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RESEARCH ARTICLE

Evaluation of Heavy Metals in Selected Medicinal Plants and their Corresponding Soils collected from Environmentally Diverse Locations of India

Kalpana P[1], Krishnamurthy Balasubramanian[2], R A Kalaivani[3]
[1]School of Basic Sciences, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Chennai, India
[2]Senior Advisor, National Agro Foundation
[3]Director, School of Basic Sciences, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Chennai, India
*Corresponding Author E-mail: **kalpsrajesh@gmail.com**

ABSTRACT:
The study aimed to determine the metal accumulation levels of *Centella asiatica* and *Bacopa monneri* from environmentally diverse regions in India. Accumulation of heavy metals in human tissues through intake of herbal preparations can cause serious impact on the health of the human beings. *Bacopa* monneri and *Centella asiatica* have been used traditionally as folk medicines. Therefore, metal accumulation of seven heavy metals (Mn, Cu, Pb, Ni , Cd, Fe and Zn) was taken up in composite (leaf, roots and stem) samples of two medicinal herbs (*Centella asiatica and Bacopa monnieri*) collected from diverse locations of Tamil Nadu, Karnataka, Assam. Background concentrations of heavy metals in soils are important due to their pollution potential and toxic effect of these metals on health aspects of human beings and the environment. Therefore, investigation of heavy metals in the soil in which the medicinal plants were cultivated was taken up in order to ascertain the contamination status.

KEYWORDS: Heavy metals, Medicinal plants, Soil contamination, Environment, Health impacts.

INTRODUCTION:
Herbal medicines are being extensively used for disease treatment, prevention and management worldwide. They, being natural, are preferred over the synthetic remedies because of their health benefits. Indians, Egyptians, Chinese and others have employed different plants and plant products for curing all kinds of ailments since ancient times. More than 25,000 plant based formulations are available in the indigenous medical texts[1]. As per World Health Organization report[2] more than 60% of the world's population depends on traditional medicine for healthcare needs primarily. In the recent years, the use of herbal medicines has come under scrutiny due to their perceived long term toxicity among other aspects.

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Toxic nature of various plants, which could be due to their chemical and mineral contents, has been attributed to the source of the material[3]. The concentration of minerals including heavy metals in the medicinal plant species used in herbal preparations have to be given due importance considering their vital role in functioning of human body. These minerals are essential for the general well-being of body[4]. But, these minerals may be toxic if the intake is more than the recommended levels per day. Medicinal herbs can cause health hazards due to the presence of heavy metals such as Cd, Ni and Pb which are harmful to humans[5]. Heavy metals affect the human health directly, when people inhale dust containing heavy metals and indirectly, due to contaminated drinking water and food[6,7,8]. Studies have reported that health consequences due to heavy metal exposure differ significantly based on the specific mineral, vulnerability of the exposed population and the pathway of attack[7,8]. Some of the factors that influence the concentrations of these metals in medicinal plants include the type of plant species, air pollution, climatic conditions and other environmental factors[9].

Bacopa monnieri (L.) Pennell is known to grow well in the areas polluted with sewage waste and industrial effluents. *Bacopa* has been recommended as an agent for phytoremediation[10,11,12]. *Bacopa monnieri* has been reported as an accumulator of copper and cadmium[11] and mercury[13]. Sinha[14] also reported that in addition to copper and cadmium this species accumulates chromium, manganese and lead from artificially contaminated soil. The ability of *Bacopa monnieri* to accumulate heavy metals has been reported and repeated cultivation of this plant in polluted areas has been recommended and practiced as a measure of phytoremediation of wetlands polluted with heavy metals[13]. In the perspective of medicinal uses, the phytoremediant property of *Bacopa monnieri* is seemed to be dangerous because this species is an important ingredient of many Ayurvedic medicines and neutraceutical products[15,16,17]. Prospects of *Bacopa* plant to accumulate As, Cr, Fe, Cd, Cu, Ni, Hg, Pb, Mn, and Zn has been studied by estimating the levels of these elements in the naturally growing plants collected from different polluted areas of Kerala[17].

Centella asiatica (Family: Umbelliferae) is extensively used in folk medicine for treatment of a wide range of sickness since ancient times[17]. It has been recommended as one of the useful medicinal herbs by WHO[18]. Only the Asiatica species of Centella genus is found in commercial drugs today[19]. Lot of studies on heavy metal bio-monitoring featuring plants had been carried out extensively in different parts of the globe[20, 21, 22].

This present study aimed to characterize the levels of seven heavy metals (Mn, Cu, Cd, ,Pb, Ni, Fe, and Zn) in composite plant samples of two medicinal plants (*Bacopa monnieri* and *Centella asiatica*) collected from different parts of India (Tamil Nadu, Assam and Karnataka states) as they are commonly used in the treatment, prevention, and management of diseases. In order to study the background contamination, the soils on which the plants were grown were also collected from the diverse regions. This study may enable to understand the importance of location in collection and ultimately the heavy metal toxicity of medicinal plants.

MATERIALS AND METHODS:

This study was taken up in three different zones viz. Assam; Mettur, Tamil Nadu and Bengaluru, Karnataka. Plant specimens of commonly used medicinal plants namely *Centella asiatica* (locally known as Vallarai) and *Bacopa monneri* (locally known as Neera brahmi) were collected. The plant samples were oven dried to achieve constant dry weights. The plant samples comprising of leaves, roots and stem were powdered and stored safely for further chemical analysis.

Acid digest of the plant species were prepared by adding 10ml of 9:4 Nitric Acid and Perchloric Acid mixture to 0.5g of powdered plant samples and digested on a hotplate till the solution becomes transparent and the volume was brought down to about 5ml. This was filtered using Whatman No. 42 filter paper and made upto 100ml in a Standard Measuring Flask and used to determine the concentration of Zinc (Zn), Iron (Fe), Nickel (Ni), Lead (Pb), Copper (Cu), Manganese (Mn) and Cadmium (Cd),

In order to study the background contamination, samples of soil in which these medicinal plants were grown were also collected from the selected regions from depth of 2 feet. Samples were transferred to polyethylene bags, labeled and shipped to the laboratory for processing and testing. Soil samples were processed by air drying at room temperature. The samples were ground and sieved using 2 mm sieve and stored for chemical analysis. Acid digest of the soil sample was prepared and used for analyzing concentration of Cadmium (Cd), Nickel (Ni), Iron (Fe), Lead (Pb), Copper (Cu), Zinc (Zn) and Manganese (Mn). Analysis was carried out using Atomic Absorption Spectrometer (Varian Model AA220). Data was reported in mg/kg.

Bio accumulation factor (BAF) of plant species (*Centella asiatica* and *Bacopa monneri*):

Bioaccumulation factor (BAF) is being extensively used as an index to measure the efficacy of plant metal translocation from soil to root and from roots to leaf [23, 24]. Metal bioaccumulation factor is the ratio of heavy metal concentration in plant to soil[25] and was calculated as follows:

$$BAF = C_{plant} / C_{soil}$$

Where

C_{plant} and C_{soil} are the concentrations (on dry weight bass) of heavy metal in plants and soil respectively.

BAF was categorized further as hyper-accumulators, accumulator and excluder to those samples which accumulated metals >10 μg g-1, >1 and <1, respectively[26]. Soil to plant metal bioaccumulation factor (BAF) is a significant indicator of metal uptake capability of plants. BAF factor values above 1 for heavy metals have been considered dangerous for plant and animal health[27].

RESULTS AND DISCUSSION:

Heavy metal concentrations in soil samples collected from different sites is furnished in Table 1.

Table 1. Concentration of metals in Soil samples collected by three different locations

S. No	Sample Location ID	Total Concentration (mg/ kg)						
		Fe	Cd	Ni	Pb	Cu	Zn	Mn
1	Site 1	2918.67	0.82	16.73	17.65	16.83	134.95	168.44
2	Site2	1596.35	0.73	17.41	12.41	15.21	26.03	85.78
3	Site3	337.98	0.64	28.81	23.48	17.23	51.06	125.78

Metal concentration in soil:

In the present study, the metal concentration (Fe, Cd, Ni, Cu, Zn, Pb, and Mn) were studied in the soil, sampled at three different locations (Assam – Site 1, Mettur – Site 2 and Bangalore – Site -3). The soil samples collected from the different geographical locations showed significant differences between the heavy metals. Among the metals studied, Zn, Fe, Cu, and Mn were found to be most predominant and also considered as essential metals for plant growth, soil microflora and living beings. However, the excess amount of these metals in soil may prove to be toxic and unsafe for human health if present more than maximum permissible limit. On the other hand, the heavy metals Cd, Ni and Pb which considered being a potential threat to a wide range of biota and a small concentration of it is hazardous to living beings were also studied in these three locations. The levels of Fe (2918.67 mg kg^{-1}), Zn (134.95 mg kg^{-1}), Mn (168.44 mg kg^{-1}) and Cd (0.82 mg kg^{-1}) are higher in the soil, sampled at Site 1 followed by Site 2 for the metal Fe (1596.35 mg kg^{-1}) and Cd (0.73 mg kg^{-1}) and then Site 3 for Zn (51.06 mg kg^{-1}) and Mn (125.78 mg kg^{-1}). The other metals such as Ni (28.81 mg kg^{-1}), Pb (23.48 mg kg^{-1}) and Cu (17.23 mg kg^{-1}) recorded its highest concentration in Site 3 followed by Site 2 for Ni (17.41 mg kg^{-1}) and Site 1 for Pb (17.65 mg kg^{-1}) and Cu (16.83 mg kg^{-1}).

In the recent years, medicinal plants are being criticized as a potential cause of heavy metal toxicity to both man and animals[28]. Among the metals studied, the most common heavy metals of great concern to human health include Pb and Cd although Ni may also cause toxicity. Organizations like WHO and Ayurvedic Pharmacopoeia of India specifies that medicinal plants which are the raw materials for the herbal formulations, should be checked for the presence of heavy metals such as Cd and Pb[29]. Similarly, metals Zn, Cu, Fe and Mn which is considered as important elements for plant growth, when in excess quantities, are toxic and cause malformation and other ailments in young children and human beings. Essential micronutrients such as Fe, Zn, Cu and Mn are affecting the human health when they are in deficient or excess levels. Effect of toxic heavy metals (Cd, Pb, Cr etc.) on human health and their interaction with essential trace elements may produce serious consequences[30]. Results of our investigation reveals that among the heavy metals Fe, Zn and Mn showed the higher levels of concentration which is more than the maximum permissible limit as compared to other metals like Cu, Cd, Ni and Pb. The concentration of all the metal studied in three regions were exceeded the maximum permissible limit as suggested by WHO organization.

Metal concentration in plant:

The total concentration of the heavy metals in plant samples of two species ie, *Centella asiatica* and *Bacopa monneri* collected from different sites are summarized in Table 2. Among the metals studied the accumulation of highest concentration of metals such as Fe (1744.20 mg kg^{-1}), Ni (8.48 mg kg^{-1}), Pb (16.76 mg kg^{-1}) and Cu (44.27 mg kg^{-1}) were observed in the plant species *Bacopa monneri* sampled at site 3. The levels of Zn (269.41 mg kg^{-1}) concentration were found to be higher in *Bacopa monneri* while the Mn (206.30 mg kg^{-1}) concentration in *Centella asiatica* sampled at Site 1 respectively. Moreover the concentration of Cd found to be in Below Detectable Level (BDL) of 0.1 mg kg^{-1} irrespective of sites and plant species studied.

Table 2. Concentration of metals in plant samples (*Centella asiatica* and *Bacopa monneri*) collected by three different locations (mg kg^{-1})

S.No	Sample Location ID	Plant species	Total concentration (mg kg^{-1})						
			Fe	Cd	Ni	Pb	Cu	Zn	Mn
1.	Site 1	*Centella asiatica*	485.70	BDL	BDL	BDL	18.82	174.99	206.30
		Bacopa monneri	841.40	BDL	BDL	BDL	15.71	269.41	44.72
2.	Site 2	*Centella asiatica*	999.26	BDL	3.72	11.03	12.73	56.68	41.09
		Bacopa monneri	407.22	BDL	4.47	7.70	16.18	99.43	67.52
3.	Site3	*Centella asiatica*	1263.53	BDL	8.05	14.77	15.00	112.01	73.76
		Bacopa monneri	1744.20	BDL	8.48	16.76	44.27	121.28	48.84

According WHO, almost 80% of the world population uses plant-based herbal medicines[2]. Plants for preparation of herbal medicines are collected in the wild mostly and only few being cultivated. Essential mineral elements important to nutrition accumulate in these plants. Other non essential elements such as Cd, Co and Pb which are not used directly by the plant and which are harmful to the human health, also accumulates in these plants[31,32]. Significant difference has been observed in heavy metal concentration among the soil and plants collected from diverse geographical locations. The comparison of metals between the soils and plant parts in the present study of *C. asiatica* and *B. monneri* shows that Cd metal concentration is Below Detectable Level (BDL) in all the three regions, while Ayurvedic Pharmacopoeia of India[29] stipulates the Maximum

Permissible limit of Cd in raw herbs is 0.3 ppm. Lead (Pb) is highly harmful for plants, animals and microorganisms. Increase in usage of chemical fertilizers, fuel combustion and sewage sludge are the major reasons leading to escalation in Pb pollution. In the present study, the concentration of Fe in the plant tissue is higher than other metal studied. However, the dietary limit of Fe in the food is 10-60 mg/day[33]. Followed by Fe, the Zn metal shows highest concentration in both c. asiatica and B. monneri plant tissue. Zn plays a major role in the plant growth as a essential micronutrient and it is also essential for human beings as it plays an important role in growth and development. Though the dietary limit of Zn is 100 ppm/day[34], except the Site 2 (Mettur, Tamil Nadu region) all the other sites Zn exceeded the maximum permissible limit.

The maximum permissible value for Cu is 0.1 mg/l (WHO), 0.20 (FAO mg/l). As per BIS, the permissible limit for Cu is 0.05 mg/l and excess limit is1.5mg/l. The Effluent Standards (CPCB) permissible limit for Cu is 3.00 ppm. In the present study, the concentration of Cu is found to be above the permissible limit in all the regions. In the study of metal concentration in plant tissues, the Ni concentration recorded BDL (Below detectable limit) in the Site 1 (Assam region), where other regions recorded, the Ni concentration ranges from 3.72 – 8.48 mg/kg. Nickel is required in minute quantity for body and plays an important role in the production of insulin. Its deficiency results in the disorder of liver[35]. EPA has recommended daily intake of Ni should be less than 1 mg beyond which it is toxic[36]. Hence, the site 2 and 3 exceeded the Ni concentration beyond the permissible limit which is considered unsafe to human being. The WHO limit for Mn in medicinal herbs has not been established yet. The results of the current study show a wide variation of manganese in different herb samples.

In the present investigation, concentrations of many heavy metals have been found to exceed the internationally accepted permissible levels. Wide variations in metal concentrations in the analyzed plants could be attributed to various factors such as metal uptake and translocation capabilities plant wise. Also, metal uptake by plants depends on several factors including the plant species and their stage of growth, the soil type, and the type of metals absorbed[37, 38]. Studies have shown wide variations in concentration factor of different metals among the plant species and sampling sites. Studies have shown that accumulation of Fe is greater compared to other essentials metals such as Cu in many species of medicinal plants.[39]

Bio accumulation factor (BAF) of plant species (*Centella asiatica* and *Bacopa monneri*)

In the present investigation, the uptake of Fe, Cd, Ni, Pb, Cu, Zn and Mn from soil to plant translocation were compared for *Centella asiatica* and *Bacopa monneri* from three different locations (Table 3).

Table 3. Bioaccumulation factor (BAF) of metals in *Centella asiatica* and *Bacopa monneri*

S.No	Sample Location ID	Plant species	Metal Bioaccumulation Factor (BAF)						
			Fe	Cd	Ni	Pb	Cu	Zn	Mn
1.	Site 1	*Centella asiatica*	0.17	NIL	NIL	NIL	1.12	1.30	1.22
		Bacopa monneri	0.53	NIL	NIL	NIL	1.03	10.35	0.52
2.	Site 2	*Centella asiatica*	0.63	NIL	0.21	0.89	0.84	2.18	0.48
		Bacopa monneri	0.26	NIL	0.26	0.63	1.06	3.82	0.79
3.	Site3	*Centella asiatica*	3.74	NIL	0.28	0.63	0.87	2.20	0.59
		Bacopa monneri	5.16	NIL	0.29	0.71	2.57	2.38	0.39

Results of the study shows that Site 1 samples have highest bioaccumulation factor (BAF) value for *Bacopa monneri* which is highest for Zn followed by Site 3 which shows highest BAF value for Fe in the plant species *Bacopa monneri*. In case of Fe, Cu, and Zn maximum BAF values were observed for *Bacopa monneri* irrespective of all three locations studied while maximum BAF value for Mn observed in *Centella asiatica* sampled at Site 1 and in all other sites BAF value were less than 1. Moreover absence and < 1 BAF values were noticed for the metal Cd, Ni and Pb all the locations. Bioaccumulation factor (BAF) and Translocation factor (TF) are the indices to measure the efficacy of plant metal translocation from soil to root and from roots to leaf[23,24]. According to the study conducted in the entire three regions, the BAF value recorded > 1 under accumulator category. Hence, the metal with higher BAF value and the extent of contamination in the soil have significant impact on the accumulated levels of the toxic metals such as Fe Cu, Zn and Mn in *Centella asiatica* and *Bacopa monneri* leaves and this could cause serious health risks through diet.[40] These reports were in accordance with the results obtained by Jena *et al.*[41]

CONCLUSION:

Most of the medicinal plants are herbs which are cultivated or naturally growing in soil have been found to be contaminated with heavy metals due to natural and anthropogenic activities. They are found to accumulate considerable quantities of toxic heavy metals. Thus, the increased levels of essential and non essential heavy metals in medicinal is impacting the public safety worldwide The high concentration of heavy metals in the medicinal plants causes health hazards in human and animals. When the raw herbs are used for human consumption, stringent quality assurance of raw materials adhering to safety standards must be followed strictly. The results from the study imply that medicinal plants used as raw materials for preparation of herbal products must be collected from an unpolluted natural habitat.

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