

COMPARATIVE ASSESSMENT OF HEAVY METALS IN *Euphorbia helioscopia* L.

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ABSTRACT

In order to ascertain accumulation of heavy metals including Fe, Co, Ni, Cu, Cr, Mn, Cd and Pb in Euphorbia helioscopia L. found in polluted and unpolluted sites of Peshawar city, investigations were performed by using atomic absorption spectrometry. Comparative assessment of heavy metal contents confirmed accumulation of iron in leaves (67.00 mg kg⁻¹) and stem (13.50 mg kg⁻¹) followed by manganese in roots (13.36 mg kg⁻¹) and stem (12.53 mg kg⁻¹). The amount of other heavy metals studied chromium, cobalt and copper were also on the higher side in roots, stem, leaves and seeds of the plant. The results showed heavy metals accumulation in plant, procured from polluted sites as compared to unpolluted sites. The main purpose of the investigation was to document evidence for the users, collectors, and practitioners of E. helioscopia obtained from the polluted areas for human consumption.

Key words: Zn, Fe, Cu, Cr, plant species, heavy metals, sun spurge.

INTRODUCTION

Euphorbia helioscopia L. (sun spurge) belonging to the family Euphorbiaceae is widely distributed throughout the world. Its juice is commonly applied to warts used to cure intolerable pain and associated with sore eyelids (Swarbrick, 1997). It is also used as hydrogogue cathartic. The leaves and stems are febrifuge and vermifuge. The oil from the seeds has purgative properties. The root is anthelmintic and the seeds mixed with roasted pepper are used in treatment of cholera. The latex is highly irritating to the mucous membrane and toxic to mammals and fish (Sosath and Hecker 1988).

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The herb is also used for humid asthma, cardiac hay fever, bronchitis and urethritis. The toxic effects of *E. helioscopia* are skin irritation, gastric and abdominal disturbances, abortion, sterility, neuralgic pains including hysteria and fatigue and also tumor promoter (Ha et al., 2001).

Herbs are easily contaminated during growing and processing. The heavy metals finally enter the human body via soil, plant, food, water etc. The level of heavy metals in plants is conditional depending upon geochemical characteristics of the soil and the ability to selectively accumulate some of these elements (Tolonen, 1990). Bioavailability of the elements depend upon the form of their bond with the constituents of the soil. Plants easily assimilate such elements through the roots, which dissolve in water and occur in ionic forms. It is believed that the great majority of heavy metals act as key components of essential enzyme systems or other proteins, e.g. the haemoprotein, haemoglobin which perform vital biochemical functions. Arsenic, Cd, Hg, Pb, etc are highly toxic for the human bio-system even at very low levels of intake and they are usually present in plants because of the increasing industrialization and associated pollution of the biosphere, taken up from the soil, water, fertilizers, pesticides treatment and anthropogenic operations (Hunt, 2003; Chen, 1992). There are no standards for herbal raw materials which establish a permissible level of metals in such materials. World Health Organization (WHO, 1998) mentions maximum permissible limits in raw materials only for arsenic, cadmium, and lead, which amount to 1.0, 0.3, and 10 mg/kg, respectively. The concentration of heavy metals is one of the criteria that makes raw plants admissible to the production of medicines due to the fact that amount taken increases with the concentration, increased by constant mass of a taken dose (Gorbanova and Gorbanov, 2004; Neil, 1993). The main purpose of the study is (i) to compare metal contents in *E. helioscopia* L. growing in polluted and unpolluted areas (ii) to avoid the use of herbal medicine containing high level of metal contaminants (iii) making awareness among the public and (iv) strict quality control and safety methods.

MATERIALS AND METHODS

Collection and Post Harvest Treatment of Plant Material

E. helioscopia L. was collected from polluted and non-polluted sites of Peshawar city. Plant parts, especially roots were washed in fresh running water to eliminate dust, dirt and possible parasites and then with deionized water and dried in shade at 25-30°C. During all these steps of sample processing necessary measures were taken in order to avoid any loss or contamination with heavy metals.

Acid Digestion of Plant Samples

Weighed quantities of crushed and powdered portion from each part of plant; root, stem, leaves and seeds were taken in a china dish and heated in an oven at 110°C to evolve moisture. Then the dried sample after charring, was heated in a furnace for 4h at 550°C. The contents of china dish was cooled in desiccator and 2.5 mL 6M HNO₃ was added to the dish to dissolve its contents. The solution was filtered and transferred to a 20 mL flask and diluted to the mark (Radojevic and Vladimir, 1999].

For estimation of heavy metals, flame atomic absorption spectrophotometer (Polarized Zeeman Hitachi, 2000) was used.

Calibration of Equipment

For the studied elements we established the following sensitivity and detection limits, respectively of the used flame atomic absorption Spectrophotometer (FAAS) apparatus. Pb 0.2 and 1.0 mg kg⁻¹, Cr 0.5 and 3.0 mg kg⁻¹, Cd 0.2 and 1.0 mg kg⁻¹, Fe 0.5 and 5.0 mg kg⁻¹, Cu 0.5 and 3.0 mg kg⁻¹, Mn 0.5 and 2.50 mg kg⁻¹, Zn 0.05 and 5.0 mg kg⁻¹, Co 1.0 and 5.0 mg kg⁻¹ and Ni 0.5 and 4.0 mg kg⁻¹

RESULTS AND DISCUSSION

Heavy metals are present everywhere in plants and soil in trace amount, required by plants and animals as micronutrients. In the present investigation heavy metals Iron (Fe), Cobalt (Co), Nickel (Ni), Copper (Cu), Chromium(Cr), Manganese (Mn), Cadmium (Cd) And Lead (Pb) were determined in roots, stem, leaves and seeds of *E. helioscopia* collected from polluted and unpolluted sites of Peshawar city. Table 1 summarizes the concentration level of heavy metals in various parts of the plant.

Copper

As can be seen from the data in Table-1, the concentration of copper was found high in the seeds 2.25 mg kg⁻¹ collected from polluted area followed by the stem (1.46 mg kg⁻¹), leaves (1.12 mg kg⁻¹) and roots (0.94 mg kg⁻¹). In case of unpolluted area, copper was found high in the leaves (1.05 mg kg⁻¹) followed by the stem (0.83 mg kg⁻¹), roots (0.66 and seeds 0.50 mg kg⁻¹). Although the concentration of copper in the polluted area is more than the unpolluted area, however it is well beyond the critical level in plants (Kabata-Pendias and Pendias, 1984). Copper toxicity is ascribed to the induction of reactive free oxygen species in the Fenton type reaction causing break down of DNA strands as well as damage to membranes and mitochondria. Copper is an essential element for plants and animals. Critical concentration for copper in plants is in between 20-100 mg kg⁻¹. Phytotoxicity can occur if its concentration in plants is higher than 20 mg kg⁻¹ dry weight (Gupta, 1975). High levels of copper may cause metal fumes fever with flue like symptoms, hair and skin discoloration,

dermatitis, irritation of the upper respiratory tract, metallic taste in the mouth and nausea (Greenwood and Earnshaw, 1986). WHO has recommended the lower limit of the acceptable range of oral intake of copper as $20 \mu\text{g kg}^{-1}$ body weight day^{-1} .

Nickel

The concentration of nickel found in plants from polluted area (Table-1) is in the order, seeds (0.40 mg kg^{-1}), leaves (0.34 mg kg^{-1}), stem (0.27 mg kg^{-1}) and roots (0.26 mg kg^{-1}), while in unpolluted area; stem (0.22 mg/kg), followed by roots (0.11 mg kg^{-1}), leaves (0.10 mg kg^{-1}) and seeds (0.04 mg kg^{-1}). High concentration of nickel was found in seeds (0.40 mg kg^{-1}), followed by leaves in plant collected from polluted areas. The higher concentration of nickel found in plants from polluted area can be explained on the basis of anthropogenic activities. Although nickel 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 (McGrath and Smith, 1990). However increased concentration has many health effects. For example nickel tends to accumulate in the kidney causing its damage. A steady exposure to nickel can cause cancer of the lungs and nasal sinus. Nickel fumes are respiratory irritants and may cause pneumonitis, chronic bronchitis, allergic reactions and skin rash (Bethesda, 1993). Environmental Protection Agency has recommended that daily intake of nickel should be less than 1 mg beyond which it is toxic (Smith, 1990).

Cobalt

Although cobalt is toxic at elevated concentration, however the body needs only in trace amount. Cobalt in the form of vitamin B₁₂ is in the physiologically active form. It is very essential to provide $2.0 \mu\text{g}$ per day in the form of vitamin B₁₂ for a diabetic individual. Soils with high cobalt concentration usually have also high arsenic and nickel which are more toxic to plants and humans than cobalt. EPA has established a chronic reference exposure level of 5 mg m^{-3} . High intake of cobalt causes vomiting, nausea, vision and heart problems and also damage of thyroid (Bethesda 1993, Smith 1990). More cobalt was found in the plants from polluted area (Table-1) where its concentration was in leaves (0.20 mg/kg) followed by stem (0.19 mg kg^{-1}) then seeds (0.17 mg kg^{-1}) and roots (0.14 mg kg^{-1}) as compared to unpolluted area, which were stem (0.13 mg kg^{-1}) roots (0.11 mg kg^{-1}), followed by the same value for leaves and seeds (0.09 mg kg^{-1}).

Manganese

The concentration of manganese found in the plants from polluted area was in the order; roots > stem > seeds > leaves which are 13.36 mg kg^{-1} , 12.53 mg kg^{-1} , 10.06 mg kg^{-1} and 9.77 mg kg^{-1} respectively, while in unpolluted area; stem > leaves > roots > seeds which are 7.37 mg kg^{-1} , 4.37 mg kg^{-1} , 1.52 mg kg^{-1} and 0.13 mg kg^{-1} , respectively. The concentration of manganese in plants from polluted

area is higher in seeds and roots as compared to unpolluted. Thus the concentration level of manganese is well below the critical level and hence acceptable, because it neither affects the plant growth nor will cause metal plant toxicity. Manganese is also an essential element for plants and animals. Its uptake is being controlled metabolically. Soil derives manganese from the parent material and its contents in the rocks are higher than the concentration of other micronutrients apart from iron (Smith, 1990). Critical manganese concentration in soil is rather high 1500-3000 mg/kg, while critical concentration in plants is in the range of 300-500 mg/kg (Kabata-Pendias and Pendias, 1992). At high levels, manganese causes damage to the brain, liver, kidneys, the developing fetus, respiratory tract and Parkinson. The National Research Council has recommended safe and adequate daily intake levels of manganese that range from 0.3 to 1 mg/day, beyond 5 mg is toxic (US Health Services, 2000).

Chromium

High concentration of chromium (Table-1) was found in leaves (0.18 mg kg^{-1}), followed by roots (0.12 mg kg^{-1}), while in stem (0.08 mg kg^{-1}) and roots was (0.05 mg kg^{-1}) of plants from polluted area as compared to unpolluted, whereas other parts of plant was below the detection limit. Chromium is one of the known environmental toxic pollutants in the world (McGrath and Smith, 1990). Besides these chromium plating and alloys in motor vehicles is considered to be a more probable source of chromium (Shaheen, 1975). An elevated concentration between $5\text{-}30 \text{ mg kg}^{-1}$ is considered critical for plants and could cause yield reduction (Kabata-Pendias and Pendias, 1992). The toxic effects of chromium intake is skin rash, nose irritations, bleeds, upset stomach, ulcers, weakened immune system, kidney and liver damage, nasal itch and lungs cancer (US Health Services, 2000).

Iron

The plant samples collected from polluted area (Table-1) showed high amount of iron in leaves $67.00 \text{ (mg kg}^{-1}\text{)}$, followed by stem $13.50 \text{ (mg kg}^{-1}\text{)}$, roots $4.90 \text{ (mg kg}^{-1}\text{)}$ and seeds $3.05 \text{ (mg kg}^{-1}\text{)}$. In case of plant samples collected from unpolluted area, high concentration was found in leaves $22.50 \text{ (mg kg}^{-1}\text{)}$, stem $11.30 \text{ (mg kg}^{-1}\text{)}$, roots (4.50 mg kg^{-1}) and seeds $2.85 \text{ (mg kg}^{-1}\text{)}$. In general the concentration of iron in plants from polluted area is higher. Thus, plants collected from polluted area are more affected by pollution, emitting more iron into the environment and to the soil. The concentration of iron in the plant is high as compared to other elements. The high iron amount in the aerial parts is also due to the foliar absorption from the surrounding air. Iron deficiency in plants produces chlorosis, however its high concentration also affects plant growth (Sakolnik, 1984). Iron together with hemoglobin and ferridoxin plays a central role in metabolism. Natural Academy of sciences has recommended maximum intake of iron 45 mg/day, beyond which

gastrointestinal effects such as constipation and nausea results (Gala, 1984; Kabata-Pendias and Pendias, 1992).

Cadmium

Cadmium is a toxic metal having functions neither in human body nor in animals or plants. Once accumulated in the kidney then it stays there, resulting in high blood pressure and kidney disease and difficult to remove by excretion. Cadmium directly damages nerve cells. It inhibits the release of acetylcholine and activates cholinesterase enzyme, resulting in a tendency for hyperactivity of the nervous system (Jones, 1987; Alloway, 1990). Critical level of cadmium in soil is 3-5 mg/kg (Kabata-Pendias and Pendias, 1992). At this level in most cases it cannot cause toxic or excessive accumulation. Concentration in plants or the lowest level of cadmium which can cause yield reduction is between 5-30 mg/kg. Surprisingly no cadmium was detected in plant samples. This may be due to a very low level (Below detection limit) of cadmium in the available soil for plant growth (Nath, 1986).

Lead

More lead was accumulated in the plant from polluted area (Table-1) whereas its concentration is; leaves (4.89 mg kg^{-1}), stem (3.30 mg/kg), roots (2.22 mg kg^{-1}) followed by seeds (1.70 mg kg^{-1}) as compared to plants from unpolluted area, which is; leaves (1.87 mg kg^{-1}), roots (1.65 mg kg^{-1}), followed by stem (1.63 mg kg^{-1}) and seeds (0.98 mg kg^{-1}). This indicates the presence of lead coming from the emission of vehicles as well as its presence in the soils polluted with wastes from different operations. High concentration of lead in the body causes anemia, pale skin, decreased hand grip strength, abdominal pain severe constipation, nausea, paralysis of the wrist joint, increases chances of miscarriage or birth defects. The central nervous system becomes severely damaged at blood lead concentration starting at 40 mcg/dL and above 70 mcg/dL causes anemia, reduction in hemoglobin levels and erythropoiesis (Gorbanova and Gorbanov, 2004; ATSDR 1993; Goyer 1988, Davies, 1990).

CONCLUSIONS

People generally use herbal medicine for prolonged period of time to achieve desirable effects. Prolong consumption of such herbal medicine might reduce chronic or subtle health hazards. Thus our findings indicate that the medicinal plant or plant parts used for different diseases must be checked for heavy metals contamination in order to make it safe for human consumption. Although the concentration of heavy metals in *E. helioscopia* L. is below the critical level, however for local or pharmaceutical purposes, it should be collected from area not contaminated with heavy metals. From the present study, it is concluded that *E. helioscopia* L. growing in polluted

area has high lead, copper, iron, chromium and manganese concentration and therefore the use should be avoided in there is fair chance of finding this plant in unpolluted areas.

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Table-1. Heavy Metals Concentration (mg kg⁻¹) in *Euphorbia helioscopia* L. growing in Polluted and Unpolluted area.

Polluted					Unpolluted			
Plant Parts					Plant Parts			
Heavy metals								
	Root	Stem	Leaves	Seeds	Root	Stem	Leaves	Seeds
Pb	2.22±0.15	3.30±0.10	4.89±0.08	1.70±0.17	1.65±0.13	1.63±0.11	1.87±0.07	0.98±0.12
Fe	4.90±0.12	13.50±0.07	67.00±0.01	3.05±0.08	4.50±0.13	11.30±0.01	22.50±0.15	2.85±0.03
Cu	0.94±0.01	1.46±0.09	1.12±0.12	2.25±0.03	0.66±0.23	0.83±0.30	1.05±0.12	0.50±0.0
Cr	0.12±0.03	0.08±0.01	0.18±0.13	0.05±0.02	nd	nd	nd	0.02±0.01
Ni	0.26±0.00	0.27±0.09	0.34±0.05	0.40±0.001	0.11±0.06	0.22±0.00	0.10±0.05	0.04±0.01
Co	0.14±0.00	0.19±0.07	0.20±0.03	0.17±0.11	0.11±0.09	0.13±0.06	0.09±0.02	0.09±0.01
Mn	13.36±0.08	12.53±0.12	9.77±0.02	10.06±0.11	1.52±0.09	7.37±0.01	4.37±0.03	0.13±0.05

± Standard Deviation, nd: not detectable, WHO permissible limits for Pb: 10 mg/kg; Cd: 0.3 mg/kg (WHO, 1998), FDA permissible limits for Cr, 120 µg (RDI); Ni: 0.1 mg/l (FDA, 1993, 1999)

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