Investigation of Heavy Metals Content in Medicinal Plant, *Eclipta alba* L.

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Summary: Heavy metal such as Cr, Fe, Zn, Mn, Ni, Pb, Cu and Cd were investigated in a medicinally important plant, *Eclipta alba* L. as well as in the soil it was grown using atomic absorption spectrophotometer. The plant samples were collected from their natural habitat at three different locations of Peshawar, Pakistan. The whole plant materials (roots, stems and leaves) were found to contain all heavy metals except Cd, which corresponds to their concentration in the soil it was grown. Among all the heavy metals, Fe was found to be at the highest level (8.95 to 27.7 mg/kg) followed by Mn (0.44 to 14.0 mg/kg) and Zn (1.04 to 4.50 mg/kg), while the rest of metals were at low concentration. The present study showed that *E. alba* L. is suitable for the control of environmental pollutants such as heavy metals, however, for medicinal purposes; it should be collected from those areas, which are not contaminated with heavy metals. The purpose of the current study was to standardize various indigenous medicinal plants for heavy metals contamination and to make awareness among the public regarding its safer use and collection areas, containing high level of heavy metals and their adverse health affects.

Introduction

Plants have been used extensively as medicine for the treatment of various ailments throughout human history and even today this trend continues. According to the World Health Organization (W.H.O.), approximately 75-80 % of the world's population use plant-based medicines. All plants may not be as useful as claimed, or may have more therapeutic properties than are known traditionally. Therefore, proper scientific knowledge is required to investigate and explore the exact standardization of such medicinally important plants [1-5].

Environmental pollution and exposure to toxic materials is a growing problem world-wide. The unscientific use of hazardous metals in agriculture and industries and its dumping has created a great risk for human, plant and animals [6]. Medicinal plants are the raw material (in the form of whole plant, crude extracts or purified constituents) for many herbal formulations and popular supplements. The assimilation of heavy and toxic metals in plants is evident [7-14]. These metals have great tendency to accumulate in living organisms including human beings if exposed for prolonged periods of time [7-14]. The presence of such metals above the permissible levels can cause various consequences. Thus, both the deficiency and excess of essential micro-minerals such as Fe, Zn and Cu are harmful to the human health. Effects of toxic metals (Cd, Cr, Pb, Hg, As, Co, Ni, etc.) on human health and their interaction with essential trace elements may produce serious health problems [7-14]. W.H.O. recommends that medicinal plants which form the raw materials for various medicines should be checked for the presence of different contaminants such as heavy/ toxic metals, pesticides, fungi and microorganisms [1-3].

Pakistan has been bestowed with the wealth of medicinal plants and crude medicinal plant materials worth more than 3 million US$ are used in Pakistan per year while large quantities of these materials are also exported to the international market (~ 6 million US$) although at cheaper prices [4, 5]. In order to make these valueless but potentially worthy products into precious one, numerous methods for the economic value addition to such products are available such as physical, chemical and biological standardizations.

Unfortunately, very little work is reported regarding heavy/toxic metals accumulation in medicinal plants or herbal drugs, especially in Pakistan. In continuation of our previous studies [9-14] regarding the standardization of various medicinal plants of Pakistan for heavy/toxic metals contamination, the authors have now screened another medicinally important plant, *E. alba* L. This study will provide useful reference data, which will

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make scientific base line values. This investigation will provide a reliable step towards the standardization of materia medica of Pakistan.

E. alba (L.) Hasskl. belongs to Asteraceae family, which find numerous application in folk medicines. It is a small, branched annual herb with white flower heads and found in the tropical and subtropical regions of the world. It is used as a tonic, diuretic, catarrhal jaundice, skin diseases, in hair oil, for increasing appetite, viral hepatitis, and to enhance memory and learning. The various extracts of this plant have shown antiviral and antimicrobial activities along with analgesic properties. This plant is also used for inflammation, minor cuts, burns and the fresh leaf-juice is considered very effective in stopping bleeding. From the whole plant, a new triterpene saponin, namely eclalbatin, together with alpha-amyrin, ursolic acid and oleanolic acid have been isolated [15]. *E. alba* L. contains coumestan-type compounds (wedelolactone and dimethyl wedelol-actone) which are used in phytopharma-ceutical formulations of medicines prescribed for treatment of cirrhosis of the liver and infectious hepatitis [16-19].

Results and Discussion

Environmental pollution due to heavy/toxic metals is not a new phenomenon, as they are present in various amounts in soil and vegetation [7-14]. In fact many of them are required as micronutrients for plants, animals, microorganism as well as human beings. The anthropogenic or man-made sources of metals contamination are mainly associated with certain industrial activities, agricultural practices, automobile emission, coal-fired power generation plants, municipal incinerator, etc [7-14]. A correct evaluation of the risk associated with heavy/toxic metals accumulation in medicinal plants as well as in the soils they are grown requires a comparison between the metals concentration estimated and reference values reported in the literature or those established by the regulatory authorities [7-14]. In the present study, heavy/toxic metals like Cr, Fe, Zn, Mn, Ni, Pb, Cu and Cd were assessed in various parts of medicinal plant, *E. alba* L. as well as in the soil it was grown to make a useful reference data (Table-1).

Chromium

The concentration of Cr in the plants collected from Spot-1 was found to be 0.19 (roots), 0.20 (stems) and 0.45 (leaves) mg/kg. Soil samples collected from this spot containing 0.57 mg/kg of Cr as shown in Table-1. The plants and soil samples collected from Spot-2 showed considerable variation in amount of Cr; *i.e.,* 0.08 (roots), 0.22 (stems), 0.31 (leaves) mg/kg and 0.42 (soil) mg/kg. Cr was also detected in the roots, stems, leaves and soil samples from Spot-3, however, the concentration is low (Table-1). The general trend of Cr level in the three spots was in order of Spot-1 > Spot-2 > Spot-3., while in the plant parts the order was; leaves > stems > root. This shows that the plant accumulates more Cr in their leaves and stems, while the lower concentration of Cr in roots can be explained by the amount of Cr present in soil and making of slow equilibrium. Concentration between 5-30 mg/kg is considered critical for plants and above this limit could cause yields reduction. The current findings show that the concentration of Cr is well below the critical limits for plants. Similar results were reported previously for other medicinal plants [9-14].

Cr is one of the known toxic pollutants in the world. The main sources of Cr contamination are tanneries, steel industries, sewage sludge applications and fly ash. Besides these, Cr plating and alloys in motor vehicles are considered to be the other prominent sources of this metal [9, 10, 20]. At an elevated level, it is toxic for plants and animals as well human beings. The problems associated with Cr exposure are; skin rashes, upset stomach ulcers, respiratory problems, weakened immune systems, kidney/liver damage, alteration of genetic materials and ultimately death [9, 10, 20].

Iron

Among the various metals studied, Fe was found to be high concentration in all samples. The plant materials collected from Spot-1 contain almost equal amount of Fe while in soil samples, its quantity was slightly higher, *i.e.,* 27.7 mg/kg. In case of plant parts and soil samples collected from Spot-2 and Spot-3, Fe was found in the range from 11.9 to 19.1 mg/kg and 8.95 to 20.0 mg/kg, respectively (Table-1). The variation in the amount of Fe in these compartments was insignificant. The results showed a general trend of Fe concentration in the plant samples in order of Spot-1 > Spot-2 > Spot-3 while in soil samples, the order was Spot-1 > Spot-3 > Spot-2. Most of Fe was accumulated in the leaves of the plant. The present results are in good agreement with the previously reported data [9-14].

Table-1: Concentration of heavy metals in plant materials (roots, stems and leaves) and soil samples.

Selected Areas	Plant Materials & Soils	Heavy Metals Concentration (mg/kg)*							
		Cr	Fe	Zn	Mn	Ni	Pb	Cu	Cd
Spot-1 (Polluted Area)	Roots	0.19	24.7	2.28	14.0	0.11	0.16	0.90	ND
	Stems	0.20	24.6	2.26	6.79	0.09	0.12	1.30	ND
	Leaves	0.45	23.9	1.61	6.83	0.16	0.41	1.21	ND
	Soil	0.57	27.7	3.87	6.54	0.79	0.55	0.34	ND
Spot-2 (Less Polluted Area)	Roots	0.08	11.9	2.55	5.76	0.08	0.08	0.83	ND
	Stems	0.22	15.6	3.47	8.13	0.09	0.03	0.82	ND
	Leaves	0.31	19.1	2.88	6.69	0.12	0.14	1.15	ND
	Soil	0.42	17.0	4.50	4.50	0.60	0.24	0.39	ND
Spot-3 (Unpolluted Area)	Roots	0.09	9.56	1.97	2.54	0.06	0.05	1.06	ND
	Stems	0.09	8.95	2.61	3.76	0.08	0.02	0.77	ND
	Leaves	0.28	16.5	1.39	6.50	0.11	0.19	0.31	ND
	Soil	0.30	20.0	1.04	0.44	0.39	0.16	0.36	ND

*on dry weight basis
ND: Not detected

Difference in the concentration of Fe in plant materials and soil samples is not because of the pollution, as Fe is very essential for plants and other living organisms. Its deficiency can cause various types of diseases; however, at high concentration it affects plant growth [9, 10, 20].

Zinc

Plant materials, *i.e.,* roots, stems and leaves collected from Spot-1 containing 2.28, 2.26 and 1.61 mg/kg of Zn, respectively, while in the soil samples its concentration was 3.87 mg/kg. In case of samples from Spot-2, the amount of Zn was 2.55 (roots), 3.47 (stems), 2.88 (leaves) mg/kg and 4.50 (soil) mg/kg. Similarly, from 1.04 to 2.61 mg/kg of Zn was found in samples from Spot-3 (Table-1). The results show that the plant accumulate high amount of Zn in roots and stems which may be due to its uptake from the available soil. Thus, as a whole the concentration of Zn in three spots was in order of Spot-2 > Spot-1 > Spot-3. An analogous order for Zn contents were reported elsewhere [9-14].

Recently, great attention have been given to Zn pollution, although it is one the essential and enzymatic elements for plants, animals and human beings. Most plant species were found to be tolerant at higher Zn concentration. The general sources of Zn contamination are; agro-chemicals, burning of fossil fuels, sewages and non-ferrous smelters [9]. Due to its importance, Zn is present in blood and about 85 % of it combines with protein for transport after absorption and its turnover is rapid in pancreas. Deficiency of Zn causes diabetic hyposomia, hypogensia or coma [8].

Manganese

Mn was found to be the second most abundant element in the present study (Table-1). The roots of plant samples of Spot-1 containing 14.0 mg/kg of Mn while the stems, leaves and soil samples have almost equal concentration of this element, *i.e.,* 6.79, 6.83 and 6.54 mg/kg, respectively. The plants collected from Spot-2 have high concentration of Mn in their stems (8.13 mg/kg) followed by leaves (6.69 mg/kg), roots (5.76 mg/kg) and soil samples (4.50 mg/kg). In the case of plant materials from Spot-3, lower concentration of Mn was recorded compared to the other two areas and its range was from 0.44 to 6.50 mg/kg as shown in Table-1. In general the concentration of Mn in the three spots was in the order of Spot-1 > Spot-2 > Spot-3. Such kind of Mn level was found in the previous studies on selected medicinal plants [9-14].

Mn is another essential element for plants and animals growth. Its uptake is controlled metabolically. Soil derived Mn from the parent materials and its content in the rocks are higher than the concentration of other micronutrients apart from Fe [8]. The main sources for Mn in soil are fertilizers, sewage sludge and ferrous smelters. Critical Mn concentration in soil is rather high 1500-3000 mg/kg and as critical concentration in plant is in the range of 300-350 mg/kg [9, 10, 20, 21]. The present results shows that the level of Mn is well below the critical values and hence acceptable at this level, because it does not affect the plants growth nor will cause environmental pollution.

Nickel

Ni was also detected in all samples as shown in Table-1. The concentration of Ni was found to be almost the same in the roots and stems from Spot-1, *i.e.,* 0.11 and 0.09 mg/kg, respectively, while in leaves 0.16 mg/kg of Ni was recorded. The soil samples from this spot containing 0.79 mg/kg of Ni. Similarly, the amount of Ni determined in the plant parts collected from Spot-2 and Spot-3 were approximately the same. However, in the soil samples obtained from Spot-2 and Spot-3 have higher amount of Ni and the values were 0.60 and 0.39 mg/kg, respectively (Table-1). Thus, the overall concentration level of Ni in the three spots was in the order of Spot-1 > Spot1 > Spot-3. Similar results were also reported previously [9-14].

Ni is an abundant element and is found primary combined with oxygen or sulfur in the environment. It is present in all soils and is emitted from volcanoes. The most common ailment arising from Ni contamination or its compounds is an allergic dermatitis known as nickel itch, which usually occurs when skin is moist. Generally, Ni and most of its salts do not cause systematic poisoning but Ni has been identified as a suspected carcinogen at elevated concentration. It also adversely affects lungs and nasal cavities [9-14].

Lead

As can be seen from Table-1, high Pb concentration was found in the plant materials (0.69 mg/kg) and soil samples (0.55 mg/kg) collected from Spot-1. Like Ni, the amount of Pb was also found to be the same in the plant parts collected from Spot-2 and Spot-3 but much lower than Spot-1, however, its concentration in soil samples obtained from Spot-2 was 0.24 mg/kg and Spot-3 was 0.16 mg/kg. The concentration of Pb in plant parts from all spots was in the order of aerial parts (stems and leaves) > roots. In the soil samples collected from three spots, the amount of Pb found in the order of Spot-1 > Spot-2 > Spot-3. Obviously the high Pb concentration in the aerial parts of plant is due to air-borne Pb which getting the fast steady state equilibrium [7, 9]. The results show that the plants from the three diverse environments accumulated different amount of Pb. The most sensitive was being Spot-1 and least one was Spot-3. The maximum acceptable Pb concentration for foodstuff is around 1 mg/kg [9]. The level of Pb found in the present study is well within the permissible limit.

Pb is considered highly hazardous for plants, animals and particularly for microorganisms. The main sources of Pb contamination are; Pb mines, fuel combustion, sewage slug applications and farmyards manure. Long-term exposure to Pb can result in a built of Pb in the body and more severe symptoms. These include anemia, pale skin, a decrease handgrip strength, abdominal pain, nausea, vomiting and paralysis of the wrist joint. Prolong exposure may also result in kidney damage [9-14]. If the nervous system is affected, usually due to very high Pb exposure, which results various affects including severe headache, coma, delirium and death [9-14]. Continued Pb exposure can cause decrease in fertility and increase the chances of miscarriage or birth defects [9-14, 21, 22].

Copper

The amount of Cu determined in the plant materials collected from Spot-1 was 0.90 (roots), 1.30 (stems) and 1.21 (leaves) mg/kg while from Spot-2, the concentration in the roots and stems were comparable and little higher quantity of Cu was recorded in the leaves, *i.e.,* 1.15 mg/kg. The plant parts; roots, stems and leaves collected from Spot-3 containing 1.06, 0.77 and 0.31 mg/kg of Cu, respectively (Table-1). The soil samples obtained from three spots have almost the same amount of Cu as shown in Table-1. The results shows that the plants grown on the three spots contained significantly different amount of Cu, however, the overall concentration of Cu in the plant material was in the order of Spot-1 > Spot-2 > Spot-3. A similar trend was found in the previous studies [9-14]. Critical concentration for Cu in plants is between 20-100 mg/kg [9-14]. In the present study, lower amount of Cu was found showing no harmful effect of this metal on plant.

Cu is one of essential elements for plants and other living organisms. The most common sources for Cu in the environment are; pesticides, fertilizers, industries and sewage sludge. Melting, grinding or cutting of Cu may produce fumes or dust, and exposure or inhalation of these fumes may produce potentially health hazards [9-14]. Fumes of Cu may cause metal fumes fever with flu like symptoms and hair and skin discoloration while dermatitis has not been reported. Systematically as well as Cu dust and fumes can cause irritation of the upper respiratory tract, metallic taste in the mouth and nausea [9-14, 22].

Cadmium

Surprisingly, Cd was not detected in the plant materials and soil samples collected from three spots (Table-1). This may be due to the less or no Cd contamination in the surrounding environment. In a similar study on *Silybum marianum*, Cd was found in the soil samples while in plant parts it was not detected [9]. Similarly, *Lepidium sativum* collected from the polluted and less polluted areas showed the presence of Cd while this metal was not found in the samples from unpolluted areas [12].

Cd is toxic metal and can cause serious health problems. More recently attention has been focused for its availability in water, soil, milk, dietary, medicinal plants and herbal drugs, etc [9-14]. The most common sources for Cd in soil and plants are phosphate fertilizers, non-ferrous smelters, Pb and Zn mines, sewage sludge application and combustion of fossil fuels [9-14]. Critical level for Cd in soil is between 3-5 mg/kg [9-14]. At this level, in most cases it cannot cause toxic or excessive accumulation concentration in plants or the lowest level of the element concentration in plants that can cause yield reduction is between 5-30 mg/kg [9-14, 22].

Experimental

Reagents and Chemicals

Analytical grade reagents, chemicals and de-ionized double distilled water were used throughout this work. HCl (37 %) and HNO_3 (65 %, extra pure) were obtained from Rd H Laborchemikalien, GmbH & Co., Germany.

Instrument

A Perkin Elmer atomic absorption spectrophotometer (AAS) (Model 3100) was used for measurements under the standard operating conditions (Table-2). The hollow cathode lamps used were made by Perkin Elmer.

Table-2: Standard operating conditions of AAS for the analysis of heavy metals in plant materials (roots, stems and leaves) and soil samples.

S. No.	Elements	λ_{max} (nm)	Slit Size (nm)	Flame Type
1	Cr	359.3	1.3	Air/Acetylene
2	Fe	248.3	0.2	Air/Acetylene
3	Zn	213.9	1.3	Air/Acetylene
4	Mn	279.6	0.4	Air/Acetylene
5	Ni	232.0	0.2	Air/Acetylene
6	Pb	283.3	1.3	Air/Acetylene
7	Cu	324.8	1.3	Air/Acetylene
8	Cd	228.8	1.3	Air/Acetylene

Sampling

The plant (*E. alba* L.) as well as the soil samples, were collected from their natural habitat at three different locations of Peshawar, Pakistan. The plant materials were divided into three parts, *i.e.*, roots, stems and leaves. A voucher specimen of the plant is authenticated at the herbarium of the Department of Botany, Kohat University of Science & Technology, Kohat, Pakistan. During the whole sampling processes, its transportation to the laboratory and storage, all precautionary measures were observed.

Extraction of Heavy Metals

The extraction of heavy metals from the plant materials (roots, stems and leaves) and soil samples was performed by the method as described in the literature [7-14]. Briefly, the plant materials (roots, stems and leaves) were washed thoroughly with tap water and finally with de-ionized double distilled water. The samples were dried at 60 °C in an oven and stored in dry clean polythene bags for further investigation. Each dried sample was converted into a finely powdered form by putting it in an agate vibratory disc mill to pass through a 1.3 mm stainless steel sieve. Dry ashing of the plant materials was done by placing 1 g sample in a pre-weighed porcelain crucible and was ignited for 6 h at 600 °C in a muffle furnace. The ashed sample was transferred to a beaker and added to it 10 mL of 20 % HCl and was digested in a water bath for 20 min. The resulting solution was filtered through an ultrafilter membrane (0.8 μm pore diameter). The filtrate was diluted to 100 mL with de-ionized double distilled water. The acid extract was directly analyzed with AAS.

1 g of air-dried and sieved (< 2 mm) soil was taken and added 15 mL of Aqua Regia (HCl :

HNO_3, 3 : 1 v/v) and swirled to wet the sample. The sample was kept overnight at room temperature and then heated at 50 °C for 30 min. The temperature was raised to 120 °C and the heating was continued for further 2 h. The mixture was cold to room temperature and added to it 10 mL of 0.25 M HNO_3. The resulting mixture was filtered through a Whatman filter paper (No. 542) and washed with small aliquot of 0.25 M HNO_3. The filtrate and washings were transferred to a 50 mL volumetric flask and leveled up to the mark with 0.25 M HNO_3 [7-14].

Conclusion

The present study showed that except Cd, all other heavy metals (Cr, Fe, Zn, Mn, Ni, Pb and Cu) were found in the selected three areas. The overall level of heavy metals was in the order of Spot-1 > Spot-2 > Spot-3. Therefore, the three spots could be categorized as polluted, less polluted and unpolluted areas, respectively for Spot-1, Spot-2 and Spot-3. The plant parts could also be considered as bioindicator for various heavy metals, for example, roots for Cr and Mn, stems for Zn and leaves for Ni and Pb. The study concluded that the plant grown on polluted areas has high risk of having the heavy metals concentration above the permissible limit for each of them as compared to the less polluted or unpolluted areas. The current findings indicate that the medicinal plant or plant parts used for various types of ailments must be checked for heavy metals contamination in order to make it safe for human consumption. In other words, for medicinal uses, it should be collected from areas not contaminated with heavy metals. The current study will also provide useful reference data for the standardization of materia medica of Pakistan, which could be employed as the scientific base line values.

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