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Effect of Ecological Variation on Heavy Metal Content of Some Medicinal Plants Used as Herbal Tea Ingredients in India

V. Naithani, P. Kakkar

Herbal Research Section, Industrial Toxicology Research Center, Post Box Number 80, M.G. Marg, Lucknow 226001, India

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In India, medicinal plants have been in use since time immemorial. They were mainly consumed in the form of Kadha (decoctions) or herbal tea (Naithani and Kakkar 2003). Herbal teas are prepared from different parts of medicinal plants and are gaining popularity all over the world as an alternative medicine. Among medicinal plants, lemongrass (Cymbopogon citrates DC. Stapf), liquorice (Glycyrrhiza glabra Linn.), holy basil (Ocimum sanctum Linn.), cloves (Syzygium aromaticum) and ginger (Zingiber officinale Rosc.) are the most popular ingredients of herbal teas in India (Naithani and Kakkar 2003; 2005). These plants are carminative, analgesic and antistress in nature and are useful in the treatment of cough, cold, sore throat, bronchitis, genito-urinary diseases, dyspepsia, flatulence, bacterial, viral and fungal diseases (Gokhale et al 2003; Shobana et al 2000). Due to the above mentioned therapeutic uses of these medicinal plants, they are extensively used as herbal tea ingredients in India. However, due to some reports of presence of heavy metals in Indian herbal formulations such as lead poisoning due to Indian herbal remedies (Keen et al.1994), their safety has been questioned. There are reports of minor and trace elements in the Indian herbal and other medicinal preparations (Samudralwas and Garg 1996), levels of heavy metals/ trace elements in some therapeutically important Indian medicinal plants (Jyothi et al, 2003; Rai et al. 2001; Haider et al. 2004), herbal teas (Naithani and Kakkar 2005) and severe lead poisoning due to Ayurvedic Indian plant medicine (Weide et al. 2003). Thus, it becomes mandatory that all herbal preparations and raw material be checked for the presence of heavy metals such as lead, cadmium, chromium and nickel. The purpose of this study was to estimate the level of these heavy metals in some medicinal plants (used as herbal tea ingredients in India), procured from cities covering different ecological zones of India.

MATERIALS AND METHODS

Five Indian medicinal plants commonly used as herbal tea ingredients, were procured from cities covering different ecological zones of India, and their identity ensured 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 Central, North, South, East and West zones of India. The samples

Correspondence to: P. Kakkar

Table 1. Medicinal plants commonly used as herbal tea ingredients.

Botanical name (Family); Common name	Part used	Medicinal properties and uses	Place of Procurement
Cymbopogon citratus DC.Stapf (*Gramineae*); **Lemon grass**	Leaves	Sudorific, analgesic, stimulant, antiperiodic and carminative; useful in catarrhal and cholera	Lucknow, Shimla, Srinagar, Dehradun, Ahmedabad, Kolkata, Rewa and Bangalore
Glycyrrhiza glabra Linn. (*Fabaceae*); **Licorice**	Stolons	Laxative, demulcent, emollient & anti-inflammatory; used in genito-urinary diseases, coughs and sore throat	Rewa, Shimla, Lucknow, Delhi, Ahmedabad, Kolkata, Bangalore
Ocimum sanctum Linn. (*Lamiaceae*); **Holy basil**	Leaves	Stomachic, anti-stress, anti-inflammatory, analgesic and anxiolytic; useful in bronchitis, chronic pulmonary tuberculosis, cancer, diabetes, allergies, arthritis, cutaneous and viral infections	Lucknow, Rewa, Shimla, Ahmedabad, Kolkata, Bangalore
Syzygium aromaticum Merr & Perry (*Myrtaceae*); **Clove**	Dried flower buds	Stimulant, aromatic, antiseptic, disinfectant and carminative; useful in flatulence, dyspepsia, asthma and toothache	Lucknow, Shimla, Rewa, Srinagar, Thiruvananthapuram, Delhi, Pune, Kolkata
Zingiber officinale Rosc. (*Zingiberaceae*); **Ginger**	Rhizome	Stimulant, carminative, expectorant, antiemetic, digestive and stomachic; useful in dyspepsia and flatulence	Lucknow, Shimla, Rewa, Srinagar, Kolkata, Bangalore, Pune

(Shobana et al 2000 ; Handa et al 2003; Gokhale et al 2003).

were dried in air and powdered prior to digestion and analysis. Table 1 shows place of procurement of samples with their therapeutic uses. All the glasswares were of Borosil "A" grade. Deionised water was used throughout the study, including rinsing of the glasswares. All the chemicals used such as concentrated HNO_3, perchloric acid etc. were of analytical (AR) grade (E.Merck). Mixed working standard (1 and 10 μg/ml) solutions were freshly prepared by diluting the stock solutions of 1000 μg/ml (Merck India). 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. 5 ml of the digestion mixture was added to it and heated on a hot plate in the fuming chamber. 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. Blanks and spiked samples were also processed and analysed simultaneously. 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 Atomic Emission Spectrometer (ICP-AES) (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 Cr 0.0061 mg/kg, Ni 0.010 mg/kg, Pb 0.042 mg/kg and Cd 0.0025 mg/kg.

RESULTS AND DISCUSSION

Cymbopogon citratus (Leaves) is extensively used for its analgesic and stimulant properties. Cd was found to be 4 folds higher in Kolkata sample of *C.citratus* than Dehradun sample (Table 2). It was below the permissible limit of 0.3 mg/kg as prescribed by WHO (1998) in all the samples. Pb level ranged from 1.45±0.35 mg/kg (Shimla sample) to 9.97±0.81 mg /kg (Kolkata sample) but was below the permissible limit of 10 mg/kg as prescribed by WHO (1998) in all the samples. Level of both Cr and Ni was found to be highest in Kolkata sample.

In *Glycyrrhiza glabra* (Stolon and roots),a medicinal plant used in cough syrups and for treatment of sore throat, Cd was 7 folds higher in Delhi sample as compared to Shimla sample (Table 3). Level of Pb was found to be highest in Kolkata sample (9.23±0.89 mg/kg) which was more than 6 folds higher than Shimla sample. Cr was detected in the range of 3.21 μγ /g to 3.72 mg/kg except in Delhi sample which had 6.4 folds more Cr level than Ahmedabad sample . Ni ranged from 1.38±0.34 mg/kg (Ahmedabad) to 5.84±0.12 mg/kg (Lucknow sample). Kolkata and Delhi samples showed higher metal content whereas comparatively low metal content was detected in samples from Shimla and Ahmedabad.

Table 2. *Cymbopogon Citratus* : Level of Cd, Pb, Cr and Ni (mg/kg) in samples from different ecological zones of India.

Region	Cd	Pb	Cr	Ni
Kolkata	0.27±0.05	9.97±0.81	3.94±0.11	3.51±0.47
Lucknow	0.17±0.05	9.37±1.84	1.68±0.33	3.37±0.52
Srinagar	0.08±0.01	2.44±0.30	1.83±0.05	2.67±0.05
Bangalore	0.25±0.06	9.68±0.55	3.53±0.42	3.34±0.54
Shimla	0.14±0.04	1.45±0.35	1.07±0.13	1.16±0.20
Ahmedabad	0.24±0.03	5.71±0.22	1.68±0.33	2.36±0.38
Rewa	0.19±0.02	4.41±0.46	1.16±0.08	1.45±0.44
Dehradun	0.07±0.01	3.46±0.35	1.50±0.37	2.90±0.15

Values are mean±S.D. of 3 determinations in each case.

Table 3. *Glycyrrhiza glabra* : Level of Cd, Pb, Cr and Ni (mg/kg) in samples from different ecological zones of India.

Region	Cd	Pb	Cr	Ni
Kolkata	0.26±0.02	9.23±0.89	3.46±0.06	2.43±0.22
Lucknow	0.06±0.02	6.84±0.04	3.72±0.19	5.84±0.12
Delhi	0.28±0.02	7.02±0.05	4.16±0.06	5.14±0.12
Bangalore	0.09±0.02	3.49±0.32	3.21±0.07	2.66±0.11
Shimla	0.04±0.01	1.46±0.40	3.87±0.05	1.80±0.04
Ahmedabad	0.24±0.02	8.64±0.55	0.65±0.06	1.38±0.34
Rewa	0.06±0.02	5.80±0.31	3.27±0.08	3.91±0.17

Values are mean±S.D. of 3 determinations in each case.

Table 4. *Ocimum sanctum* : Level of Cd, Pb, Cr and Ni (mg/kg) in samples from different ecological zones of India.

Region	Pb	Cd	Cr	Ni
Kolkata	9.05±0.61	0.29±0.03	4.19±0.36	2.72±0.21
Lucknow	8.30±1.46	0.07±0.02	3.50±0.40	4.62±1.54
Bangalore	9.04±1.00	0.29±0.06	3.45±0.7	2.23±0.35
Shimla	2.42±0.29	0.05±0.01	1.26±0.26	1.22±0.14
Ahmedabad	5.41±0.69	0.25±0.03	3.25±0.43	1.95±0.17
Rewa	4.23±0.53	0.28±0.01	2.10±0.11	3.65±0.16

Values are mean±S.D. of 3 determinations in each case.

Table 5. *Syzygium aromaticum* Linn. : Level of Cd, Pb, Cr and Ni (mg/kg) in samples from different ecological zones of India.

Region	Cd	Pb	Cr	Ni
Kolkata	0.28±0.06	9.95±1.10	3.10±0.39	4.15±0.36
Lucknow	0.25±0.05	9.21±1.37	1.90±0.03	2.51±0.28
Delhi	0.20±0.05	8.80±1.55	2.89±0.06	2.36±0.30
Srinagar	0.15±0.02	9.55±1.32	1.67±0.55	1.73±0.27
Shimla	0.17±0.04	5.25±0.32	0.69±0.03	1.10±0.06
Thiruvananthapuram	0.06±0.01	2.45±0.29	1.53±0.36	1.23±0.11
Rewa	0.20±0.06	9.30±1.49	1.86±0.07	2.62±0.43
Pune	0.07±0.02	8.21±1.72	0.95±0.03	1.59±0.34

Values are mean±S.D. of 3 determinations in each case.

Table 6. *Zingiber officinale* Rosc. : Level of Cd, Pb, Cr and Ni (mg/kg) in samples from different ecological zones of India.

Region	Cd	Pb	Cr	Ni
Kolkata	0.29±0.05	8.83±1.30	3.83±0.72	2.68±0.54
Lucknow	0.27±0.03	8.56±1.47	2.72±0.90	3.69±0.23
Srinagar	0.18±0.02	4.85±0.43	2.11±0.05	1.48±0.18
Bangalore	BDL	5.56±0.24	0.82±0.02	1.61±0.40
Shimla	0.18±0.06	3.44±0.37	1.36±0.41	1.10±0.45
Rewa	0.25±0.04	4.06±0.41	3.27±0.23	3.55±0.12
Pune	0.27±0.07	5.51±0.55	2.04±0.42	2.34±0.29

BDL : Below Detection Limit of the instrument.
Values are mean±S.D. of 3 determinations in each case.

Table 7. Mean concentration of metals in different parts of plants (mg/kg).

Part	Cd	Cr	Ni	Pb
Leaves	0.19	2.51	2.66	6.11
Stolons	0.15	3.19	3.31	6.10
Dried flower buds	0.17	1.82	2.16	7.84
Rhizome	0.20	2.30	2.35	5.83

Ocimum sanctum (leaves), an extensively used herb for nutraceuticals as well as herbal teas, showed highest level of Cd in Kolkata and Bangalore samples which had 5.8 folds more Cd than Shimla sample although below the permissible limit of 0.3 mg/kg WHO (1998). Pb content of Kolkata sample was found to be 3.7 folds more than Shimla sample. Cr ranged from 1.26±0.26 mg/kg (Shimla sample) to 4.19±0.36 mg/kg (Kolkata sample) (Table 4). Thus Kolkata sample showed highest metal content amongst all the samples tested.

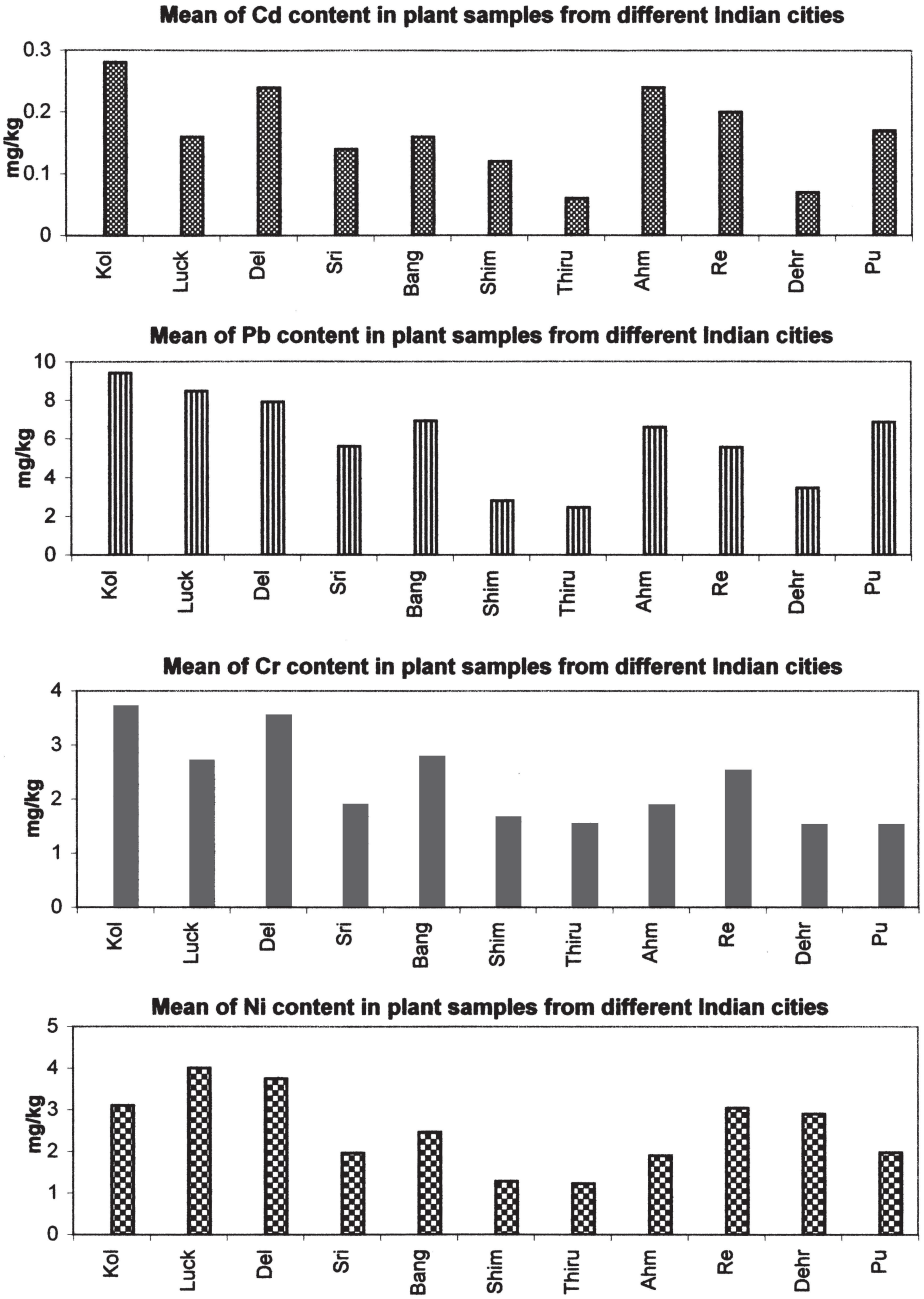

Figure 1. Mean of heavy metals in plant samples from different Indian cities.
Kol:Kolkata; Luck:Lucknow; Del:Delhi; Sri:Srinagar; Bang:Bangalore
Shim:Shimla; Thiru:Thiruvananthapuram; Ahm:Ahmedabad; Re:Rewa
Dehr:Dehradun; Pu:Pune

In *Syzygium aromaticum* (Dried flower buds), a spice and flavouring agent, Cd ranged from 0.06±0.01 mg/kg (Thiruvananthapuram sample) to 0.28±0.06 mg/kg (Kolkata sample) thus making a variation of 4.7 folds between the highest and lowest level of Cd detected (Table 5). Highest level of Pb was detected in Kolkata sample which was 4 folds higher than Thiruvananthapuram sample but below the WHO permissible limits. Cr was detected 4.5 times higher in Kolkata sample than Shimla sample (0.69±0.03 mg/kg). Highest level of Ni was detected in Kolkata sample and lowest in Shimla sample. Thus the data indicate higher metal content in samples from Kolkata and Delhi whereas comparatively low metal content was seen in samples from Pune, Shimla and Thiruvananthapuram.

In *Zingiber officinale* (Rhizome), an integral part of spicy food in India, highest level of Cd was detected in Kolkata sample and lowest in Shimla sample (Table 6). Pb ranged from 3.44±0.37 mg/kg (Shimla sample) to 8.83±1.30 mg/kg (Kolkata sample). Kolkata sample had 4.7 times more Cr as compared to the Bangalore sample (Table 6). Level of Ni was highest in Lucknow sample (3.69±0.23 mg/kg) making it 3 times more than Shimla sample (1.10±0.45 mg/kg) (Table 6).

The mean concentraton of metals in different parts of plants taken up for the study was also compared (Table 7) and it was found that Cd accumulation was highest in rhizome followed by leaves , dried flower buds and stolons . Accumulation of Cr was found to be highest in stolons followed by leaves , rhizome, and dried flower buds. Level of Pb ranged from 5.83 (mg/kg) (rhizome) to 7.84 mg/kg (dried flower buds). Pb level was found to be very much close to each other in leaves, stolons and rhizome. Over all the study showed higher metal accumulation in stolons, leaves, rhizome and comparatively less metal content in bark samples indicating uptake of metals from soil and water as well.

Metal content in samples from different regions (cities) of India was also compared (Fig. 1) and highest level of metal was detected in samples from Kolkata, followed by Bangalore, Delhi and Lucknow. Comparatively, less heavy metals were detected in samples from Ahmedabad, Rewa and Pune. Samples from Srinagar, Dehradun, Shimla and Thiruvananthapuram showed lowest level of metal contamination. Level of Ni and Cr were highest in Lucknow and Kolkata samples and least in Thiruvananthapuram samples (Fig. 1).

Mean of Cd and Pb found in the samples of 5 medicinal plants studied was 0.18 mg /kg and 6.39 mg /kg respectively which is below the WHO permissible limit of 0.3 mg/kg and 10 mg/kg respectively. The mean value of Cr and Ni was found to be 1.42 and 2.63 mg/kg respectively which is below the Acceptable Daily Intake (ADI) and Provisional maximum Tolerable Daily Intake (PMTDI) as given by WHO (1989 ,1997 ,1998) and JECFA (1993). Overall study showed higher metal contamination in samples from Kolkata, Bangalore, Delhi and Lucknow and comparatively, low metal contamination in samples from Thiruvananthapuram, Shimla, Srinagar and Dehradun. All the medicinal plants studied were found to have heavy metal content below the ADI and PMTDI.

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REFERENCES

AOAC (1998) Wet digestion for non-volatile metals in : AOAC official methods of analysis 16th edition, 4th revision, vol. 1, chapter 9

Gokhale SB, Kokate CK, Purohit AP (2003) Pharmacognosy. 18th edn., Nirali Prakashan, Pune, India

Handa SS, Kapoor VK (2003) Textbook of pharmacognosy. Second edition, Vallabh Prakashan, Delhi, India, pp 1-9, 325-345

Haider S, Naithani V, Barthwal J, Kakkar P (2004) Heavy metal content in some therapeutically important medicinal plants. Bull Environ Contam Toxicol 72 : 119-127

JECFA (1993) Evaluation of certain food additives and contaminants. Forty-first report of the Joint FAO/WHO expert committee on food additives, Geneva, Switzerland

Jyothi NV, Mouli PC, Reddy SR (2003) Determination of zinc, copper, lead and cadmium in some medicinally important leaves by differential pulse anodic stripping analysis. J Trace Elem Med Biol 17 : 79-83

Keen RW, Deacon AC, Delves HT, Moreton JA, Frost PG (1994) Indian herbal remedies for diabetes as a cause of lead poisoning. Postgrad Med J 70: 113-114

Naithani V, Kakkar P (2003) Herbal tea : An introduction. Vish Vigyan Sandesh 9 : 20-21 (In Hindi)

Naithani V, Kakkar P (2005) Evaluation of heavy metals in Indian herbal teas. Bull Environ Cont Toxicol 75 : 197-203

Rai V, Agarwal M, Khatoon S, Rawat AK, Mehrotra S (2001) Estimation of Co and Mn in some medicinal plants. Bull Environ Contam Toxicol 66 : 427-432

Samudralwar DL, Garg AN (1996) Minor and trace elemental determination in the Indian herbal and other medicinal preparations. Biol Trace Elem Res 54 : 113-121

Shobana S, Naidu KA (2000) Antioxidant activity of selected Indian spices. Prostaglandins Leukot Essent Fatty acids 62 : 107-110

Weide R, Engelhart S, Farber H, Kaufmann F, Heymanns J, Koppler H (2003) Severe lead poisoning due to Ayurvedic Indian plant medicine. Dtsch Med Wochenschr 128 : 2418-2420

World Health Organization (1989) Evaluation of certain food additives and contaminants, Thirty-third report of the joint FAO/WHO expert committee on Food Additives. WHO Technical Report Series number 776. WHO, Geneva, Switzerland

World Health Organization (1997) Guideline for drinking water quality. second edition, vol. 1, Recommendations. WHO, Geneva, Switzerland

World Health Organization (1998) Quality control methods for medicinal plant materials. WHO, Geneva, Switzerland