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Evaluating heavy metals Contents in medicinal plant *Mentha longifolia*

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Abstract
Toxic heavy metals were investigated in the medicinal plant *Mentha longifolia*. The plant samples were collected from different locations of Chhattisgarh, Central India. The plant parts including root, stem and leaves were found to have the quantity of heavy metals. Plant ability to accumulate and translocate the toxic metals i.e. Cr, Mn, Fe, Cu, Zn, and Pb were estimated by use of bioaccumulation factor (BAF) and translocation factor (TF). These results may be useful for the evaluation of the quality of medicinal plants.

Keywords: Medicinal plant, Bioaccumulation factor, Translocation factor

Introduction
Medicinal plants are the raw material for many herbal formulations and popular supplements. The use of herbal medicines has been on the rise in recent years due to their low prices. There is a common concept among people that herbal medicines have no side effects and that "being natural in origin, herbs are safe". The assimilation of heavy metals in plants is obvious because of widespread heavy metals in the soil due to geo-climatic conditions [1-5]. Heavy metals have a greater tendency to accumulate in human organs over prolonged periods of time. The presence of heavy metals beyond the permissible limits can cause metabolic disturbances. Thus both the deficiency and excess of essential micronutrients such as Fe, Zn and Cu are harmful to the human health. Effects of toxic metals (Cd, Cr, Pb, etc.) on human health and their interaction with essential trace elements may produce serious consequences [6]. The World Health Organization (WHO) recommends that medicinal plants which form the raw materials for the finished products may be checked for the presence of heavy metals, pesticides, bacterial or fungal contamination. *Mentha longifolia* (family: *Lamiaceae*, Class: *Magnoliopsida* Sanskrit: *Aswagandha*;) is an important drug in the ancient system of Ayurveda used to cure a variety of ailments and is widely distributed in India, Pakistan, Sri Lanka, Greece, Egypt, China, Mediterranean regions, Canaries, S. Africa, Iraq, Iran, Syria and Turkey [7-12]. Heavy metal deposition in plants from anthropogenic sources has increased the attention on inorganic pollution and established plants as passive bio-monitors [13-14]. A variety of plant species have been used as biological monitors since, they have a tendency to assimilate metals from the surrounding environment [15-16]. Metal toxicity was found to have a significant relationship with factors controlling metal tolerance, including available uptake sites, chemical interaction and ionic speciation. Many plants that accumulate >1000 mg kg^{-1} or >10000 mg kg^{-1} of trace metals are categorized as metal hyper accumulators. Most plants translocate inorganic and nutrient constituents from roots to leaves [17-20]. Based on the above studies, the aim of the present study was to determine (1) the heavy metal concentrations in the leaves, and roots of *Mentha longifolia* with relation to its surrounding soil, (2) Bio-accumulation factor (BAF) and Translocation Factor (TF).

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2. Materials and Methods

2.1. Sampling area

The study area is different sites of Chhattisgarh India including its capital city, Raipur. All the sampling sites are from the Chhattisgarh. The state hosts a wide variety of minerals found associated with igneous, sedimentary and metamorphic rock formation. Large deposits of Coal, Iron ore, Limestone, Dolomite and Bauxite are located in parts of the State.

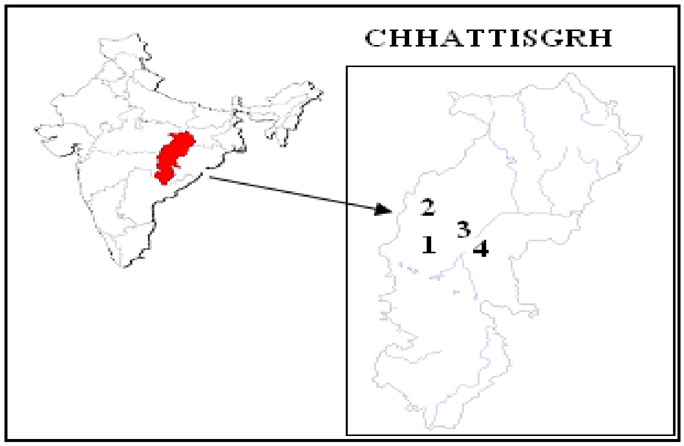

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Figure 1: Location of Sampling Sites

2.2. Sampling of soil and plant material

The different parts of *Mentha longifolia* (i.e. leave, stem, root etc.) and rhizospheric soil were collected from contaminated sites of Chhattisgarh state, Central India Figure 1. The plants were washed thoroughly with deionized distilled water, dried in a shed, and compressed into a powder with the help of a manual grinder and sieved out the particles of mesh size, < 0.1mm.

2.3. Chemical and Reagents

The Analytical Reagent Grade chemicals were used for digestion of the soil and plant samples. The ICP multi-element standard was used for preparation of the calibration curve.

2.4. Soil and plant sample analysis

The Plant and Soil samples air-dried, weighed and placed in a dehydrator at approximately 80°C from 48 to 72 hours depending on sample size. The samples were ground to a fine powder with mortar and passed through a sieve of <0.1 mm mesh size. The weighed amount (0.500 g) of the sample was digested with 5 ml HNO_3 + 2 ml $HClO_4$ + 1 ml HF in the closed microwave oven [21]. An instrument inductively coupled plasma- atomic emission spectrometry (ICP-AES) Ultima-2 from Jobin Yvon, France equipped with parallel flow nebulizer and the cyclonic spray chamber was used for the monitoring of the heavy metals (i.e. Cr, Mn, Fe, Cu, Zn, Pb). The flow rate of argon plasma, sheath and nebulizer gas maintained was 12, 0.2 and 0.8 l min^{-1}, respectively at sample uptake of 1 ml min^{-1}. Soil pH was measured in a 1: 5 (ww^{-1}) soil to distilled water mixture. The leaves of the plants collected in the field were rinsed in distilled water and dried overnight at 70°C. Plant (root and leaf) samples were wet-ashed as described earlier.

Trace metals translocation in plant from root to leaf was measured using TF which is given below:

$$TF = C_{pp} / C_r$$

Where, C_{pp} = metal concentration in plant tissue, mg kg^{-1} fresh weight

C_r = metal concentration in soil, mg kg^{-1} dry weight.

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

C_p and C_{soil} are metal concentrations in aerial parts of the plant (mg kg^{-1}) and in soil (mg kg^{-1}), 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 [18].

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3. Results and discussion

Total metal contents were determined in soil samples and different parts of *Mentha longifolia* collected from different site are summarized in Table 1 to Table 3.

Table 1: Soil and Plant Morphology

S.N	Site	Types of bedrock	Site characteristics	pH of the soil	Age of plant (days)	Water percentage
1	Site1	Limestone, Dolomite, Quartzite	Sewage blackish soil	6.8	30-40	85
2	Site2	Bauxite, Limestone	Red soil	6.0	40-45	91
3	Site3	Types of bedrock	Red soil	7.1	30-35	86
4	Site4	Limestone, Dolomite, Quartzite	Brownish soil	6.3	40-45	76

Table 2: Concentration of metal in different part of *Mentha long folia* collected from different sites, mg kg^{-1}

Metal	Site 1			Site 2			Site 3			Site 4		
	Soil	Root	Leaf	Soil	Root	Leaf	Soil	Root	Leaf	Soil	Root	Leaf
Cr	85	23	43	65	21	32	80	24	29	50	22	28
Mn	1100	198	276	656	12	32	820	115	65	990	46	11
Fe	43500	5689	4980	45212	1072	1458	22000	3321	1120	25500	1450	2750
Cu	55	26	32	61	18	24	54	34	10	34	26	25
Zn	96	59	81	170	58	32	190	112	126	145	88	112
Pb	21	11	09	18	07	03	49	32	12	41	21	12

Table 3: Bioaccumulation Factor (BAF) and Translocation Factor (TF) of Heavy metals in *Mentha longifolia*

Metal	Site 1		Site 2		Site 3		Site 4	
	BAC	TF	BAC	TF	BAC	TF	BAC	TF
Cr	0.51	1.87	0.49	1.52	0.36	1.21	0.56	1.27
Mn	0.25	1.39	0.05	2.67	0.08	0.56	0.01	0.24
Fe	0.12	0.88	0.03	1.36	0.05	0.34	0.11	1.89
Cu	0.58	1.23	0.39	1.33	0.18	0.29	0.74	0.96
Zn	0.84	1.37	0.19	0.55	0.66	1.12	0.77	0.77
Pb	0.43	0.82	0.17	0.43	0.24	0.38	0.29	0.57

*Bioaccumulation factors mean the ratio of metal concentration in aboveground fronds to that in soils
**Translocation factors mean the ratio of metal concentration in aboveground fronds to that in belowground rhizoids;

3.1. Chromium

Soil samples collected from different Sites showed significantly different amounts of chromium. In the case of the plants, high chromium amounts were found in plants collected from Site 1. For example, in the case of Site 1, a high concentration was found in the leaves (43 mg kg^{-1}) followed by the roots (23 mg kg^{-1}). Although chromium was also present in the roots and leaves in Site 2 and 3, however, their concentration is not significant except for the leaves, i.e., chromium is present in low concentrations. Thus in general the concentration in the four Sites was in the order: Site 1 > Site 2 > Site 3 > Site 4 while among the plant parts leaves > root. This also shows the excreting channel of the chromium from the plant body through the leaves, while the greater concentration of chromium in roots can be explained by the presence of chromium in the soil and the making of slow equilibrium.

3.2. Manganese

Manganese is another essential element for plant and animal growth. The main sources for manganese in soil are fertilizers, sewage sludge and ferrous smelters. Critical manganese concentration in plants is in the range from 300 to 350 mg kg^{-1}. The leaves and root of Site 1 high amounts of manganese (276 and 198 mg kg^{-1}) compared to other Site. Thus the concentration level of manganese is well below the critical level and hence acceptable at this level, because it does not affect the plant growth nor will it cause pollution.

3.3. Iron

Iron is another essential element for plant and animal growth. Its deficiency can cause various types of diseases; however, its high concentration also affects plant growth. The soil samples collected from the different Site showed significant differences between the heavy metals (Table 2 to Table 3). The plant samples collected from the four Sites have different amounts of iron. For example, a high amount of iron was found in the soil (45212 mg kg^{-1}) followed by root (5689 mg $kg^{-1}$and leaf (4980 mg kg^{-1}) in the plant samples collected from Site 1. Thus in general the order of iron in soil was Site1>Site2>Site4>Site 3.In addition to this iron is found in leaf and root of the four Site in excessive amount. The amount of iron in root of *Mentha longifolia* follows following order: Site1>Site3>Site4>Site2. While in case of leaf of this entire Site it follows order: Site1>Site4>Site2>Site3.

3.4. Copper

Copper is one of essential elements for plants and animals. The most common sources for copper distribution in soils are pesticides, fertilizers, industry and sewage sludge. The concentration of copper was found to be higher in the soil from Site 1 and Site 2 than Site 3. Plants grown in the three Sites contained significantly different amounts of copper, due to the difference in the concentrations of copper in the soils of the three Sites. The level of copper concentration in roots, leaves and soil were found to be higher in Site 1 than Site 2 that has in fact a greater concentration than Site 3. Leaves accumulated significantly more copper (0.58 mg kg^{-1}) in Site 1. A similar trend was found in site 4, in which the leaves have 0.79 mg kg^{-1} while the concentration of copper was found lower in Site 3 (Table 1). Thus the copper concentration in plant parts was in the order: leaves > roots. The internal concentration of the Cu varies less, although the soils differ strongly with respect to metal concentration and soil properties.

3.5. Zinc

Zinc is one of the important metals for normal growth and development in human beings. A wide range of Zn concentration was observed among the soil collected at the four Site (from 96 to 190 mg kg-1).The concentration of zinc was found to be higher in the soil from Site 2 and Site 3 than Site1. The level of zinc concentration in roots and leaves were found to be higher in Site 3 than Site 4 that has in fact a greater concentration than Site 3. Leaves accumulated significantly more zinc (0.84 mg kg-1) in Site 1 followed by Site 4 (0.77 mg kg-1). Thus the zinc concentration in plant parts was in the order: leaves > roots.

3.6. Lead

Lead is regarded as highly hazardous for plants, animals and particularly for microorganisms. The main sources of lead pollution in agriculture and plants are lead mines, fuel combustion, sewage sludge applications and farmyard manure. As can be seen from Table2, high lead concentration was found in the root and leaf of the plants collected from Site3 and Site4, respectively. Due to soil and air pollution, lead concentration in plant parts was in the order: soil>root> leaves. Obviously the high lead concentration in the above ground parts is due to air borne lead and also due to getting the fast steady state equilibrium. The plants from the three different environments accumulated different amounts of lead. The most sensitive was Site3 and the least sensitive was Site2.

The results demonstrated a broad intra-specific and metal-specific variation in metal tolerance in *Mentha longifolia*, the pattern of which corresponded with that of the soil metal composition at the Site 1 and Site 3 (Table 2). Both on the Site 1 and Site 3 were much more tolerant to Fe and Mn than the Site 2 and Site 4, respectively. Moreover, the Site 1 and Site 3 are more tolerant to Cr than any of the other Sites. This implies that *mentha longifolia*, in spite of its apparent constitutive ability to tolerate high rates of accumulation of particular metals, is not necessarily tolerant to high concentrations of the same metals in the root environment [1-2, 5].

Bioaccumulation factor (BAF) and Translocation factor (TF) has been used as an index to measure the effectiveness of plant metal translocation from soil to root and from roots to leaf [1, 8]. In this study the uptake of Cr, Mn, Fe, Cu, Zn and Pb from soil to root and root to leaf translocation were compared in *Mentha longifolia* from different soil types (Table 3). *Mentha longifolia* concentrated >50% of Cr in their leaves. TF was estimated not only for frond / root, but also for root/ soil to better understand Chromium distribution in the plants. Translocation of chromium (Cr) in *Mentha longifolia* collected from different sites follow the order: Site 1 > Site 2 > Site 4 > Site 3. So that in all Sites Chromium (Cr) is accumulated in root from the soil and then it is uptake by the plant leaf. The *Mentha longifolia* plant removes ≈50 % of the Cr present in the soil in it grown. The trait of effective translocation of Chromium to the leaf from the roots in hyper-accumulators has been observed [22]. The translocation factor for *Mentha longifolia* collected from different Site shows a high degree of correlation, it means that *Mentha longifolia* can be a hyper-accumulator of Cr from soils. In all the samples collected from different types of soils (Table 1), *Mentha longifolia* shows highest uptake of metals Mn for Site 2, which is red soil, acidic in nature and the leaf contents maximum percentage of water. Results show that samples collected from Site 1 has highest bioaccumulation factor (BAF) and translocation factor (TF) is also highest for Mn. Translocation factor for Mn in *Mentha longifolia* for all samples collected vary in the range from 0.24 to 2.67 and BAF follow the range from 0.01 to 0.25. So it can be concluded that Mn is not accumulated in the root, but it transfers toward the leaf of *Mentha longifolia*. BAF and TF for iron in *mentha longifolia* are found in the range from 0.03 to 0.12 and from 0.34 to 1.89

Results from our investigation suggested that the root of *Mentha longifolia* acted as a primary sink as well as a source of Cr and Zn to the leaf. Iron metal is not accumulated in the root, but it moves towards the leaf in mentha longifolia with high efficiency compare to the root. BAF and TF for Copper are found in the range from 0.18 to 0.74 and from 0.29 to 1.33. Copper has highest translocation factor (TF) for Site 2. A sample collected from Site 3 has highest BAF for Fe and Pb. Site 4 shows highest BAF for Cr and Cu while highest TF for Fe. Hence *Mentha longifolia* can be used as hyper accumulator plant as it has mean translocation factor at all sampling Site is >1. In the case of Zn, *Mentha longifolia* also accumulates and translocate within the plant. In the order of accumulation and translocation of Zn on the four Site follows: Site 1 > Site 4 > Site 3 > Site 2 and Site 1 > Site 3 > Site 4 > Site 2. In case of Fe, compare to accumulation, translocation of Iron is higher in *Mentha longifolia* plant, which follow the order: Site 4 > Site 2 > Site 1 > Site 3. All other three metals i.e. Mn, Cu, Pb are also accumulated and translocated in *Mentha longifolia* plant, but in less compare to Cr, Fe and Zn. Based on the accumulation and translocation data obtained in this study *Mentha longifolia* can be used more effectively in removing Cr, Fe and Zn from the contaminated fields [23-25]. The contents of these metals are found higher if the metals availability in the soil is high. The variation of Zn in soil, root and in leaf of *Mentha longifolia* plant is higher. Trace metal concentrations in soil were comparatively higher than in roots but lower than that of the leaves in the sampled plants. The mobilization of trace metals from soil through the roots to the leaves of the plants was also found in the literature [1, 27-29].

Mean BAF of metals Cr, Mn, Fe, Cu, Zn, and Pb are 0.48, 0.10, 0.08, 0.47, 0.62, and 0.28 whereas their mean TF are 1.5, 1.2, 1.1, 0.95, 0.95, and 0.55 (Table 3). Highest mean BAC is obtained for metals Zn and highest TF is for Cr. TF value for Zn is also high, this indicate the metal Zn is highly mobile in nature [30-32]. The overall TF value of Cr, Mn, Fe, Cu, Zn, and Pb are significant and it supports that accumulation of Mn, Fe, and Pb is comparatively less while that of Cr, Cu, and Zn is more in *Mentha longifolia*.

Conclusion

The present study showed that plants grown in contaminated areas have a high risk of having heavy metal concentrations beyond the permissible limit for each of them as compared to the less contaminated areas. The concentrations of the elements in the plant tissues were affected by the concentrations of the heavy metals in the soil. In the contaminated soils, however, different trends were observed. From the tables it is evident that the roots absorb high concentrations of metals from the soil. The quality of soil and the extent of contamination the soil exert major and significant impact on the accumulated levels of the toxic metals such as Cr, Mn Fe Cu, Zn and Pb in the *Mentha longifolia* leaves, which could cause health risks through diet [32-35]. *Mentha longifolia* absorbs a high concentration of all the elements i.e. Cr, Mn Fe Cu, Zn, Pb etc. Of the heavy elements, Fe tends to be the highest accumulated in the root of the *Mentha longifolia* plant followed closely by Mn then Zn and Cu. Similar high concentrations are observed in the leaves of the *Mentha longifolia* plant with Fe being present in the highest concentration at this stage. Pb also tends to accumulate in the leaves of the Mentha longifolia plant. Based on the accumulation and translocation factors obtained in this study, *Mentha longifolia* plant is effective in removing Fe, Mn, Zn and Cu from the fields. The present study gives a new perspective about the presence of some trace metals in some indigenous medicinal herbs and the oils they grow in.

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