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Toxic and some essential metals in medicinal plants used in herbal medicines: A case study in Pakistan

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The levels of some heavy metals in eight different medicinal plant samples along with soils were collected from two different locations from salt range of Punjab, Pakistan in order to evaluate those vital metals involved in human health implications. These plant species were: *Dodonaea viscosa, Withania somnifera, Solanum nigrum, Calotropis gigantea, Mentha spicata, Paganum hermala, Cannabis sativa, Adhatoda vasica,* especially those used in the treatment of diseases and their long term usage. The evaluated metals were cadmium (Cd), Nickel (Ni), Chromium (Cr) and lead (Pb). Atomic absorption spectrophotometry (wet digestion) was used for the analyses, and content of metals per sample was expressed as mg/kg. The analysis of variance revealed that there were significant effects of site variation on medicinal species in Ni and Cr contents while the opposite were observed in case of Pb and Cd at location I. The Ni concentrations found in medicinal plants varied significantly while Pb, Cd and Cr were evident as non significant at location II. There was positive non significant correlation of Pb and Cr among soil and plant while negative non significant for Ni and Cd at both locations. The findings suggest that the use of these plant species for the management of diseases will not cause heavy metal toxicity and may be beneficial to the users in cases of micronutrient deficiency, as these metals were found to be present in readily bioavailable form.

Key words: Toxicity, heavy metals, atomic absorption spectrophotometer, medicinal plant.

INTRODUCTION

Medicinal plants play a major role in the health care sector of developing nations for the management of diseases, and almost 80% population of the world directly or indirectly depends on medicinal plants and their products. Thus herbal preparations, drugs and medicines have a prominent role in the pharmaceutical markets and health care sector of the 21st century (Annan and Houghton, 2008). Heavy metals are important factors for the proper functioning of vital organs in the body. Iron is a component of hemoglobin and other compounds used in respiration. Heavy metals are widespread in soil as a result of geo-climatic conditions and environmental pollution even in wilderness areas of the world. Therefore, their assimilation and accumulation in medicinal plant species is obvious. Together with other pollutants, heavy metals are discharged into the environment through industrial activities, automobile exhaust, heavy-duty electric power generators, municipal wastages, refuse burning and pesticides used in agricultural practices (Jarup, 2003).

The extract of some medicinal plants like fresh juice of onion is able of reduce both acute and chronic pain as well as irritation, with a more burly effect towards inflammation (Nasri et al., 2012). Human beings, animals and plants take up these metals from all possible environments that is, through soil, air and water. Heavy metals have the tendency to accumulate in various vital plants and as well as in human organs. Since plants and animals are essential sources of micronutrients for man, either through dietary sources and various herbal preparations, so it becomes necessary to monitor the levels and fluctuations in biological mediums that are explored by man for both dietary and medicinal purposes, because deficiencies or excesses of micronutrients can be the possible threats for disease generation. Although a lot of phytochemical and bioactivity related studies have been carried out on a number of medicinal plants, however not much has been reported on different aspects of heavy metal contents of these plants (Bayor et al., 2009).

Peganum harmala L. is recognized as Syrian rue, Wild rue and Harmal. *P. harmala* extracts are considered vital for drug development because they are stated to have frequent pharmacological activities in the Middle East, particularly in Iran and Egypt. For a long time, *P. harmala* has been used in conventional medicines for the liberation of pain and as an antiseptic cause (Asgarpanah and Ramezanloo, 2012). Higher amount of different heavy metals in various plants and ultimately in animals can cause a variety of complications in animals and human beings (Parmeggiani, 1983).

The *n*-hexane fraction of root was disastrous to fabricate any insecticidal effect, while the chloroform fraction was useful against *Callosbruchus analis* and *Rhyzopertha dominica* with 20% mortality. The crude ethanolic extract and its ethyl acetate fraction illustrate 40% mortality *C. analis* and *R. dominica* (Alam et al., 2012). Various doses of heavy metals have also been reported in plants to decline because their accumulation trends are positively correlated with loss of flora. The heavy metals in higher amount cause toxicity which may go undetected without systematic scientific studies and procedure of their monitoring.

Although the level of heavy metal has been evaluated in plants, it is easily taken up by public due to the use of plants in different local herbal medicine. These medicinal plants have been widely employed to treat different problems in human beings and other organism (Ravindran et al., 2005). Trace element ingestion, even in very small quantity, can affect not only the physiological function and health of living organisms but also demographic, distribution and reproduction, as well as survival of organism. Toxic elements like Pb and Cd are widely distributed and mobilized in environment, and human exposure to these non-essential elements has been consequently elevated (Lansdown and Yule, 1986).

At higher level of exposure of human beings, there is damage to almost all organs and organ system, most notably central nervous system, kidneys and circulatory system, leading to death at excessive levels. At very low level, biochemical, psychological and neuron, which are the heavy oral-processes, are influenced mostly (Goldstein, 1992). Pb persists to be a significant public health problem in various countries in Asia, not only between human but also among a variety of species of other organism (Hsu et al., 2006). It is necessary to know about the level of diverse heavy metal in medicinal plants used in herbal medicine. Some of the heavy metals may be lethal and these are frequently present in environmental matrices. They are establishing in soil, water and plant and readily mobilize by human activities as waste material (Larison et al., 2000).

The present study was assumed to estimate some heavy metals in generally used medicinal plants as raw drugs and other herbal medicine. Therefore, heavy metals cause a potential threat to different organism including human health (Hsu et al., 2006). Heavy metal concentrations are eminent in all the environment that is, soil, water and air in the last decades (Nriagu and Pacyna, 1988). The toxicity of heavy metal to aquatic organisms has been the focus of interest to scientist for numerous years. Important toxic metal includes Cd, Hg, Pb, Cu, Cr, Ni, Mn, Ca, Au, Ag, Li, Ce, Ga and Fe. The heavy metals find their means to water bodies through wastewater from diverse industries (Meena et al., 2008).

In view of the fact that micronutrients can be good, toxic or lethal depending on the quantity, the study also appraise the health implications of the heavy metals, based on the suggested daily intake of medicinal plant decoctions (Anonymous, 2007). Keeping in view the extensive use of medicinal plants in herbal medicine and destructive effects of heavy metals on public health, this study therefore required to set up the presence, quantity and occurrence of six heavy metals (Ni, Cr, Cd, and Pb)

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Table 1. The samples with symbols.

Symbols	Plant species
PS1	*Dodonaea viscosa*
PS2	*Withania somnifera*
PS3	*Solanum nigrum,*
PS4	*Calotropis gigantea*
PS5	*Mentha spicata*
PS6	*Paganum hermala*
PS7	*Cannabis sativa*
PS8	*Adhatoda vasica*

Table 2. The mean square values of Pb, Ni, Cd and Cr in selected soil samples.

Metals	Sites			
	Soil		Plant	
	Location I	Location II	Location I	Location II
Pb	0.312*	0.843**	0.124^{ns}	0.064^{ns}
Ni	0.003^{ns}	0.005**	0.073***	0.086**
Cd	0.499*	0.218^{ns}	0.003^{ns}	0.004^{ns}
Cr	0.011^{ns}	0.021^{ns}	0.736***	0.077^{ns}

in eight medicinal plants frequently used for the treatment and prevention and execution of diseases in various regions of the world.

MATERIALS AND METHODS

Area of study

The present investigation was conducted in the sub-urban area of Kalar Kahar, Chakwal, Pakistan. Kallar Kahar is a union council and subdivision of Chakwal District in Punjab, Pakistan. It is a tourist destination located 25 km southwest of Chakwal along the motorway. It is notable for its natural gardens, peacocks and a saltwater lake. Geographically, the valley is located between coordinates 32.26' 11" to 32.41' 18" North and 71.50' 33" to 72.30' 07" East. The present experimentation was carried out in an area of 200 acre. Different types of plants are planted in this land like *Dodonaea viscosa, Withania somnifera, Solanum nigrum, Calotropis gigantea, Mentha spicata, Paganum hermala, Cannabis sativa* and *Adhatoda vasica.*

Plants

A total of 8 different medicinal plant species used in the study were collected from their natural habitat and authenticated at the Department of Botany, University of Sargodha, where voucher specimen of each plant species is deposited. The leaves were air-dried for four days and later pulverized.

Sample collection and processing

Five composite samples of 8 different plants were obtained from the vicinity of Kallar Kahar, Punjab Pakistan in September, 2012 (Table 1). During present investigation, two locations were selected for the collection of soil and plant samples from 20 km radius of Kalar Kahar Lake to evaluate variation in metals from different locations associated with health implications. The samples were stored in plastic food grade containers kept at room temperature until analysis. Samples were dried at 70°C for 48 h in a hot air oven and ground prior to chemical analysis. The soil samples were collected following the procedures described by Sanchez (1976). These collected samples were air dried, stored in labeled sealed brown bags and placed in oven for 48 h at a temperature of 72°C. The plant samples were also taken from the same locations from where soil samples were collected by sterilized equipments. The collected medicinal plant samples include *D. viscosa, W. somnifera, S. nigrum, C. gigantea, M. spicata, P. hermala, C. sativa,* and *A. vasica* (Table 1). Twenty four samples of these plants were taken during sampling from each location (3 replicates of each plant). The selected samples were washed with distilled water and diluted HCl to remove dust particles and other contaminants.

Sample preparation for analysis

Heavy metal analysis was done according to Association of Analytical Communities (AOAC) (1995) for non-volatile heavy metals. For this, 1 g powder of each sample was digested in HNO_3 and $HClO_4$ (9:1) using the wet digestion method by heating slowly on hotplate in fume hood chamber until clear solution was obtained. The final volume of solution was made up to 25 ml with deionized water. All necessary precautions were adopted to avoid possible contamination of sample. The heavy metals under study were Lead (Pb), Nickel (Ni), Cadmium (Cd) and Chromium (Cr). Analysis was done using atomic absorption spectrophotometer AAS 6300 (Shimadzum Japan). Standard reference material of all metals (E. Merck) was used for calibration and quality assurance.

Statistical analysis

Data for different attributes were subjected to a statistical analysis using the statistical package for social sciences (SPSS) software for correlation and one-way analysis of variance worked out. Statistical significance between the mean was tested at 0.05, 0.01 and 0.001 level of probability as suggested by Steel and Torrie (1980). The bio concentration factor from soil to forage was calculated using Microsoft excel by following the formula stated by Cui et al. (2004).

Bio concentration factor = Mean metal concentration of plant / Mean metal concentration of soil

RESULTS

Soil metals

The mean square values of Pb, Ni, Cd and Cr in selected soil samples are presented in Table 2. Analysis of variance showed significant ($P < 0.001$) effects of site on soil Pb and Cd while its reverse was true in case of Ni and Cr at location I. The Pb and Ni concentrations found in soil varied significantly ($P < 0.001$) while Cd and Cr non significantly in soil samples at location II. The mean range concentrations (mg/kg) in soil were: Pb (6.80 to 7.83), Ni (0.701 to 0.78), Cd (3.45 to 4.63) and Cr (0.028 to 0.041) at location I while Pb (6.25 to 7.86), Ni (0.59 to

Table 3. The order of accumulation of heavy metals by medicinal plants from soil.

Metals	Order of accumulation in medicinal plants
Pb	$PS_4 > PS_1 > PS_5 > PS_2 > PS_8 > PS_3 > PS_7 > PS_6$
Ni	$PS_6 > PS_7 > PS_4 > PS_3 > PS_5 > PS_2 > PS_8 > PS_1$
Cd	$PS_3 > PS_5 > PS_6 > PS_3 > PS_4 > PS_7 > PS_1 > PS_8$
Cr	$PS_1 > PS_2 > PS_8 > PS_7 > PS_5 > PS_3 > PS_4 > PS_6$

Table 4. Mean metal concentrations (mg/kg) in soil.

Soil	Site I				Site II			
	Pb	Ni	Cd	Cr	Pb	Ni	Cd	Cr
S1	7.200	0.730	4.050	0.028	7.333	0.686	4.050	0.040
S2	7.383	0.710	4.483	0.030	7.500	0.603	4.400	0.030
S3	7.466	0.706	3.716	0.035	7.600	0.676	3.933	0.035
S4	6.800	0.715	4.283	0.036	6.250	0.655	4.266	0.036
S5	7.050	0.766	3.450	0.033	6.816	0.705	3.683	0.040
S6	7.600	0.701	4.400	0.041	7.366	0.641	4.366	0.038
S7	7.833	0.711	3.900	0.035	7.716	0.628	4.283	0.038
S8	7.400	0.786	4.633	0.033	7.866	0.590	4.466	0.040

0.70), Cd (3.68 to 4.46) and Cr (0.030 to 0.040) were at location II (Table 3).

Medicinal plants metals

Analysis of variance of data for heavy metals in plants showed significant ($P < 0.001$) effects of site on plants Ni and Cr while its opposite results were observed in case of Pb and Cd at location I. The Ni concentrations found in medicinal plants varied significantly ($P < 0.001$) while Pb, Cd and Cr non significantly at location II (Table 2). The mean concentration ranges (mg/kg) in soil were: Pb (5.78 to 6.27), Ni (3.25 to 3.64), Cd (0.025 to 0.040) and Cr (3.40 to 4.65) in medicinal plants at location I while Pb (6.28 to 6.69), Ni (3.20 to 3.62), Cd (0.066 to 0.086) and Cr (3.87 to 4.27) were at location II (Table 4).

DISCUSSION

The soil Pb levels ranged from 5 to 25 mg/kg as reported by Hayashi et al. (1985). These concentrations of soil Pb were lower them those earlier researches reported by Oluokun et al. (2007) in Nigeria, but higher than those found by Aksoy et al. (1999) in Turkey while studying on bio monitoring of heavy metal pollution in that region. The lead level in location soil also depend on intrinsic nature of soil and extrinsic factors through anthropogenic activities, leading to increased level of Pb far above the background level (Osweiler, 1996). The values found during the present study were at both ends higher than the critical values of 0.85 mg/kg as reported by Adriano (1986).

The present findings were much lower than values established by Govil (2001). So, there is no danger of its toxicity for medicinal plants growing therein. The recommended values of Cd in soil are 3 to 8 mg/kg (Ross, 1994). In the present study, the values of soil cadmium were found to be higher than those reported previously by Miller (1983), and almost similar values of soil cadmium have been reported by Pierce et al. (1982). Although the level of soil Cd in current investigation were lower than the toxic level suggested for plants, but the toxicosis by this element can be anticipated in this studied area. The soil chromium values were higher than the critical level of 0.02 mg/kg as reported by Anderson et al. (1973) but lower than those reported by Bergmann (1992) as 2 to 50 mg/kg. Soil Cr content reported during present study was higher than those reported in some earlier studies (Obiajunwa et al., 2002), and there is no danger of toxicity for medicinal plants in this area by this element in soil.

Mean concentration of Pb was greater than the critical level of 0.05 mg/kg reported by Tokaliogla et al. (2000). According to World Health Organization (WHO) (1998), permissible limit for Pb contents in herbal medicine is 10 ppm. The permissible limit set by Food and Agriculture Organization (FAO)/World Health Organization (WHO) (1984) in plants is 0.43 ppm. The lowest level of Pb which can cause yield reduction is 5 to 30 ppm, while the maximum acceptable concentration for food stuff is around

1 ppm (Neil, 1993). Pb concentration of 80 mg/kg in plants caused deleterious effects in human. The mean Pb value in plant samples were lower than those values described earlier by Oluokun et al. (2007).

The Ni concentration in the present study was below the limit described by Abassi et al. (2007). The permissible limit set by FAO/WHO (1984) in plants was 1.63 ppm. Ni is required in minute quantity for body as it is mostly present in the pancreas and hence plays an important role in the production of insulin. Its deficiency results in the disorder of liver (Kabata-Pendias and Pendias, 1992). Environmental Protection Agency (EPA) has recommended that the daily intake of Ni should be less than 1 mg beyond which it is toxic (McGrath and Smith, 1990). However, for medicinal plants, the WHO (2005) limits have not yet been established for Ni. Ni toxicity in human is not a very common occurrence because its absorption by the body is very low (Onianwa et al., 2000). Nickel exerts a potent toxic effect on peripheral tissues and on the reproductive system (Vukadinovre and Bertie, 1988). It also causes dose-related decreases in bone marrow cellularity and in granulocytomacrophage and pluripotent stem cell proliferative responses. Pb and Nickel were present in lower amount in the plants studied as compared to the other heavy metals in medicinal plants. There, levels were lower than the permissible limits which is 10 mg/kg for Pb (WHO, 1993), and 5 to 15 mg/kg for Ni per day (Barceloux, 1999).

Cd level was much higher than the critical level suggested by National Research Council (NRC) (1980). Cadmium accumulates in human body and damages mainly the kidneys and liver. The lowest level of Cd which can cause yield reduction is 5 to 30 ppm, while the maximum acceptable concentration for food stuff is around 1 ppm (Neil, 1993). The permissible limit set by FAO/WHO (1984) in edible plants was 0.21 ppm. The obtained cadmium levels in samples in this study were below the provisional tolerable weekly intake (PTWI) presented by WHO (60 µg/day/60 Kg) (Herrman and Walker, 1999).

Cadmium is a non essential trace element with uncertain direct functions in both plants and humans (Cui et al., 2004). It is however reported that the lowest level of cadmium which can cause yield reduction in plants is 5 to 30 µg/g, and the maximum acceptable concentration for foodstuff is about 1 µg/g (Hayashi et al., 1985). The results of this study indicated that about 80% of the plant species had cadmium content above 30 µg/g which is essential for improved yield. Cd in all the medicinal plants was lower than the toxic limits which is generally accepted and the normal Cd levels in medicinal plants are between 0.2 to 0.8 mg/kg, and toxic levels of Cd were describe as 1 to 30 mg/kg (Kabata-Pendias, 1992). It has also been reported that provisional tolerable weekly intake are of Cd is 7 mg/kg/body weight/week (WHO, 1993). The values found in our study were much lower than the above described values. Higher level of Cr in plants could cause toxicities in human. The permissible limit set by FAO/WHO (1984) in edible plants was 0.02 ppm. In present study, Cr level was low from the toxic level observed in salt range of Pakistan (Ahmad et al., 2009). The toxic effects of Cr intake is skin rash, nose irritations, bleeds, upset stomach, kidney and liver damage, nasal itch and lungs cancer (McGrath and Smith, 1990). The daily intake of Cr (50 to 200 µg) has been recommended for adults by US National Academy of Sciences (Watson, 1993). Cr was found below their recommended daily allowances that are 18 mg/day given by National Research Council (Anonymous) (1989). The contents of Cr range from 3.40 to 4.65 mg/kg in different plant sample.

Weak levels were similar to the results previously reported by various researchers (Koc and Sari, 2009; Maiga et al., 2005; Sheded et al., 2006). These plant cadmium were lower the Food and Drug Administration (FDA) recommended daily intake of this element for dietary sources which is 0.12 mg/g (Haider et al., 2004). This study was an attempt to enrich knowledge about the mineral contents of some medicinal plants and soil, and it may help in formulation of chemically pure medication. In this study, all of the determined values are below the WHO's permissible levels, except Cr, and may not constitute a health hazard for consumers, so it can be recommended that medicinal plants growing in this specific area of Pakistan are suitable for phytotherapeutical uses.

Bioconcentration factor

The bioconcentration factor for Pb, Ni, Cd and Cr from soil to medicinal plants ranged from 0.8471 to 0.932, 4.367 to 5.189, 0.0159 to 0.021 and 102.79 to 149.57, respectively (Table 5). The lowest value showed that the plant may be resistant to metals while the highest value indicated highly sensitive nature of plant (Alam et al., 2003). The low concentrations may be due to the presence of some other metals in the soil and this can suppress the uptake of metals by studied medicinal plants (Lokeshwari and Chandrappa, 2006). The bio concentration of metal depends upon the bioavailability of metals. The current investigation also supports the results that accumulation of Pb and Ni is comparatively less than that of Cd and Cr in medicinal plants (Olaniya et al., 1998).

Correlation

There was positive non significant correlation of Pb ($r = 0.081$) and Cr ($r = 0.146$) among soil and plant with negative non significant for Ni ($r = -0.236$) and Cd ($r = -0.372$) at both locations (Sinha et al., 2006). These results

Table 5. Mean metal concentrations (mg/kg) in medicinal plants.

Medicinal plants	Site I				Site II			
	Pb	Ni	Cd	Cr	Pb	Ni	Cd	Cr
Dodonaea viscosa	6.241	3.641	0.035	3.825	6.696	3.180	0.066	4.141
Withania somnifera	5.970	3.485	0.025	3.505	6.478	3.200	0.080	3.875
Solanum nigrum	5.788	3.378	0.035	3.400	6.381	3.475	0.078	4.073
Calotropis gigantea	5.970	3.196	0.028	3.791	6.343	3.566	0.073	3.933
Mentha spicata	5.761	3.258	0.040	4.456	6.283	3.543	0.071	3.816
Paganum hermala	6.270	3.290	0.031	4.636	6.435	3.621	0.086	4.276
Cannabis sativa	6.093	3.263	0.033	4.651	6.663	3.561	0.066	4.176
Adhatoda vasica	5.780	3.196	0.035	3.931	6.431	3.446	0.073	3.966

showed the mineral imbalance of Pb and Cr that had strong relation among soil and medicinal plants (Fytianos et al., 2001). Considering the Ni and Cd, there was weak association between soil and medicinal plants at both locations. The overall results indicated clearly that heavy metals are present in Pakistan medicinal plants and that the contents of these metals except iron were within acceptable and safe limits. Therefore, herbal formulations of these plant species can also be beneficial sources of appropriate and essential trace elements, though care must be taken to avoid iron toxicity, especially in higher doses.

Conclusion

The present study gives a new perspective about the presence of some heavy metals (Pb, Ni, Cd and Cr) in the medicinal plants and their corresponding soil. The lead, nickel and cadmium concentrations during the present study were below the WHO permissible levels and may not constitute a health hazard for consumers in this area. The current investigation showed that the Cr concentration is above the standard level and it can be toxic for humans. Effluents can be a source of this increase. Most of the plant species contained safe levels of the heavy metals analyzed and hence may have no adverse effects normally associated with heavy metal toxicity on people who patronize these products for their health needs.

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