (Hug et al., 2011; Neumann et al., 2011), the use of fertilizers and pesticides fortified with As (Renner, 2004; Islam et al., 2014), lead smelting, emission from vehicles and other industrial activity in the area of cultivation (Islam et al., 2015).

However, Galfati et al. (2011) reported that some plants can tolerate high levels of heavy metals. Cu content in the leaf extract of *A. paniculata* was less than previously studied by Mythili et al. (2011), 1.0 mg.kg^{-1}. Leaf extract of *A. paniculata* was also found to be higher in Cr content (5.7 mg.kg^{-1}) rather than other parts of the plant (root: 14.45 mg.kg^{-1} and stem: 14.9 mg.kg^{-1}) (Mythili et al., 2011). Although the contents of some heavy metals were comparatively higher in *A. paniculata* tissues, yet it is still safe for consumption since it is below the safe levels prescribed by relevant authorities (Table 3). This is consistent with previous reports that plants can alter the solubility of metals (McLaughlin et al., 1999), primarily through the production and secretion and modification of soil microbial activity (Galfati et al., 2011).

Table 3. Permissible limits of heavy metals in finished herb/food products.

Heavy metal	Concentration (mg kg^{-1}; mg L^{-1} or ppm)	References
Al	10	European Commission[a]
Cd	0.3	World Health Organization[b]
Cr	2	
Cu	150	
Ni	100	
Pb	10	
Hg	0.2 - 0.5	
As	2 - 5	
Zn	50	World Health Organization[c]
Sources: [a] Walker (2011), [b] WHO (2007), [c] WHO (2005)		

4. Conclusion

Nowadays, the impact of economic development activities had contributed to the high levels of heavy metal contamination on the environment. Hence, *A. paniculata* which recognized as one of the herbal medicine could be contaminated to heavy metals directly through soil and water. The tolerance to heavy metals in *A. paniculata* tissue collected from different planting locations were presented a variety of elements such as aluminium (Al), arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), nickel (Ni), lead (Pb) and zinc (Zn). Heavy metals in the soils and plant tissues vary by location. The results found that level of heavy metals in soil collected from *A. paniculata* plantation area does not exceed the international safety levels. Instead of eight heavy metals detected in the soil, only Al, Zn, Cr, Cu and Ni were found to accumulate in the tissues of *A. paniculata* and it also below the allowable limits as issued by WHO.

Acknowledgement

The project was funded by the NKEA Research Grant Scheme (NRGS), Ministry of Agriculture Malaysia. The authors wish to thanks to all staff in Department of Crop Science, Universiti Putra Malaysia for all the cooperation given while the project is implemented.

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