Investigation of Heavy Metals Cu, Pb, Cd, Mn, Cr, Fe and Ni in *Ammi visnaga*

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Summary: Heavy metals Cu, Pb, Cd, Mn, Cr, Fe and Ni were investigated in the medicinal plant *Ammi visnaga* using atomic absorption spectrophotometer. The plant was collected from three different regions including polluted, less pollute and relatively unpolluted area of Peshawar. The importance of this study was to investigate the effect of heavy metal on *A. visnaga*. The local peoples use A. visage for different body disease and for stomach pain too. The study will be of particular importance for public awareness about the heavy metals contents and their possible health effects.

Introduction

Herbal drugs are used in recent years for the treatment of various diseases due to their lesser side effects and acceptability to the majority of the third world countries. Heavy metals are widely distributed in soil due to the geoclimatic condition and environmental pollution, therefore, the assimilation in plants is obvious. Heavy metals along with pollutants are discharged into the environment through industrial activity, auto mobile exhaust, heavy duty electric power generator, municipal wastes, refuse burning and pesticides used in agriculture etc.

The toxicity of heavy metals depends on the chemical form of the element, i.e. speciation. The most devastating forms of the metals are,

1) Those that causes immediate sickness or death (e.g. a sufficient large dose of arsenic oxide). And (2) that which can pass through the membrane protecting barrier that protects the developing fetus.

Heavy metals are dangerous in the form of their cations, and are highly toxic when bonded to short chains of carbon atoms. Biochemically, the mechanism of the action arises from the strong affinity of the cation for sulfur. Thus sulfhydryl groups, -SH, which commonly occur in the enzymes that control the speed of critical metabolic reaction in the human body, readily attach themselves to ingest heavy metals cation. Due to this reason, the resultant metal-sulfur bonding affects the entire enzyme, it cannot act normally and as a result human health is adversely affected.

Heavy metals have great significance due to their tendency to accommodate in human organs over a prolong period of time. The presence of heavy metals beyond the allowed upper and lower limits can cause metabolic disturbances. Thus both the deficiency and excess of heavy metals may produce undesirable effect [1]. Effect of toxic metals (Cd, Cr, Pb, Ni, etc) on human health and their interaction with essential trace elements may produce serious consequences [2].

Environmental impact of heavy metals such as Cd, Ar, Pb, Cr, Mn, Co and Fe as well as their heath effect has been the major concern. High level of Hg causes disfunction of central nervous system (CNS). Since Hg compound can pass to the fetus, the newly born baby shows symptoms of mental retardation. Seizures, moter disturbances and even paralysis. Hundreds of death in Iraq in 1950, 1960 and 1972 and a few in china resulted from the consumption of bread made from seed grain that had been treated with mercury based fungicides. The minimata disease [3] killed and affected thousands of people in1950s, caused after eating Hg contaminated fish.

Cadmium is reported to cause osteomalacia and pyelonephrites and Pb may cause renal tumors and other Carcinoma [4]. Out break of itai itai in Japan due to the consumption of rice, containing high level of Cd [5]. Men, animals and plants through air, water and food take up these metals from the environment. Medicinal plant which are the raw material for many of the herbal formulation and popular nutrient supplements, or sold all over the country.

In the recent past there has been a steady growth in the number of patients visiting practitioners of complementary system of medicine. This growing popularity is partly due to the popular concept that "being natural in origin, herbs are safe". WHO [1998] [6] recommends that medicinal plants which form the raw material for the finished products may be checked for the presence of heavy metals, pesticides, bacterial or fungal contamination. The aim of the present study was to investigate the effects of heavy metals polluted soil on the cellular and acellular parts of ammi visnaga. The study will be useful for public awareness about the use of such medicinal plants for various types of diseases containing high level of heavy metals and which may create problem in the long run.

Results and Discussion

Ammi visnaga belong to the Umbelliferea or parsley family. Its seeds are used as (a) diuretic (b) antispamodic with specific action as a coronary vasodilator, brochodilator and urinary tract anti-spamodic. Ammi visnaga improves blood supply to the myocardium and makes myocardial metabolism more efficient. The antispasmodic activity may be partially due to the calcium antagonist effect. Ammi visnaga is used to treat mild forms of angina, mild obstructive pulmonary diseases and as an anti-spamodic for interstitial cystitis.

It contains cumarins, furanochromones and a small amount of volatile oil. Cumarin has been used in treatment of skin diseases like vitiligo and psoriasis. It also decreases the toxicity of the cardiac glycoside, digitoxin, due to the coronary vasodilator and antiarrhythmic effects. Ammi visnaga is also contraindicated in the pregnancy due to the emmenagogue and uterine stimulating activity. Public use *A. visnaga* is for gas trouble and stomach pain

Heavy metal as environmental contaminant is not a new phenomenon. They are present everywhere in plants and soil in trace amount. In fact many are required by plants and animals as micronutrients. In the present study heavy metals like Cu, Pb, Cd, Cr, Mn, Fe, and Ni were determined in roots, stems seeds and their soil of *A. visnaga*. The plants were collected from polluted, less polluted and unpolluted area of Peshawar. Heavy metals levels were also determined in the soil on which the plants are grown. Table-1 summarizes the concentration level of heavy metals in various parts of the plants.

Copper

Copper is one of essential elements for plants and animals. The most common sources for copper distribution on soils are pesticides, fertilizers, industries and sewage sludges. Critical concentration for copper in plants is in between 20-100 mg/kg [7]. Melting, grinding or cutting of copper may produce fumes or dust, and exposure or inhalation of these fumes may produce potentially health hazards. Fumes of copper may cause metal fumes fever with flu like symptoms and hair and skin discoloration while dermatitis has not been reported. Systematically as well, copper dust and fumes can cause irritation of the upper respiratory tract, metallic taste in the mouth and nausea.

The concentration of copper was found high in samples from spot 1 than spot 2 and 3. Plants growing on the three spots contained significantly

Table-1 Concentration of heavy metals in roots, stems, seeds and soils. mg/kg

Spot-1	Plants parts	Cu	Pb	Cd	Cr	Mn	Fe	Ni
1	Roots	1.01±0.01	01 ±0.03	nd	0.07±0.04	3.59±0.06	22.99±0.49	0.14±0.07
2	Stem	0.92±0.03	0.05±0.02	nd	0.09 ±0.01	2.05±0.03	9.59 ±0.13	0.13±0.06
3	Seeds	1.03±0.00	0.01 ±0.05	nd	0.1 ±0.04	8.65±0.01	16.85±0.01	0.26±0.00
	Soil	0.32±0.21	0.30±0.20	0.03±0.02	0.12±0.01	1.22±0.83	10.38±0.15	0.06±0.08
Spot-2								
1	Roots	0.90±0.03	nd	nd	0.04 ±0.01	2.53±0.07	14.94±0.31	0.06±0.01
2	Stem	0.76 0.02	0.03±0.01	nd	0.05 ±0.03	2.00±0.01	6.59±0.06	0.09±0.05
3	Seeds	0.94±0.01	nd	nd	0.07 ±0.02	5.39±0.09	15.60±0.13	0.20±0.01
4	Soil	0.22±0.01	0.18±0.12	nd	0.06±0.08	0.98±0.05	8.26±0.43	0.43±0.01
Spot-3								
1	Roots	0.79±0.02	nd	nd	0.03 ±0.00	1.68±0.01	1197±0.07	0.05 0.04
2	Stem	0.58±0.01	0.01 ±0.05	nd	0.04 ±0.02	0.81±0.01	3.10 ±0.16	0.08 0.05
3	Seeds	0.90±0.01	nd	nd	0.07 ±0.01	5.21±0.01	13.91±0.12	0.17±0.11
4	soil	0.28±0.02	0.14±0.02	nd	0.05±0.01	0.78±0.50	10.6±0.50	0.03±0.01

nd = not detected , ± = Standard Deviation.
WHO permissible limits for Pb: 10 mg/kg; Cd: 0.3 mg/kg (WHO 1998)
FDA permissible limits for Cr; 120 ug (RDI); Ni: 0.1 mg/l (FDA 1993, [12], 1999 [13]).

different amount of copper, due to the difference in the concentration of copper in the soil of the three spots. The level of copper concentration in roots, stem and seeds were found to be higher in spot 1 than 2 which in fact has more concentration of copper than spot 3. In spot 1, seeds accumulated significantly high copper 1.03 mg/kg followed by roots, which is 1.01 mg/kg. A similar trend was found in spot 2, in which the seeds have 0.94 mg/kg followed by the roots which is 0.90 mg/kg, while the level of copper was found lower in spot 3 Table-1. Thus the copper concentration in plant parts were in the order seeds > roots > stem.

Lead

Lead is regarded as high hazardous for plants, animals and particularly for microorganisms. The main sources of lead pollution of agricultural and plants are lead mines, fuel combustion, sewage sluge applications and farmyard manure [8]. The maximum acceptable concentration for foodstuff is around 1mg/kg [7]. Long term exposure to lead can result in a built of lead in the body and cause more severe symptoms. These include anemia, pale skin, a decrease hand grip strength, abdominal pain, nausea, vomiting and paralysis of the wrist joint. Prolong exposure may also result in kidney damage. If the nervous system is affected, usually due to very high exposure, the resulting affects include severe headache, coma, delirium and death. Continued exposure can lead to decreased fertility and/or increase chances of miscarriage or birth defects.

As can be seen from Table-1, high lead concentration was found in spot 1, followed by spot 2. In all spots on an average stem accumulated high concentration of lead in all three spots. In spot 1, root accumulated high level of lead 0.1 mg/kg followed by the stem 0.05 mg/ kg. The lead concentration was in the order of roots > stem > seeds. In spot 2, high concentration of lead was found in stem 0.03 mg/kg followed by the roots, however no Pb was detected in their roots. While in spot 3, considerably low concentration of lead was found Table-1. Due to air and soil pollution lead concentration was in the order stem > roots > seeds. Obviously, the high level of lead in the stem is due to air borne lead [9]. The plants from three different environments accumulated different amount of lead. The most sensitive was being spot1, and least one was spot 3. Although Lead was detected in the studied plant, however its concentration is well beyond the critical level.

Chromium

Chromium is one of the known environmental toxic pollutants in the world. The main sources of chromium contamination are tanneries, steel Industries, and sewage sludge application and fly ash [10]. Besides these chromium plating and alloys in motor vehicles is considered to be a more probable sources of chromium [11]. At an elevated concentration it could be toxic for plant and animals. Concentration between 5-30 mg / kg is considered of critical for plants and could cause yields reduction [7]. The problems that are associated with chromium exposure are skin rashes, upset stomach ulcers, respiratory problems, weakened immune systems, kidney liver damage, alteration of genetic material, lung cancer and ultimately death.

Soils samples collected from three different parts Table-1 showed significantly different amount of chromium. In case of the plants high chromium was found in plants collected from spot 1 and spot 2 Table-1. For example in case of spot 1, high concentration was found in seeds 0.1 mg/kg followed by stem 0.09 mg/kg. Although chromium was also present in the seeds, stem and roots in spot 2 and 3, however their concentration was not significant i.e. present in low concentration (Table-1). Thus in general the concentration in the three spots was in the order spot 1 > spot 2 > spot 3. While among the plant parts seed> stem > roots.

Manganese

Manganese is another essential element for plant and animal growth. Its uptake is controlled metabolically. Soil derived manganese from the parent material and its content in the rocks are higher than the concentration of other micronutrients apart from iron [12]. The main sources for manganese in soil are fertilizers, sewage sludge and ferrous smelters. Critical manganese concentration in soil is rather high 1500-3000 mg/kg [8] and as critical concentration in plants is in the range of 300-350 mg/kg.

In all the three spots, seeds accumulated high concentration of manganese followed by the roots. In spot 1, high concentration of manganese was found in seeds 8.65 mg/kg followed by the roots 3.59 mg/kg. Similarly in spot 2, high concentration was found in seeds 5.39 mg/kg followed by roots 2.53 mg/kg. The spot 3 have comparatively lower concentration of chromium than spot 1 and 2. In all the three spots the concentration of chromium occur in order of seeds > roots > stem. The concentration of manganese in all the three spots is well below the critical level and hence acceptable at this level because at this level it will not affect plant growth nor will cause manganese pollution.

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 three spots showed significant difference in heavy metals concentration. For example, high concentration of iron was found in roots of spot 1, 22.99 mg/kg followed by the seeds 16.25 mg/kg. In case of spot 2, high concentration of iron was found in the seeds 15.62 mg/kg followed by the roots 14.94 mg/Kg. The concentration of iron was found lower in all the parts of spot 3 plants Table-1. In general except from sample-1 the seeds accumulated high concentration of iron.

Nickel

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

In all the three spots, the plant seeds accumulated high concentration of nickel. In the plant of spot 1, seeds accumulated high concentration of nickel 0.26 mg/ kg followed by the roots 0.14 mg/kg. In spot 2, the plant seeds accumulated 0.20 mg/kg of nickel followed by 0.09 mg/kg in the stem. In spot 3 the concentration of nickel was comparatively low than the spot 1 and 2. Thus in general the concentration of nickel in the three spots was in the order spot 1 > spot 2 > spot 3. The level of heavy metal in *A. visnaga* is well beyond the critical level and hence is not contaminated. Unfortunately we were unable to compare our results and recommended level of heavy metals with our studies carried out in Pakistan, because no such study is on record.

Experimental

The combined effects of heavy metals on growth, productivity and medicinal contents of A.visnaga polluted areas were investigated.

Sampling areas

Spot 1 (Polluted area)

In this area the plant is exposed to both polluted soil and air pollution.

Spot 2 (Less polluted area)

This area is situated at a distance of 3 km from polluted area; the plant is exposed to less polluted soil and air.

Spot 3 (Relatively unpolluted area)

This area is located at a distance of 4 km from polluted area; the plant is growing relatively in unpolluted air and soil.

Post harvest treatment of plant material.

Plant samples were taken from the three areas. Plant parts like roots, stems leaves and seeds were rinsed with de ionized water, dried, crushed and powdered. The dried samples were stored in bottles for further processing.

Soil samples were taken in plastic envelopes, dried and stored. 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 soil samples. (Aqua Regia Digestion)

1.0 g of Weighed air-dried and sieved (< 2 mm) soil was taken in a flask. 15- ml of Aqua Regia was added and swirled to wet the sample. It was kept overnight. The next day the flask was heated at 50 Ċ for 30 minutes. The temperature was raised to 120 Ċ and the heating was continued for two hours. The flask was cooled and added 10- ml of 0.25 M HNO_3, [13].

The solution was filtered through a whatman no. 542 filter paper. The flask and filter paper were washed with small aliquots of 0.25 M HNO_3. The filtrate and washings were transferred to a 50-mL flask and made up to the mark with 0.25 M HNO_3.

Acid digestion for plant samples.

1.0 g of Weighed crushed powdered part from each part of plant like root, stem, and seed was taken in a china dish and was heated in the furnace for 4 hours keeping the temperature at 550 °C. The contents of the china dish were cooled in a dessicator. Then 2.5- mL 6 M HNO_3 solution was added to the dish to dissolve its contents. The solution was transferred to a 20-mL flask and was diluted to the mark of 20-mL [13]. The analysis for heavy metals was done by Polarized Zeeman Atomic Absorption Spectrophoto-meter Hitachi-2000.

Samples were analyzed for HNO_3 extractable, Cu, Pb, Cd, Cr, Mn, Fe, and Ni by using Polarized Zeeman Atomic Absorption Spectrophotometer Hitachi-2000.

For the studied elements we established the following sensitivity and detection limits respectively of the used polarized Zeeman atomic absorption spectrophotometer hitachi-2000 apparatus.

Pb	0.2	1.0	mg/kg
Cr	0.5	3.00	
Cd	0.2	1.0	
Fe	0.5	5.0	
Cu	0.5	3.0	
Mn	0.5	2.50	
Ni	0.5	4.0	

Conclusions

The study showed that plant grown on polluted area has high concentration of heavy metals than less polluted and unpolluted area. The population generally uses 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 finding indicate that the medicinal plant or plant parts used for different types of diseases must be checked for heavy metal contamination in order to make it safe for human consumption. Although the concentration of heavy metals in A.visnaga is well below the critical level, however for local or pharmaceutical purposes it should be collected from area not contaminated with heavy metals.

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