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Environmental Contamination and Toxicology

Heavy Metal Content in Some Therapeutically Important Medicinal Plants

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Medicinal plants are the basic raw material for many of the herbal formulations and popular nutrient supplements sold over the counter. Thus, it becomes absolutely essential to ensure the quality of the plant material and detect presence of contaminants. In recent years, there has been a tremendous growth in the visits of patients to practitioners of complementary system of medicine (Engel and Straus 2002). This growing popularity is partly due to the assumption that herbs which are natural in origin are safe and without any side effects. However, concern has been raised equally by researchers and regulatory agencies regarding safety of herbs. Apart from some medicinal plants containing toxic glycosides or alkaloids (Arzt and Mount 1999) which may be harmful if overdosed, adulteration and contamination also poses challenges in their use. WHO (1998) recommends that medicinal plants which form the raw material for the finished product must be checked for presence of heavy metals, pesticides, bacterial or fungal contamination. Environmental impact of heavy metals such as Cd, Pb, Hg and As as well as their health effects have been the source of major concern. Outbreak of Itai-itai disease (Nogawa 1981) in Japan due to consumption of rice containing high levels of cadmium; the minamata disease (Gary and Philips 2000) caused after eating methyl mercury contaminated fish are some of the examples of the ill effects of environmental pollution due to toxic metals. Cadmium is reported to cause osteomalacia and pyelonephritis and Pb may cause renal tumors (Schumacher et al 1991). Heavy metal contamination of crops and its effect on growth of plants has been studied in detail in our lab (Shukla *et al.* 2002; Shukla *et al.* 2003). Our recent studies have shown that heavy metal contamination also affects nutrient uptake in wheat during early growth period (Shukla and Kakkar 2002). In the present study, attempts have been made to study the level of heavy metals such as copper, chromium, manganese, nickel, zinc, cadmium and lead in some medicinal plants collected from different ecological zones of India.

MATERIALS AND METHODS

Medicinal plant samples were collected from different ecological zones of India and identified with the help of experts. Market samples sold in medicinal plant stores were also procured from different regions. Care was taken to procure samples from North, South, East and West zones of India. The samples were dried in air prior to digestion and analysis.

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All chemicals used in the study were of analytical grade (E.Merck) Deionised water was used throughout the study, including rinsing of the glasswares. A digestion mixture comprising of conc. HNO_3 and perchloric acid (ultrapure grade) in the ratio of 6:1 was used for wet digestion of the samples. Mixed working standards (1 and 10 µg/ml) solutions were freshly prepared by diluting the stock solutions of 1000 µg/mL (Merck India). Blanks and spiked samples were also processed and analysed simultaneously.

The dried samples were powdered in a mixer grinder taking care not to overheat the samples. One gram of each powdered sample was accurately weighed on an electronic balance (Shimadzu LIBROR AEX-200G). The samples were then put in a 100 ml digestion flask and 5 ml of the digestion mixture was added to it and heated on a hot plate in the fuming chamber. The flasks were heated slowly first and then vigorously till one ml remained at the bottom. If the solution turned brownish, another 5 ml of digestion mixture was added and the process repeated till a white residue was obtained. The residue was dissolved and made up to 10 ml with 0.1N HNO_3 in a volumetric flask (grade one).

The solutions were then analysed on Inductively Coupled Plasma Emission Spectrometer (8440 Plasma Labtam). All necessary precautions were adopted to avoid any possible contamination of the sample as per the AOAC guidelines (1998). The detection limit of the instrument for each metal was Cu 0.0054 µg/g, Cr 0.0061 µg/g, Mn 0.0014 µg/g, Ni 0.010 µg/g, Zn 0.0018 µg/g, Pb 0.042 µg/g and Cd 0.0025 µg/g.

RESULTS AND DISCUSSION

Selection of the plant parts used for this study was based on their extensive use in traditional system of medicine in India. Table 1 summarizes the botanical as well as common name of the plant, its part used, the place of collection, major chemical constituents and medicinal uses. As is evident from the table, efforts were made to procure samples from different ecological zones comprising of Tamil Nadu in South, Assam in East, Jaipur in West and Tarikhet in North. The medicinal uses of these plants in Ayurveda covers a number of ailments including hypertension, neurological disorders, asthma, immuno-stimulants, antibacterial, menstrual disorders, rheumatism and urinary tract infection etc.

Analysis for seven heavy metals namely Cd, Cr, Cu, Mn, Ni, Pb and Zn was performed in a total of 23 samples procured from different regions. In *Cardiospermum halicacabum* L. (Table 2) Cd was 0.23 µg/g in Assam sample and 0.49 µg/g in Tamil Nadu making it two fold more than Assam sample. Similarly Cr in Tamil Nadu sample was 3 times more than the Bhubaneshwar sample. Levels of Cu, Mn and Zn were more or less similar in all the three samples from different zones (Fig. 1a). Content of lead differed in the three samples but was lower than the WHO prescribed limit (1998) for lead content in herbal medicines (10 mg/kg). The same amount of Ni was found in Assam and Bhubaneshwar samples but they were 1.76 times higher in the Tamil Nadu sample as compared to that from Bhubaneshwar.

In roots of *Coix lachryma jobi* L., levels of cadmium in all the samples were higher than the WHO prescribed limit of 0.3 µg/g (1998). Cadmium being a known nephrotoxic metal, the data has significance as this medicinal plant is extensively used for urinary tract infections. Level of Cr ranged from 4.22 to 12.58 µg/g. The highest

Table 1. Plants analyzed in the study.

Plant species	Common name	Part used	Place of collection	Chemical constituents	Medicinal uses
Cardiospermum halicacabum L.	Balloon vine	Seed	Tamil nadu Bhubaneshwar Assam	Cyanogenic glycosides	Hypotensive, anti-bacterial and Blood Pressure depressant; Useful in rheumatism, lumbago and nervous diseases.
*Coix lachryma-*jobi L.	Gavadhuka	Root	Assam Jhansi Meghalaya Tarikhet	Benzoxazo-linones	Menstrual disorders, air passage and urinary tract.
Cymbopogon citratus (DC.)	West Indian Lemon grass	Leaves	Patiala Lucknow (Market) Lucknow (Authentic)	Alpha and beta citrals, cymbopogone, cymbopogonol, geraniol etc.	Sudorific, stimulant and antiperiodic; Useful in catarrhal
Cyperus rotundus L.	Nut grass	Tubers	Bangalore Lucknow Punjab	Essential oil containing pinene, traces of cineole, sesquiterpenes and isocyperol; rotundone and saponins	Diuretic, emmen-gogue, anthelmintic, diaphoretic, astrin-gent and stimulant; useful in disorders of the stomach and irritation of the bowels
Hyoscyamus niger L.	Henbane	Seed	Tarikhet Bhubaneshwar Assam	Hyoscyamine and Atropine	In asthma, whooping cough and neuro-logical disorders
Piper longum L.	Pippali	fruit		Pipernonaline and piperundecalid ene	Alterative tonic; useful in chronic bronchitis, cough, cold and as antidote to snake-bite and scorpion sting
Sisymbrium irio L.	Hedge mustard	Seed	Jaipur Delhi Jhansi Aligarh	Isorhamnetin	Expectorant, stimulant and restorative; Useful in asthma

(Chopra *et al* 1956; Rastogi *et al.* 1995)

Table 2. Heavy metals content (µg/gm) in some medicinal plant samples used in traditional system of medicine.

Plant	Place	Cd	Cr	Ni	Pb
Cardiospermum halicacabum L.	Assam (A)	0.23±0.08	3.49±0.80	5.80±0.45	5.79±0.41
	Bhubaneshwar (A)	0.37±0.08	3.19±0.85	5.46±0.55	9.30±1.31
	Tamil nadu (A)	0.49±0.04	9.64±1.18	9.64±1.37	7.11±1.10
Coix lachryma jobi L.	Assam (A)	0.43±0.02	12.58±0.89	8.18±0.04	9.32±0.26
	Meghalaya (A)	0.72±0.06	8.05±0.96	6.34±1.48	12.40±2.07
	Jhansi (A)	1.30±0.38	4.94±0.44	6.41±0.77	11.25±1.24
	Tarikhet (A)	1.71±0.14	4.22±0.22	12.2±1.23	13.87±1.40
Cymbopogon citratus (D.C.)	Lucknow (M)	0.23±0.09	5.30±0.32	6.23±1.84	8.51±0.42
	Lucknow (A)	0.17±0.05	1.68±0.33	5.37±0.52	12.23±1.70
	Patiala (A)	0.29±0.02	3.53±0.45	5.71±0.56	12.17±0.76
Cyperus rotundus L.	Bangalore (A)	0.38±0.03	9.71±1.60	4.82±0.86	18.47±1.36
	Lucknow (A)	0.32±0.14	1.61±0.24	3.74±0.66	18.33±2.20
	Punjab (A)	1.40±0.43	22.17±1.08	15.03±1.53	14.87±2.25

Values are ±S.D. of three determinations in each case.
M- Market; A- collected from authentic source
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)

level of Pb was 13.87 µg/g, which is very close to the permissible limit by WHO. Cu was 5.76 in Jhansi sample and 26.66 in Meghalaya sample whereas Zn was 18.4 and 46.2 respectively (Fig. 1b). Copper being an essential trace element, it is required above 4 µg/g for proper growth and above 20 µg/g has been cited as toxic by Stevensen (1986). Mn ranged from 48 to 192 µg/g in the samples analysed.

Cymbopogon citratus DC.(lemon grass) used extensively as a herbal tea ingredient was procured from market as well as from an authentic source from Lucknow. Pb content in Lucknow as well as Patiala samples was found to be close to the permissible limit whereas Cd was within the permissible limit. Cr was higher in the market sample (Lucknow) as compared to authentic. Levels of Cu and Ni were similar in both the samples whereas the Patiala sample has approximately two times higher Cu. Zn was 18.13 µg/g in the Lucknow sample and 36.6 µg/g in Patiala sample. Mn was 2 times higher in the Lucknow market sample as compared to the authentic and 2.5 times in Patiala sample (Fig. 1c).

Cyperus rotundus L. (nut grass), used in rheumatism and as a diuretic and immuno-stimulant, was found to have Cd within permissible limits except for the Punjab sample which had 1.4 µg/g, four times more than the other samples analysed. Ni, Mn and Cu were found to be much higher in the Punjab sample as compared to the other two samples. High levels of Zn were found in both the Bangalore and Punjab samples (Fig. 1d). Cr was 1.61 µg/g in Lucknow sample and 22.1 µg/g in Punjab sample but was within the FDA prescribed limit of 120 µg (RDI) for food and feeds (FDA, 1999). Since the permissible limit for the presence of metals in raw medicinal plants

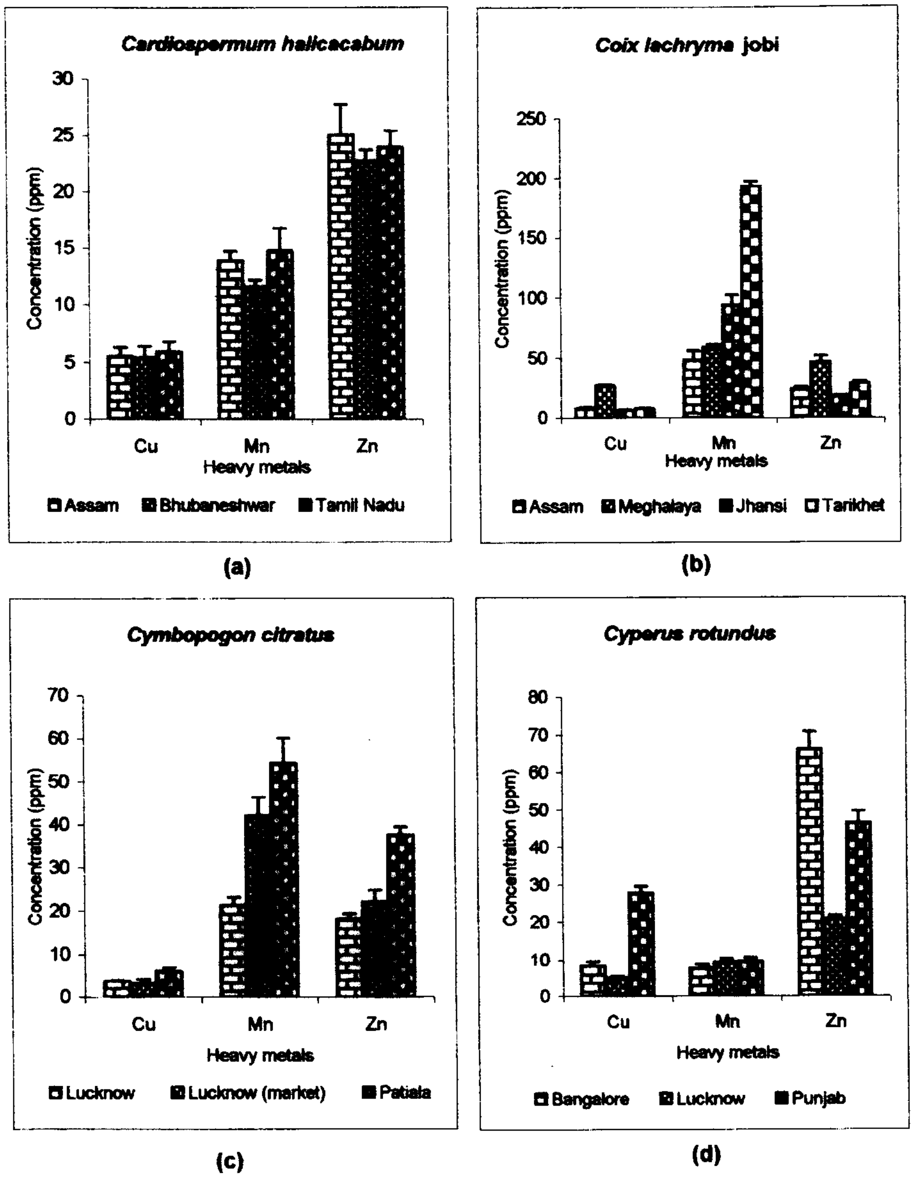

Figure 1. Level of essential trace elements Cu, Zn and Mn in some important medicinal plants. Values are ±S.D. of three determinations in each case.

or herbal finished products is not available except for Pb and Cd (proscribed by WHO), it is difficult to envisage the potential toxic effects of these metals at the levels found in our study. However, a regional variation in their levels due to geoclimatic conditions or industrial activities is clear from the data presented here.

Hyoscyamus niger L. (Henbane) as such and its active constituent hyoscyamine have been extensively used in neurological disorders, age-related dementia and depression (Asolkar *et al.* 1992). Levels of cadmium and lead were found to be below the permissible limit in all the 3 samples analysed (Table 3). Cr was found to be much below the FDA prescribed limit. Ni was approximately 4 μg/g in the Delhi and Lucknow samples and 2.6 μg/g in the Tarikhet sample. Level of Mn was found to be very high showing 47 μg/g (Fig. 2a) in the Delhi sample. Zn was highest in the Lucknow sample whereas Cu was within toxic limits in all the samples.

Samples of *Sisymbrium irio* L. from Aligarh, Delhi, Jaipur and Jhansi contained Cd, Pb and Cr well below the permissible limit. The level of Ni ranged from 1.5 μg/g to 5.4 μg/g. As the seeds of *S. irio* are extensively used for asthma and other bronchial disorders (Mabberley 1990), the low levels of heavy metals make it safe for use. Cr has been shown to be instrumental in causing respiratory disorders in stainless steel welders, chrome platers and chromate production workers (Anatoly *et al.* 1998), thus it is imperative that the medicine being used for such ailments has Cr contamination much below the permissible limit. The Levels of Mn and Zn (Fig. 2b) was found to be high in samples collected from Bangalore, a southern city of India. This could be due to a large number of industrial units in the surrounding areas.

Table 3. Heavy metals content (μg/gm) in the medicinal plant samples from different ecological zones.

Plant	Place	Cd	Cr	Ni	Pb
Hyoscyamus niger L.	Delhi (M)	0.02±0.01	3.20±0.76	4.75±0.45	2.60±0.47
	Lucknow (A)	0.03±0.02	3.82±0.25	4.59±0.99	2.36±0.57
	Tarikhet (A)	0.04±0.01	2.52±0.43	2.68±0.75	1.42±0.15
Piper Longum L.	Punjab (A)	ND	1.72±0.49	5.96±0.41	ND
	Delhi (M)	ND	1.09±0.11	2.75±0.97	3.87±1.44
	Lucknow (A)	ND	4.66±0.14	9.81±0.79	ND
Sisymbrium irio L.	Aligarh (M)	0.26±0.02	4.03±0.52	5.49±0.77	5.49±0.77
	Delhi (M)	0.12±0.02	4.95±0.54	3.85±0.77	2.75±0.27
	Jaipur (A)	ND	1.56±0.44	1.50±0.47	2.39±0.49
	Jhansi	0.11±0.01	2.49±0.18	3.23±0.24	6.16±0.60

Values are ±S.D. of three determinations in each case.
ND: Not detectable
M- Market; A- collected from authentic source
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)

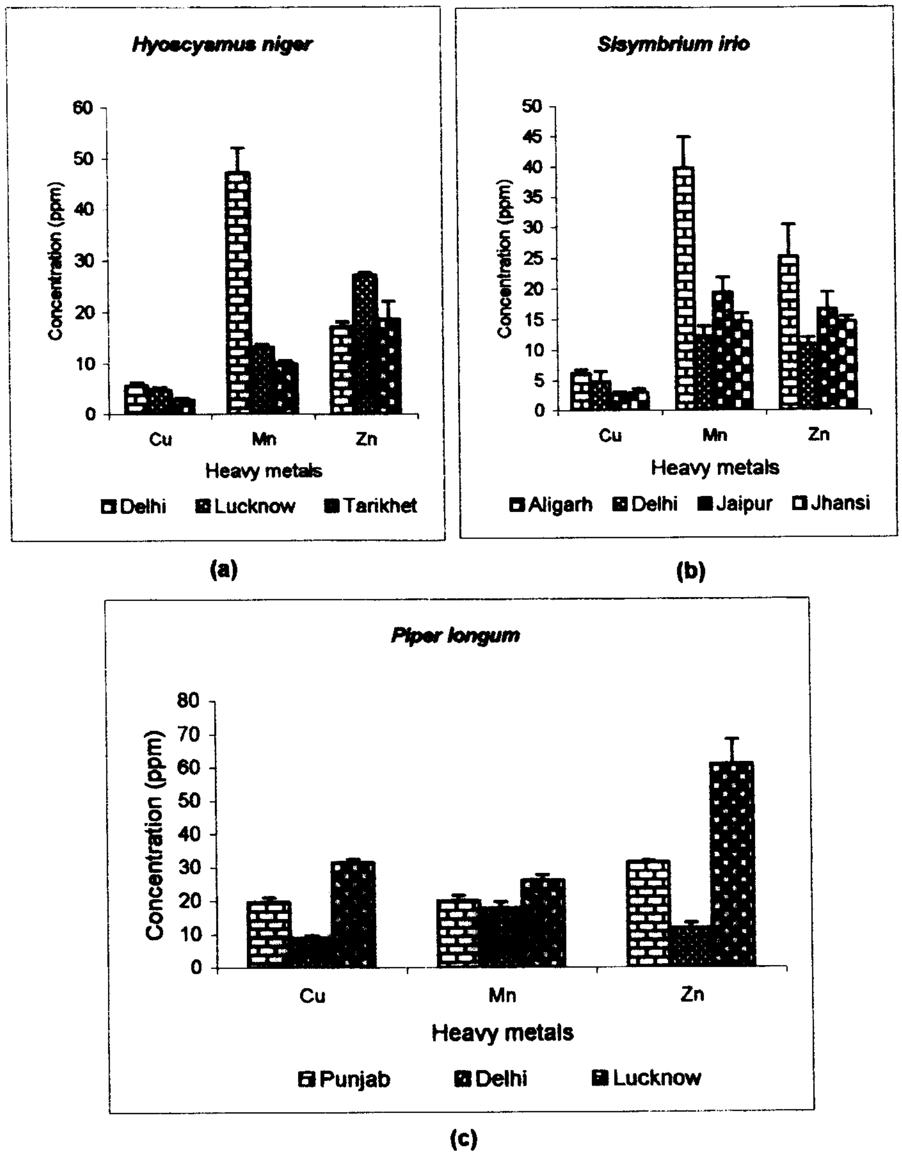

Figure 2. Comparison of Cu, Zn and Mn content in some medicinal plants procured from different ecological zones. Values are ±S.D. of three determinations in each case.

In *Piper longum* L., Cd and Pb were undetectable except for Delhi sample which had 3.87 µg/g Pb. Cr was 4 times higher in the Lucknow sample as compared to the Delhi sample whereas Ni was 3.5 times higher. It seems the fruits of this particular plant, (an ingredient of a number of herbal formulations) being a good adaptogen, do not accumulate the common heavy metals tested here making it safe for use. Apart from being an ingredient in finished products, this is also popularly used in herbal tea and as an immuno-stimulant given to mothers post-partum. Cu levels in the Lucknow sample were 31.4 µg/g which could be growth inhibitory for the plant. Zn level (Fig. 2c) was found to be 60 µg/g in the Lucknow sample. The Punjab sample also showed more than 20 µg/g.

Thus, it is evident from the data that the level of essential trace elements as well as heavy metals in medicinal plants depends on geoclimatic conditions of the region, anthropogenic activities such as chemical industries, plant species and plant part used. The data clearly shows that two species collected from same region have different heavy metal content which depends on many compounding factors including chemical constituents of the plant. The data has significance as efforts are being made world over to set up a permissible limit for heavy metals in herbal medicine.

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