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ACCUMULATION OF TRACE ELEMENTS IN MEDICINAL PLANTS IN MANGALORE ENVIRONS OF COSTAL KARNATAKA

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Abstract: The concentration of trace elements Cr, Ni, Cu, Zn, Rb and Cd were studied in *Azadirachta indica* and *Ocimum sanctum*, collected from Mangalore region of coastal Karnataka, by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Higher levels of Zn and Cr were observed in both the plants. The Cd content in both the plants was low. Soil samples collected from rooting zone of these two plants were also analyzed for the presence of these elements. The soil to plant transfer factor was found to be higher for Zn and Rb in *Azadirachta indica* and for Cu and Cr in *Ocimum sanctum*. Results were discussed in the light of values reported for these plants in other environs of the world.
Key words: *Azadirachta indica*, *Ocimum sanctum*, ICP-MS, trace elements.

1. Introduction

The coastal region of Karnataka is heading to become a region of major industrial activities with chemical and fertilizer factories, oil refineries, super thermal power stations and a host of other industries. Some of these industrial activities release trace elements to the environment of the region. In the absence of proper assessment, trace elements released from these industrial activities may cause harmful effects on the population and environment of the region. In view of this, the concentrations of Cr, Ni, Cu, Zn, Rb and Cd were studied in commonly found medicinal plants in the region *Azadirachta indica* and *Ocimum sanctum* and in soils in the rooting zone of these plants. Medicinal plants are important for pharmacological research and drug development, not only when plant constituents are used directly as therapeutic agents, but also when they are used as starting materials for the synthesis of drugs. Therefore concentration of trace elements in medicinal plants assumes great significance. As the soil is the major reservoir for trace elements, the transfer of trace elements from soil to medicinal plants was also studied. *Azadirachta indica* (Neem tree) is in the family Meliaceae and kingdom plantae. The plant parts used for the medicinal purpose are bark, leaves, flowers, seeds and oil [1]. The plant *Ocimum sanctum* (Holy basil) is in the family Lamiaceae and kingdom plantae. The whole plant is used for the medicinal purpose.

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Trace elements in medicinal plants are responsible for their medicinal as well as toxic properties [2]. Therefore, quantitative estimation of various trace element concentrations is important for determining the effectiveness of the plants in treating various diseases and to understand their pharmacological action.

2. Materials and methods

2.1. Instrumentation

A VG Plasma Quad PQ II (V.G. Elemental, Winsford, Cheshire, England) ICP-QMS was used for the determination of trace elements. Sample introduction was carried out by pneumatic nebulization using a Meinhard concentric nebulizer, a double-pass Scott-type spray chamber cooled to 10^0C, and a Fassel-type torch. The solution flow rate was controlled by a peristaltic pump (sample uptake rate: 1 mL min^{-1}). The ICP-QMS operating conditions were optimized daily for maximum sensitivity and calibrated using a 100 ng mL^{-1} tuning solution of Li, Be, Al, Ti, Co, Sr, In, La, Bi, Th, and U. A quadrupole mass analyzer having a mass range of 1-300 amu. with a resolution of 0.3 amu has been employed.

2.2. Reagents and Solutions

High purity reagents were used for preparation of the standards. All solutions were prepared in de-ionized water obtained from a Milli-Q™ system (18 MΩ, MilliQ system, Millipore, Bedford, MA, USA). Stock solutions of thirty individual elements each of 1000 mg L^{-1} ((BDH, Leicestershire, UK) were mixed together and diluted daily with 1 mol L^{-1} HNO_3 (65% Suprapur®, E. Merck, Darmstadt, Germany) to obtain the working solutions. Instrument calibration standard solutions of diverse elements were prepared by dilutions from 1000 mg L^{-1} stock solutions. All solutions for analysis were made up in Class I, Grade A volumetric flask (Vensil, Bangalore, India). Whatman 541 filter paper (Maidstone, England) and micron filter assembly using 0.45 μm Millipore filter paper (Pall India, Mumbai, India) were used for filtration of solutions prior to analysis by ICP-MS. For dissolution of the samples Hydrofluoric acid (40% Suprapur®, E. Merck, Darmstadt, Germany), Perchloric acid (70% Suprapur®, E. Merck, Darmstadt, Germany) were used.

2.3. Sample collection, dissolution and analysis by ICP-MS

The plant samples of *Azadirachta indica* and *Ocimum sanctum* and representative soils from the region were collected and analyzed after dissolution and matrix separation by ICP-MS. Approximately 2 kg of each plant (leaf, stem and root) were collected in a

polythene bag, brought to the laboratory, washed under running water to remove adhered matter if any and then cut into pieces. The samples were then ashed at 500^0C in a muffle furnace. The ashed sample (~0.5 g) was taken in a platinum crucible and digested with a mixture of HNO_3 and $HClO_4$ (2:1 v/v) on a hot plate to remove the organic matrix from the sample. The resultant solution was evaporated to near dryness followed by addition of 1mL of HNO_3 and again evaporated to near dryness. Two such evaporation steps were carried out and finally the solution was made in 1% HNO_3 and quantitatively transferred into a 25 cc volumetric flask. The sample solution was then filtered through a micro filter assembly using 0.45 μm Millipore filter paper to remove any minute particles prior to analysis by ICP-MS.

The upper 20cm layer of the top soil of each of the plants was sampled separately. About 1 kg of soil sample was taken and brought to the laboratory. All the samples were carefully processed following standard procedure [3]. The oven dried soil samples were ground and powdered using an agate mortar and pestle and sieved through an ASTM test sieve No. 230 (<63μm). The fine powder of samples (~ 0.1gm) was taken in a platinum crucible and digested with a mixture of HNO_3, $HClO_4$ and HF on a hot plate to remove the silicate and organic matrix. The resultant solution was evaporated to near dryness followed by addition of 1mL of HNO_3 and again evaporated to near dryness. Two such evaporation steps were carried out and finally the solution was made in 1% HNO_3 and quantitatively transferred into a 25 cc volumetric flask. The sample solutions were filtered through Whatmann 541 filter paper before performing ICP-MS analysis.

Both the plant and soil samples were subjected to analysis by ICP-MS using the two point external calibration technique. Standard solutions of 100 ppb and 500 ppb were used for the quantitative determination of elements. One of the calibration standards (200 ppb) was employed as a sample to account for any variations in the plasma operating conditions. A minimum of two runs were made for all the plant and soil samples.

3. Results and Discussion

The results of concentration of trace elements in plants and associated soils are given in Table 1. In *Azadirachta indica* the concentration of elements are 115.0 μg g^{-1}, 7.2 μg g^{-1}, 63.5 μg g^{-1}, 200.8 μg g^{-1}, 51.7 μg g^{-1} and 0.6 μg g^{-1} for Cr, Ni, Cu, Zn, Rb and Cd respectively. The concentrations in associated soil are 142.4 μg g^{-1}, 140.1 μg g^{-1}, 61.6 μg g^{-1}, 119.0 μg g^{-1} and 38.1 μg g^{-1} for Cr, Ni, Cu, Zn and Rb respectively. The concentration of Cd in soil was below detection level even though the plant has detectable level of Cd

concentration. In *Ocimum sanctum* the concentrations of elements are 212.2 μg g^{-1}, 27.1 μg g^{-1}, 129.2 μg g^{-1}, 254.5μg g^{-1} , 22.5 μg g^{-1}, 192.7 μg g^{-1} and 1.1 μg g^{-1} for Cr, Ni, Cu, Zn, Rb and Cd respectively. The concentrations in associated soil are 121.2 μg g^{-1}, 60.8 μg g^{-1}, 10.4 μg g^{-1}, 180.8 μg g^{-1}, 16.2 μg g^{-1}, and 1.1 μg g^{-1} for Cr, Ni, Cu, Zn, Rb and Cd respectively. The results obtained in the present study for the plants have been compared with those reported by other investigators for the same plants in Table 2. From the table it is clear that Cr, Cu and Zn values obtained in the present study are higher compared to the values reported in the literature for the plants [4,5]. The variation in the trace elemental concentration in different environs can be attributed to several factors like the climatic conditions of the region, mineral composition of the soils in which the plant grows, the preferential uptake of the particular plant for certain elements, the species and the release from industrial activities.

Table 1 also lists the transfer factor for each of the six elements. The transfer factor, defined as the ratio of the concentration of the element in plant to the concentration in soil was calculated in order to understand the elemental uptake by the plants from soil. The uptake of elements within the soil to plant is a part of the biochemical cycling. The mobility and availability of trace elements depend on several factors such as geochemical, biological and climatic conditions. The soil to plant transfer ratios for the plant *Azadirachta indica* are 0.8, 0.1, 1.0, 1.7 and 1.4 for Cr, Ni, Cu, Zn and Rb respectively. The ratios for the plant *Ocimum sanctum* are 1.8, 0.4, 12.4, 1.4, 1.4 and 1.0 for Cr, Ni, Cu, Zn, Rb and Cd respectively. The variability of soil to plant transfer ratio is expected as this macroscopic parameter integrates a number of soil chemical, biological, hydrological, physical and plant physiological processes, each of which shows its own variability and in addition may be influenced by external factors such as climate, development, industrialization and human agricultural practices. According to Ehlken and Kirchner [6] the concentration of trace elements accumulating in plants may not primarily depend on its absolute concentration in the soil-plant system but on the concentration ratio to other micro and macro nutrients. The higher transfer factor observed for the element Cu in the plant *Ocimum sanctum* can be attributed to the redistribution of the element within the soil profile. For deep rooting plants the redistribution of the contaminant within the soil profile may even cause an increase of the transfer factors with time. The influence of the non uniform root densities and concentrations of trace substance on root uptake can cause significant variation in transfer factor.

4. Conclusion

The elemental content in the medicinal plants and associated soils showed wide variation. The concentration of Cr, Cu and Zn were considerably high in *Azadirachta indica* and *Ocimum sanctum* compared to the values reported for the same species in other regions of the world. This can be attributed to the various industries operating in the region. The level of trace elements in plants will be affected by the geochemical characteristics of the soil and by the ability of plants to selectively accumulate some of the elements. The uptake of some of the elements by plant was insignificant even though the soil on which the plant grows has significant amount.

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Table 1. Trace element concentration in medicinal plants and associated soils (ppm)

	Azadirachta indica			*Ocimum sanctum*		
Trace Element	Soil	Plant	Transfer factor	Soil	Plant	Transfer factor
Cr	142.4	115	0.8	121.2	212.2	1.8
Ni	140.1	7.2	0.1	60.8	27.1	0.4
Cu	61.6	63.5	1.0	10.4	129.2	12.4
Zn	119.0	200.8	1.7	180.8	254.5	1.4
Rb	38.1	51.7	1.4	16.2	22.5	1.4
Cd	BDL	0.6	-	1.1	1.1	1.0

Table 2. Comparison of elemental concentration in medicinal plants common to present study and other similar studies (ppm)

	present study	Naga Raju et. al., (2006)	Ray et. al., (2004)	present study	Naga Raju et. al., (2006)	Ray et. al., (2004)
Cr	115	48	3.2	212.2	38.8	4.7
Ni	7.2	21.7	-	27.1	24.1	-
Cu	63.5	6.4	12.0	129.2	19.4	12.6
Zn	200.8	20.6	17.2	254.5	58.1	28.2
Rb	51.7	8.1	45.9	22.5	-	34.4
Cd	0.6	-	-	1.1	-	-