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Original Research Paper

Effect of Regional Variation on The Heavy Metal Content of *Achyranthes Aspera* Using Atomic Absorption Spectroscopic Technique

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ABSTRACT

Many mineral elements occur in plant and animal tissues in very minute quantities that earlier scientists were unable to measure their precise concentration with analytical methods then available. Modern analytical technique like Atomic Absorption Spectroscopy has the ability to measure all trace elements in the smallest of biological samples with great precision and accuracy. There is also a need for heavy metal analysis to be an integral part of the standardization of herbal medicines. *Achyranthes aspera* Linn. is a wild weed growing throughout India. It has been used against various ailments of which anti-diabetic, antiviral and jaundice are a few to name. Six common heavy metals viz., Pb, Zn, Cr, Fe, Ni and Cu were analysed by Atomic Absorption Spectroscopy. Among the 6 metals analysed in whole plant powder of *Achyranthes aspera* Fe showed maximum concentration while Ni showed lowest concentration.

INTRODUCTION

Various mineral elements occur in plant and animal tissues in such minute amounts that early workers were unable to measure their precise concentrations with analytical methods then available. They were, therefore, described as occurring in trace quantity, hence the term 'trace element'. This is still in use despite the development of modern analytical laboratory techniques such as atomic absorption spectrometry and neutron activation analysis, which have an ability to measure all trace elements in the smallest of biological samples with great precision and accuracy. In fact, it could be argued that since the development of these highly sophisticated techniques, the term 'trace' has become scientifically obsolete (Sauberlich & Johnson 1982, Wolf 1982).

A trace element is considered as essential for both man and animals if it meets the following criteria: It is present in all healthy tissues; its concentration from one species to the next is fairly constant; depending on the species studied, the amount of each element has to be maintained within its required limit if the functional and structural integrity of the tissues is to be safeguarded, and the growth, health, and fertility to remain unimpaired; its withdrawal induces reproducibly the same physiological and/or structural abnormalities; its addition to the diet either prevents or reverses the abnormalities (Cotzias 1967).

Several trace elements are known to fulfil this criteria, of which the most well known are iron, zinc, manganese, selenium, chromium, copper, cobalt, nickel, molybdenum, lead and iodine. Heavy metals are a matter of concern in herbal drugs, especially as certain plants have the tendency of storing them from the soil, polluted water and atmosphere (Newall et al. 1996, Baker et al. 1994).

Although there has been considerable research on the response of plants to heavy metals, the mechanism, which helps plants to survive in metal contaminated environments, is still not very clear. This is particularly evident with respect to the plants having long life span like trees. Most of the research carried out so far on heavy metals has been on herbaceous or short lived plants (Borovik 1990, Passaw 1978, Ross 1994). Studies carried out on higher plants reveal that they can be used as accumulative monitors of many metal elements in polluted areas (Al-Shayeb 2002). *Achyranthes aspera* is a weed growing throughout India. It has been used against various ailments of which antidiabetic and jaundice are few to name (Hayashi et al. 1990, Ambasta 1986, Ayensu 1981, Kritikar & Basu 1984). Six common heavy metals viz., Pb, Zn, Cu, Fe, Ni and Cr were analysed by atomic absorption spectroscopy.

MATERIALS AND METHODS

Achyranthes aspera whole plant was collected from various geographical regions namely Mumbai, Kolhapur and Lucknow in the flowering season. Herbarium of the plant was authenticated from NBRI (National Botanical Research Institute), Lucknow. After collection of the plant, it was carefully segregated, washed and dried at 40°C to constant weight. The dried plant free of moisture was powdered and sieved through a BSS Mesh No. 85 sieve and stored in an air tight pearlpet container. It was then analysed for its heavy metal content by using AAS.

Atomic Absorption Spectroscopy is one of the most advanced techniques for the trace element analysis as well as major and minor constituents of aqueous and non-aqueous solutions as it has a wide linear dynamic range unlike ICP-AES. The AAS was provided with 100 mm burner. Air-acetylene flame was used for most of the elements. For chromium estimation, reducing flame was required. Absorbance readings for Fe, Cu, Zn, Pb, Ni and Cr were recorded on Chemito model to get precision. Accurately weighed 5 g of the dried material was taken in a porcelain/silica dish and ignited with the help of a bunsen burner for about 1 hr. The porcelain dish was kept in a muffle furnace at 450°C ± 20°C till grey ash was obtained. The ash obtained was dissolved in 40 mL aqua regia. It was digested at low temperature on the hot plate, concentrated to about 20 mL and diluted to 100 mL with distilled water. After attaining room temperature, it was filtered through Whatman filter paper no. l. The absorbance reading was taken on the Atomic Absorption Spectrophotometer (AAS) at respective wavelength by using appropriate hollow cathode lamp. Various experimental readings of the AAS for different metal ions are given in Table 1. Blank solution was aspirated before each sample solution.

Working standards were prepared from 1000 ppm stock standards of individual metal ions supplied by M/s Merck India Ltd. Absorbance of different concentrations of working standards of each element was recorded and the standard curve was drawn. For each element, the calibration curve was drawn using standard solution of each element in the appropriate range. The instrument reading directly displayed the concentration of metal ion in the unknown solution on the basis of previously recorded calibration curve. The concentration of element in the sample was calculated in ppm and was recorded as mg/l00g. Concentration of element below 0.1 ppm was considered as Below Detectable Level (BDL). Average of concentration was reported as the concentration of individual element.

RESULTS AND DISCUSSION

The normal range of concentration of six heavy metals namely Fe, Cu, Pb, Zn, Ni and Cr in plants is presented in Table 2. The results of the heavy metal analysis are presented in Table 3. The concentra-

tion of Ni was minimum (8.23 ppm) in Kolhapur while maximum in Mumbai (9.53 ppm). The concentration of Fe was lowest (75 ppm) in Lucknow and highest in Mumbai (90 ppm). In case of Cu, the concentration was minimum in Kolhapur (45.6 ppm) and maximum in Mumbai (47.46 ppm). The concentration of Pb was minimum (19.3 ppm) at Kolhapur while maximum at Mumbai (21.47 ppm). The concentration of Cr was minimum (0.67 ppm) in Kolhapur and maximum (1.01 ppm) in Mumbai. The concentration of Zn was minimum (30.23 ppm) in Kolhapur and maximum in Mumbai (32.53 ppm).

Amongst the six metals analysed namely, Fe, Cu, Pb, Zn, Ni and Cr from *Achyranthes aspera,* Fe was found to be in maximum concentration and Cr in minimum concentration in plants of all regions. There was no significant variation observed in the total metal concentrations between various geographical regions. However, individual metal concentration varied in plant powders from different regions. The six metals analysed by AAS showed maximum concentration collected from Mumbai region. As iron was found to be in excess amount (75 ppm) in *Achyranthes aspera,* it may impair the function of organs especially liver, pancreas, heart, joints and pituitary. On the other hand iron defi-

Table 1: Various experimental conditions of AAS model, AA-203 for different metal ions.

Element Name	Fe	Cu	Zn	Pb	Ni	Cr
Lamp current (mA)	6.00	4.00	5.00	4.00	4.00	7.00
Wavelength (nm)	248.34	324.58	213.84	217.12	217.12	358.02
Slit width (nm)	0.30	0.50	1.00	1.00	1.00	0.20
PMT voltage (volts)	-4.63	-290	-365	-499	-365	-350
Fuel flow (1pm)	2.13	1.79	2.03	1.94	2.03	2.33
Air flow (1pm)	6.00	6.00	6.00	6.00	6.00	6.00
Burner height (mm)	3.40	3.40	3.40	3.40	3.40	3.40

Table 2: Typical concentrations of six metals in plants.

Metal	Normal range in plant material (ppm)	Concentration in contaminated plant (ppm)
Fe	50-300	300-400
Cu	4-15	20-100
Pb	0.1-10	30-300
Zn	3-400	100-400
Cr	1-10	20-100
Ni	0.02-5	10-100

Table 3: Metal concentrations in *Achyranthes aspera* collected from different regions.

Metal	Concentration (ppm)		
	Kolhapur	Mumbai	Lucknow
Fe	80	90	75
Cu	45.6	47.46	43.2
Pb	19.3	21.47	20.5
Zn	30.23	32.53	27.8
Cr	0.67	1.01	0.90
Ni	8.23	9.53	7.12

Note: Each reading is the mean of three values.

ciency may lead to anaemia, fissures at corner of mouth and koilonychia. As the concentration of Cu was found to be in excess (47.46 ppm) in *Achyranthes aspera*, it may lead to nausea, diarrhoea, hepatic failure, mental deterioration and renal dysfunction, whereas Cu deficiency causes anaemia, growth retardation, osteopenia, defective keratinzation, pigmentation of hair and scurvey. The concentration of lead was found to be maximum 21.47 ppm. If the concentration is in excess amounts, it causes abdominal pain, headache, irritability, joint pain, fatigue, anaemia, peripheral motor neuropathy, while deficiency of lead shows signs of anorexia, convulsions, coma, death due to generalized cerebral oedema and renal failure. Subclinical lead poisoning shows mental retardation, selective deficiency in language balance, behaviour and school performance. The concentration of Zn was maximum in Mumbai (32.53 ppm). If the concentration of Zn is higher, then it causes gastritis, sweating, fever, nausea, occupational respiratory distress, pulmonary fibrosis and chronic large doses depresses immune function and cause hypochronic anaemia. Whereas, Zn deficiency leads to growth retardation, alopecia dermatitis, diarrhoea, gonadal atrophy, immunological dysfunction, impaired spermatogenesis and congenital malformation. The concentration of Cr was maximum in Mumbai (1.01 ppm). Excess of Cr leads to occupational renal failure, dermatitis and pulmonary cancer, whereas deficiency of Cr leads to impaired glucose tolerance, peripheral neuropathy and confusion.

There was no significant variation in the total metal concentration between various geographical regions. Thus, *Achyranthes aspera,* as investigated in the current study, does not provide ample evidence to indicate pollution related accumulation of heavy metals. Since, the levels of these heavy metals do not vary with the geographical regions of collection, it may suggest that these higher levels could be related to the normal physiology of the plant.

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