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HEAVY METAL ANALYSIS IN FREQUENTLY CONSUMABLE MEDICINAL PLANTS OF KHYBER PAKTUNKHWA, PAKISTAN

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Abstract

Plants are extensively consumed for their medicinal and aromatic properties either in fresh or dry form. Their medicinal properties are hidden in the active ingredients of the plant parts. These active ingredients sometime linked in their structure to some non-essential elements or heavy metals. Excess of non-essential elements and/or presence of heavy metals are toxic to human health in long run. The present study was carried out to evaluate the selected medicinal plants for their endogenous lead, arsenic, cadmium, mercury and zinc contents. We observed higher Pb contents in *Cucumis sativus* (0.229 µg/g), followed by *Malus baccata* (0.191 µg/g) and *Geranium wallichianum* (0.038 µg/g). Arsenic contents were higher in *Portulaca oleraca* and *Geranium wallichianum* (0.542 µg/g), while 0.308 µg/g arsenic was recorded in *Monotheca buxifolia*. Lower arsenic contents were found in *Malus baccata* (0.139 µg/g) and *Saxifraga flagellaris* (0.33 µg/g). The Cadmium contents were 0.036 µg/g in *Cucumis sativus* and *Monotheca buxifolia*, while 0.03 µg/g in *Malus baccata*. Mercury contents were 0.0436 µg/g in *Portulaca oleracea*, 0.041µg/g in *Cucumis sativus* and 0.038µg/g in *Saxifraga flagellaris*. Highest Zn contents were recorded for *Geranium wallichianum* 0.209 µg/g, followed by *Cucumis sativus* (0.187 µg/g), *Purtulaca oleracea* (0.149 µg/g), *Malus baccata* (0.135 µg/g) and *Saxifraga flagellaris* (0.118 µg/g). Our current findings suggest that the medicinal plants contains permissible quantity of heavy metals and their use is thus beneficial for curing diseases and coping with micronutrient deficiency.

Key words: Medicinal plants, Heavy metal, Zinc, Lead, Mercury, Arsenic, Cadmium, Atomic Absorption Spectroscopy

Introduction

Traditional medicines are natural and still ideal to a major unit of the world (Ahmad *et al*., 2016; Tariq *et al*., 2016). The main advantage of traditional medicine are their apparent effectiveness, low side effects and of low cost. Plants are sensitive to climatic situations and accumulate different metals in their different parts and this can change the overall essential composition of the plant (Behera & Bhattacharya, 2016; Zahra *et al*., 2016). Medicinal plants are natural antioxidants and used for the cure of diseases all over the world (Shaban *et al.,* 2016; Habiba *et al*., 2016). Wild plants are the raw material for the manufacture of a wide range of local medication and are used without any superior management (Buialska *et al.,* 2015; Ikram *et al*., 2015). The ecological influence of lethal metal pollution associated with health problems persist a great area of distress. Trace amounts of some heavy metals are beneficial to human health; but their presence out of certain limits is injurious, causing continued cell poisoning. Plants are potentially active to accumulate metals (heavy and essential) in concentrations much higher than metal concentrations in the environment. This is due bio-accumulation and bio-concentration in plants increases their harmful effects. For example when excessive consumption of lead (Pb) can cause high blood pressure long weakening effects to organs like kidney and brain. Cadmium (Cd) causes injury to respiratory system, renal and cardiac complications. Even though excessive amounts of an essential mineral, like zinc can cause temperature, vomiting and overall faintness. Iron deficiency leads to anemia, in large amounts iron is risky and might cause abdominal and skin difficulties (Mustapha *et al.,* 2016; Nkansah *et al.,* 2016; Adnan *et al*., 2014). Medicinal plants contaminated with heavy metals are reported in many earlier works from different regions of Asia, America, and Africa herbal (Dghaim *et al*., 2015). About safety of traditional medicinal plants and presence of heavy metals in them, the available information is very less. The present study aims at determining and documenting the level of heavy metals in some commonly used medicinal plants in comparison to the World Health Organization (WHO) standard limits.

Materials and Methods

Collection and sample preparation of medicinal plants: Medicinal plants were collected, washed, air dried and powdered using grinding machine for further use. 3g of powdered samples were used according to the AOAC method (1990; 1995).

Heavy metal analysis was done according to AOAC (1990; 1995) using flame atomic absorption spectrometer (Perkin Elmer AA Analyst 700) and wet digestion method adopted from Meena *et al.* (2010). Standardization curve was established using working standards for each metal.

The experimental data was replicated thrice and analyzed statistically and mean values with standard errors were obtained.

Results and Discussion

Plant based medicines are said to be comparatively free from cross effects like allopathic drugs but the toxic metals in these stuffs causes health problems (Kulhari *et al.,* 2013; Hussain *et al*., 2014). Amongst the elements, presence of heavy metals (a.w. 63.5-200.6 g/mol and s. gravity more than 5 g/cm), which are universal in nature cause severe damage to living beings particularly humans (Behera & Bhattacharya, 2016). Human are inspired to consume more veggies, fruits and herbs, which are the springs of vitamins, minerals, fiber and valuable for health. The plants have both toxic and non-toxic metals at different concentration. Plants absorbed water along minerals and metals from soil and deposits it in different plant parts. Medicinal plants are used generally in cure of numerous diseases in herbal, Ayurveda, Unani and homeopathic systems of medicines in multiple ways (Shinwari *et al.*, 2013; Khalil *et al.*, 2014). Plants comprise secondary metabolites which not only have ingredients (alkaloids) but they also adulterated with toxins like heavy metals, which are risky to living entities (Cereda & Mattos, 1996). The opportunity of the toxic heavy metals that can be transferred to humans and animals through herbs is alarming for raw, traditional and herbal medicines. For required healing profits, raw material of good quality should be chosen free of heavy metal. WHO reports that medicinal plants and their products should be checked qualitatively and quantitatively for analysis of heavy metals (Singh *et al.*, 2014). Chen *et al.* (2005) reported presence of cadmium, cobalt, copper, iron, manganese, nickel, lead, zinc and mercury in 42 Chinese herbal medicinal plants. All samples had relative higher concentrations of iron, manganese, and zinc. A few of them were found to have fairly higher concentrations of the lethal metals such as cadmium, lead, and mercury. This was perhaps caused by adulteration during air-drying or preservation.

The most common heavy metals involved in human poisoning contain lead, mercury, arsenic, and cadmium. The world health organization thus recommends that those medicinal plants, which form the raw materials for herbal tonics should be tested for the presence of heavy metals. Yet, bulks of people living in remote rural regions collect them for their own use without any testing for harmful metals. The general concept that medicinal plants are harmless and lack heavy metals may be misinterpreted (Annan *et al.,* 2013). Sudha & Vivek (2014) reported that copper and zinc were present in high concentrations related to other metals in *Ceropegia juncea* (Roxb.). Cu and Zn are useful because these are indispensable elements in trace amounts for body metabolism. The others like arsenic, cadmium, lead and mercury are non-essential and found in low levels.

Plants absorbed lethal and non-toxic metals over a varied range of concentration. It is well known that plants absorbs metals (heavy + essential) from soil and accumulate them in their various parts. Current study was conducted to determine the heavy metal concentration in selected medicinal plants used as herbal medication by the local inhabitants. The concentration of lead (Pb), Arsenic (As), Cadmium (Cd), Mercury (Hg), and Zinc (Zn) in medicinal plants (*Cucumis sativus, Purtulaca oleracea, Malus baccata*, *Sexifraga flagellaris*, *Gerenium wallichianum* and *Monotheca buxifolia*) are given (Table 3; Fig. 1). Chen *et al.* (2005) reported that the concentration of metals in medicinal plants was comparable to that of several East Asian vegetables and fruits. Man and animals both intake poisonous heavy metals from vegetation used raw as fodder and in medication (Dwivedi & Dey, 2002).

Lead and its complexes are lethal to many body's tissues such as heart, blood, generative system, digestive tract, kidney and nerves. Lethal doses cause severe illness leading to permanent difficulties with cerebral problems. Lead toxicity cause stomach pain, nuisance, anemia, peripheral neuropathy and irritability. In severe conditions, it can cause seizure, coma and finally death (Bellinger, 2008; Singh *et al.*, 2014). The concentration of Pb is 0.229 µg/g in *Cucumis sativus*, which is highest among the selected medicinal plants. The Pb contents in *Malus baccata* is 0.191 µg/g, 0.038 µg/g in *Geranium wallichianum* and 0.017 µg/g in *Purtulaca oleracea*. *Saxifraga flagellaris* and *Monotheca buxifolia* showed no Pb presence (Fig. 2). The permissible amount of lead is 0.2 µg/g which revealed that all medicinal plants contain lower concentrations than the permissible quantity (Table 1). Similar to our current work, Sindhu & Beena (2016) reported the lower lead contents were present in medicinal plants as compared to universal standard.

Table 1. Permissible limits (FAO/WHO 1999) of the heavy metals (µg g^{-1}).

Permissible limits	Cadmium	Lead	Arsenic	Mercury
	0.1	0.2	0.1	0.03

Table 2. Local uses of selected medicinal plants.

S. No.	Botanical name	Family	Part used	Local uses
1.	*Cucumis sativus*	Cucurbitaceae	Seeds	Nutritive, Demulcent
2.	*Purtulaca oleracea*	Portulacaceae	Seeds	Expectorant
3.	*Malus baccata*	Rosaceae	Fruits	Dysentery, Stomachache, Nutritive
4.	*Saxifraga flagellaris*	Saxifragaceae	Rhizome	Kidney stone
5.	*Gerenium wallichianum*	Geraniaceae	Roots/Rhizome	Toothache, anti-rheumatic agent
6.	*Monotheca buxifolia*	Sapotaceae	Leaves	Purgative, vermifuge, in throat infections

Table 3. Concentration of heavy metal in selected medicinal plants.

S. No.	Medicinal plant	Lead (Pb) µg/g	Arsenic (As) µg/g	Cadmium (Cd) µg/g	Mercury (Hg) µg/g	Zinc (Zn) µg/g
1.	*Cucumis sativus*	0.229 ± 0.11	Not detected	0.036 ± 0.002	0.041 ± 0.01	0.187 ± 0.002
2.	*Purtulaca oleracea*	0.017 ± 0.01	0.542 ± 0.198	Not detected	0.0436 ± 0.009	0.149 ± 0.020
3.	*Malus baccata*	0.191 ± 0.104	0.139 ± 0.009	Not detected	Not detected	0.135 ± 0.040
4.	*Saxifraga flagellaris*	Not detected	0.330 ± 0.198	Not detected	0.038 ± 0.021	0.118 ± 0.008
5.	*Gerenium wallichianum*	0.038 ± 0.021	0.542 ± 0.19	Not detected	Not detected	0.209 ± 0.13
6.	*Monotheca buxifolia*	Not detected	0.308 ± 0.171	0.030 ± 0.001	Not detected	0.370 ± 0.12

Lead (Pb) mostly affects the nervous system, bones and kidneys as failure of kidney function and abnormalities of nervous system function is reported (Obi *et al.*, 2006; Khan *et al*., 2016; Singh *et al.*, 2014). Pb intoxication leads to nausea, headache, constipation fatigue, muscular aches and anemia. Rattan *et al*. (2005) reported that heavy metals were present in higher contents than in plant parts studied. However, accumulation of heavy metals varied from plant to plant. Pb was the highest in *Calotropis procera* root and the lowest in *Peristrophe bicaliculata* whole plant, and was lower in inhabited area than in traffic flow area.

Arsenic is non-essential toxic metal. Arsenic exhibits its poisonous effects by deactivating up to 200 functional enzymes. Acute poisoning starts with sickness, vomiting, belly pain, severe diarrhea, encephalopathy and marginal neuropathy. Chronic effects fallouts multi-system diseases with carcinogenic effect on most parts of the body (Singh *et al.,* 2014; Shinwari *et al*., 2013). Arsenic disrupts ATP production in Krebs cycle. At the level of the citric acid, arsenic inhibits pyruvate dehydrogenase and by competing with phosphate, it uncouples oxidative phosphorylation, thus inhibiting energy-linked reduction of NAD+, mitochondrial respiration, and ATP synthesis. Hydrogen peroxide production is also increased, which might form reactive oxygen species (ROS) and oxidative stress. These metabolic interferences led to death from multi system organ failure probably from necrotic cell death, not apoptosis (Chiou *et al*., 1997).

Arsenic concentration was 0.542 µg/g *in Purtulaca oleracea* and *Geranium wallichianum*, while 0.308 µg/g arsenic content was recorded in *Monotheca buxifolia*. *Malus baccata* showed 0.139 µg/g and *Saxifraga flagellaris* showed 0.33 µg/g while no traces of arsenic was found in *Cucumis sativus* (Fig. 3). Similar work was presented by Sindhu & Beena (2016), who reported that arsenic level is below detection limits in medicinal plant. Arsenic are often fatal, their signs consist of polyneuropathy, liver illness, optical disorder and finally loss of sight (Annan *et al.*, 2013).

Cadmium is a trace element with no defined direct roles in plants and humans, however the low level of Cd can cause reduced biomass in plants and extreme acceptable Cd contents for food and fodder is about 1 µg/g (Annan *et al.*, 2013; Singh *et al.*, 2014). Cadmium is a lethal metal and cause severe health problems. In recent times, focus has been made on Cd presence in water, soil, milk, dietary products, medicinal plants, herbal drugs, etc. The most common sources for cadmium in soil and plants are combustion of fossil fuels, phosphate fertilizers, lead and mines, non-ferrous smelters and sewage sludge application (Jarup *et al.,* 1998). Cadmium causes a disease Itai-itai showing symptoms like unstiffening of skeleton, anemia, renal failure and eventually death (Singh *et al.*, 2014).

In current study, Cd was recorded in *Cucumis sativus* and *Manotheca buxifolia* which was 0.036 µg/g and 0.03 µg/g respectively. *Purtulaca oleracea, Malus baccata*, *Saxifraga flagellaris* and *Geranium wallichianum* showed no Cd presence (Fig. 4). Our results indicated that selected medicinal plants have much lower Cd contents in their parts than the permissible limits of WHO or FAO. Similar work was also reported by Sindhu & Beena (2016), who reported the presence of Cd below detection levels in medicinal plant.

Mercury (Hg) causes problems in renal and nerve-system and can snapped the placental barrier with bad effects on the fetus. Hg disclosure may occur mostly from fish ingesting and dental mixture fillings of teeth (Singh *et al.,* 2014). Mercury poisoning causes well-known disease hydra-rgyria. The effects may include brain injury, kidney and lungs failure in addition to its pink disease acrodynia, Hunter-Russell syndrome, and Mina Mata disease (Clifton, 2007; Atsdr, 1999).

Portulaca oleracea showed 0.0436 µg/g of Hg, followed by *Cucumis sativus* and *Saxifraga flagellaris* i.e. 0.041µg/g and 0.038µg/g respectively. *Malus baccata*, *Geranium wallichianum* and *Monotheca buxifolia* showed no Hg results (Fig. 5). Previously researchers, Sindhu & Beena (2016) reported the presence of mercury below detection levels in medicinal plants. High levels of Hg is associated with male infertility in human, inhibition of antioxidant enzymes, and harm to brain (Choy *et al*., 2002; Annan *et al.,* 2013). All plants in current study contained permissible levels of mercury.

Zinc is essential for healthy life but excess of zinc can be harmful and cause zinc toxicity. Such toxicity have been observed to occur at Zn ingestion of more than 225 µg/g of Zinc. Zn is an important portion of several metallo-enzymes, particularly of the enzymes which play a vital role in nucleic acid metabolism. Zn acts as a membrane stabilizer and immune response stimulator. Nausea, vomiting, diarrhea, fever and laziness are the indicators of acute zinc toxicity. The probable harmless and satisfactory regular ingestion of zinc is in the range of 10,000 and 20,000 µg/day (Annan *et al.,* 2013).

In current study, the level of Zn was higher in *Geranium wallichianum* rhizome (0.209 µg/g), which was followed by *Cucumis sativus* seeds with 0.187 µg/g, while in *Purtulaca oleracea* seeds 0.149 µg/g, *Malus baccata* fruit 0.135 µg/g, *Saxifrga flagellaris* rhizome 0.118 µg/g of zinc was recorded. *Monotheca buxifolia* leaves showed lowest Zn levels among the selected medicinal plants (Table 3; Fig. 6). Similar report was presented by Sindhu & Beena (2016), who reported Zn below detection levels in medicinal plants.

Zn is one of the most essential trace nutrients present in muscles and bones (85%), 11% in the skin and the liver while the rest is distributed in all other tissues of the body (Tapiero & Tew, 2003). Excessive amounts of zinc can cause abdominal pain, nausea, vomiting and diarrhea. Continued contact of Zinc causes copper shortage (Anonymous, 2001).

The plants have the tendency to accumulate some of the metals which are not linked directly to their survival like Cd, Co and Ag (Ajasa *et al*., 2004). In human, trace elements play a pivotal role both as preventive and as curative agents against various diseases. However, the contamination of heavy toxic metals in plants due to any factor could develop serious health problems because there is a narrow concentration range between the deficiency and toxicity levels of heavy metals in humans (En *et al*., 2003; Shaban *et al*., 2016).

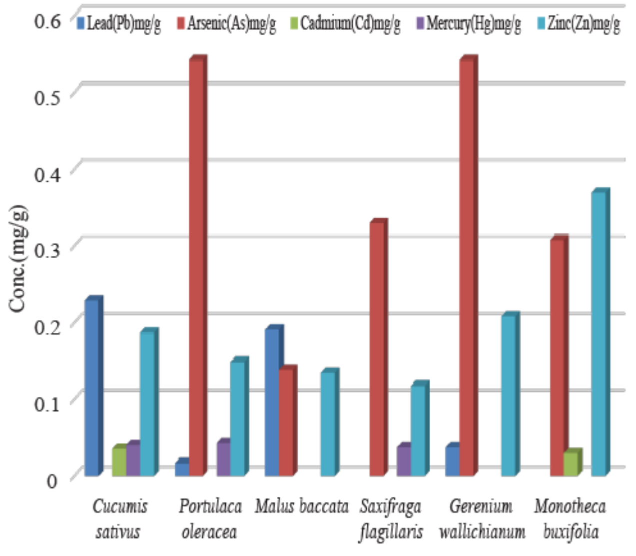

Fig. 1. Concentration of various heavy metals in the selected medicinal plants.

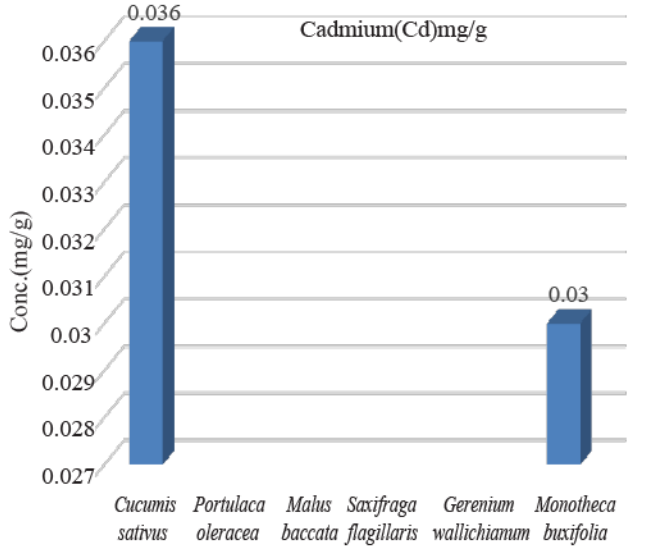

Fig. 4. Cadmium concentration of selected medicinal plants.

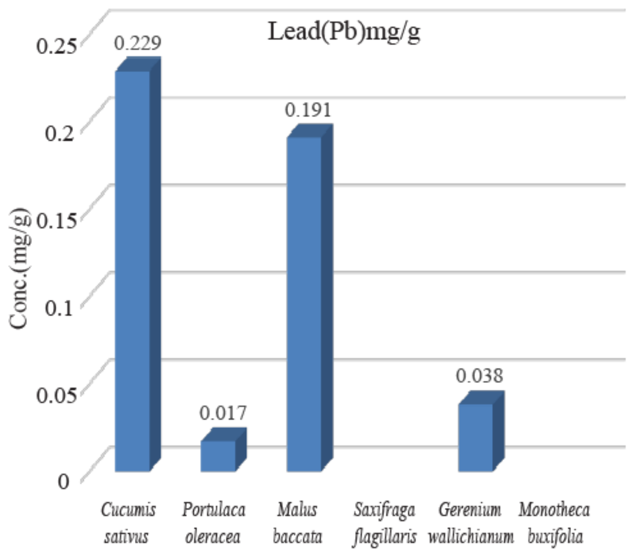

Fig. 2. Lead concentration of selected medicinal plants.

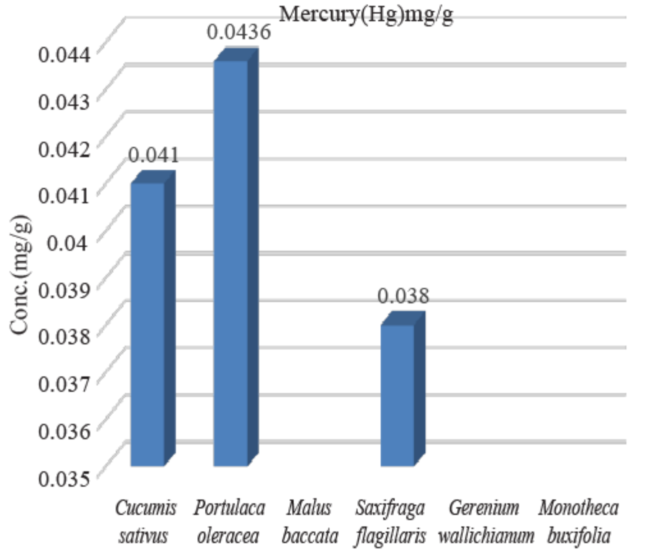

Fig. 5. Mercury concentration of selected medicinal plants.

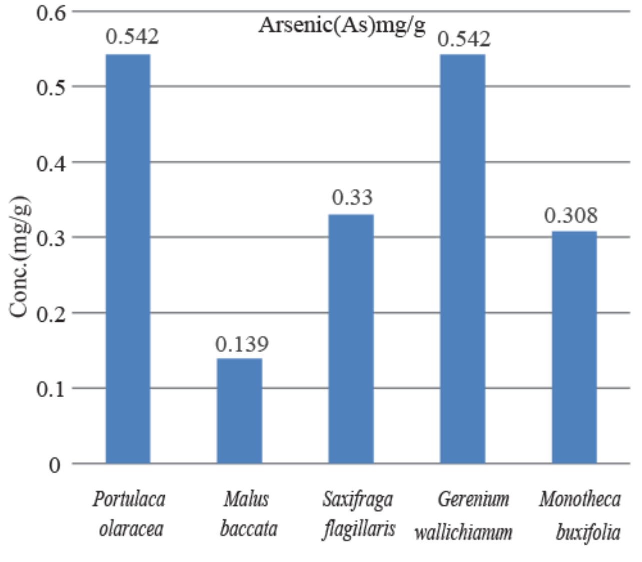

Fig. 3. Arsenic concentration of selected medicinal plants.

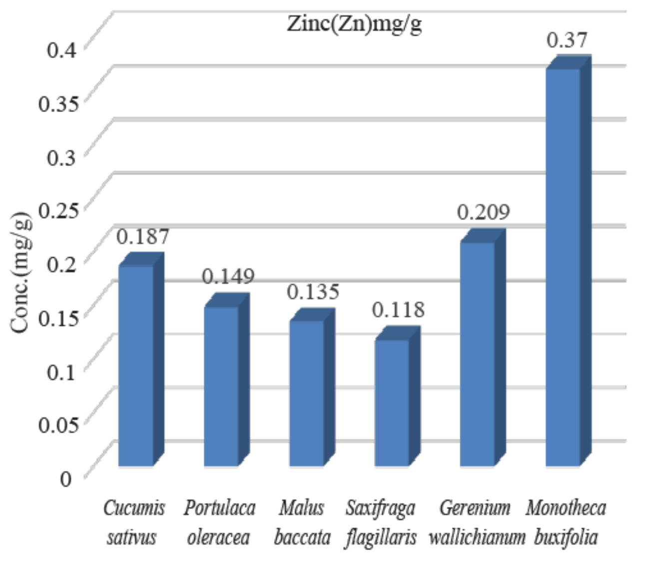

Fig. 6. Zinc concentration of selected medicinal plants.

Conclusion

Medicinal plants are sources of active ingredients for the treatment of diseases ranging from common cold to toxic ailments like cancer. Pakistan has rich medicinal plants flora, which are used as medication as well as food supplements. Raw herbs are accessible to everyone but their use should not be continued for longer time. The heavy metal analysis of these medicinal plants showed that the level of studied heavy metals are very low in general as compared to the heavy metal levels toxic to human body. Taking in consideration the chronic lethality of these heavy metals suggest evaluation of more medicinal plants as the levels of heavy metals may vary among different plant species.

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