

DETERMINATION OF TOXIC METALS IN SOME HERBAL DRUGS THROUGH ATOMIC ABSORPTION SPECTROSCOPY

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ABSTRACT

This study presents a picture of occurrence of heavy metals (Pb, Cd, Cu, Cr, Co, Fe, Ni, Zn) in some selected valuable herbal drugs (*G. glabra*, *O. bracteatum*, *V. odorata*, *F. vulgare*, *C. cuminum*, *C. sativum*, and *Z. officinalis*) purchased from three different zones (southern, eastern, and western) of Karachi city using atomic absorption spectrophotometer. Heavy metal concentrations in these drugs were found in the range of: 3.26-30.46 for Pb, 1.6-4.91 for Cd, 0.65-120.21 for Cu, 83.74-433.76 for Zn, 1.61-186.75 for Cr, 0.48-76.97 for Ni, 5.54-77.97 for Co and 65.68-1652.89 µg/g for Fe. Percentage of heavy metals that were found beyond the permissible limits were: 71.4% for Pb, 28.51% for Cd, 14.2% for Cu, and 9.5 % for Cr. Significant difference was noticed for each heavy metal among herbal drugs as well as their zones of collection using two way ANOVA followed by least significant (LSD) test at $p < 0.05$. Purpose of this research is to detect each type of heavy metal contaminant of herbal drugs by environmental pollution, as well as to highlight the health risks associated with the use of such herbal drugs that contain high levels of toxic heavy metals.

Keywords: Atomic absorption spectrophotometer, environmental pollution, heavy metal toxicity, herbal drugs.

INTRODUCTION

Medicinal plants are being widely and successfully utilized in various disciplines of natural health care systems including Unani, herbal, ayurvedic, and homeopathic systems of medicines in the form of single herbs, polyherbal formulations and standardized extracts. It is a common misperception that medicines of natural substances cannot be toxic, but according to the advanced researches it has been documented that plants not only contain toxic secondary metabolites, but they are also contaminated with environmental pollutants specially heavy metals, which pose a great health risks to all living organisms upon long term exposures (Arpadjan *et al.*, 2008; Itanna, 2002; Lasisi *et al.*, 2006; Obi *et al.*, 2006). Heavy metals are classified in two main categories i.e. essential and toxic heavy metals. Essential heavy metals (Cu, Zn Cr, Fe, and Co) are required in very trace quantities for the proper functioning of enzyme systems, hemoglobin formation, and vitamin synthesis in men and for the growth and development and photosynthesis in plants. Metabolic disturbances are encountered in case of both deficiency and excess of these essential metals. On the other hand toxic metals for example Pb, Cd, As, and Hg are not required by the body and they produce deleterious effects upon exposure even at very low concentrations (Abu-Darwish *et al.*, 2009; Friberg *et al.*, 1986; Islam *et al.*, 2007; Singh and Garg, 2006). Heavy metals are persistent in the environment and are subjected to bioaccumulation in food chains (WHO 2007). Anthropogenic processes, involving the application of synthetic fertilizers, lime, organic manure, industrial

residues contribute various amounts of heavy metals to the ecosystem (Annan *et al.*, 2010; Kos *et al.*, 1996; Martins *et al.*, 2008). Metal contamination in herbal drugs remain continue during their transportation and storage at herbal shops where these are exposed to environmental pollution, dust, and heavy metals because of unhygienic storage conditions, and become toxic in nature. Such contaminated herbs are one of the major potential sources of heavy metal accumulation in the human organs and systems, because these are not only utilized as herbal medicines and food supplements, but many of them are consumed as condiments in daily routine.

Unfortunately less attention is paid towards the legislation and quality control parameters of herbal drug industry as compare to allopathic system of medicines. FAO/WHO has highlighted this critical issue and strongly recommends heavy metal analysis in the herbal medicines along with other necessary biological, chemical, and environmental analysis in their guidelines (WHO 1989, 1993, 1998, 2005), and also documented the dietary allowances, absorption, elimination, and toxic profiles of heavy metals.

Karachi city is counted as one of the most crowded and polluted city of the world. Environmental pollution, industrialization, and automobile smoke are increasing day by day, resulting in the increase load of heavy metals on the ecosystem. This research is based on two major phases. First one is to study the existence of heavy metals in the herbal drugs which are being sold at the local herbal markets of Karachi, Pakistan in order to check their purity for human consumption. In second phase a comparative data of heavy metal contents of the same

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herbal drug purchased from three different zones of the city, exposed to varying degrees of pollution was also done to check the effect of pollution on the heavy metal concentration of herbal drugs.

MATERIALS AND METHODS

Materials

Seven (1-7) selected medicinal plants, listed in table 1 were purchased from three different herbal markets situated at southern (zone A), eastern (zone B) and western (zone C) of Karachi city. Each herb was procured from three zones making a total of twenty one samples for comparative study. The collected samples were stored in airtight glass bottles throughout the study. These medicinal plants were identified by Prof. Dr Ghazala .H. Rizwani, Department of Pharmacognosy, University Of Karachi. Their voucher specimens were deposited in the Pharmacognosy museum, faculty of pharmacy, university of Karachi. Table 1 contained the information of local and botanical names, part used, and medicinal uses of the tested plant materials (Bown, 1995-2002, Duke *et al.*, 2002, Monograph on Unani Medicine (2003), Youngken, 2004). Dried sample of each plant drug (100g) was pulverized twice into fine state using grinder (Panasonic MX-J220P), and passed through 60 mesh sieve in order to get uniform particle size of pulverized material. Each pulverized material was kept in clean, dry, labeled, and Stoppard glass vials.

Analytical grade Nitric acid (HNO₃) and 70% Perchloric acid (HClO₄) supplied from Merck Germany were used as reagents for wet digestion of samples. Solutions were

prepared in distilled deionized water. All glass wares (Pyrex) were cleaned and rinsed with distilled water prior to use.

The standard solutions of elements Cu, Co, Cr, Cd, Ni, Fe, Pb, and Zn (May and Baker Rhone) were prepared in three different concentrations to obtain calibration curve by diluting stock solutions of 1000 ± 5 ppm of each element.

Method of analysis

Powder of herbs was digested through wet digestion method (Perkin Elmer, 1991). Briefly, 1 gm sample of each herb was weighed in a beaker on electrical balance (Sartorius CP 3245), 10 ml of concentrated HNO₃ was added and allowed to stand overnight. Solution was heated carefully in a water bath (Gerhardh Schuttewasserbad SW20) until the production of red nitrous oxide fumes had not been ceased. Beaker was allowed to cool at room temperature. 4 ml of 70% HClO₄ was added and mixture was heated again to evaporate into a small volume and filtered through Whatman filter paper NO.42, transferred to a 50 ml volumetric flask and made up the volume with distilled water.

Heavy metal analysis of all plant drugs were performed on atomic absorption spectrometer (Perkin Elmer 3030B). Standard operating parameters for working elements were set, given in table 2. The hollow cathode lamps for Cu, Cr, Cd, Co, Ni, Pb, Zn, and Fe (Buck Scientific) as radiation source and fuel was air acetylene (BOC Karachi) were used. The concentrations of analytes were directly obtained from calibration graphs and all

Table 1: Pharmacognostic features of tested medicinal herbs.

S. No.	Herbal drugs	Family	Local names	Part used	Medicinal uses
01	<i>Glycyrrhiza glabra L.</i>	Papilionaceae	Mulithi	Root	laxative, tonic demulcent, cough, cold, expectorant, Bronchitis
02	<i>Onosma Bracteatum Wall.</i>	Boraginaceae	Gule-e-gaozaban	Flower	Fever, tuberculosis, bronchial, mouth, and throat infection, pleurisy, rheumatism and skin conditions
03	<i>Viola odorata L.</i>	Violaceae	Gul-e-Banafsha	Flower	Cold and cough, lung troubles, cancer of lung and gastrointestinal tract.
04	<i>Foeniculum Vulgare Mill.</i>	Apiaceae	Saunf	Fruit	Indigestion, formerly used in chronic cough, carminative
05	<i>Cuminum cyminum L.</i>	Apiaceae	Zeera	Seed	Common cold, cough, stomach diseases, indigestion
06	<i>Coriandrum sativum L.</i>	Apiaceae	Dhania	Fruit	Anxiety, insomnia, cold and cough, diuretic, carminative
07	<i>Zingibar officinalis Roscoe</i>	Zingiberaceae	sont	Rhizome	Motion sickness, cold, and cough, morning sickness, nausea, influenza, shock, digestive disturbance

Table 2: Working parameters of atomic absorption spectrophotometer

S. No.	Elements	Weave length (nm)	Slit width (nm)	Lamp current (A)
01	Pb	217.0	0.7	10
02	Cd	228.8	0.7	08
03	Cu	324.8	0.7	10
04	Zn	213.9	0.7	10
05	Cr	352.9	0.7	12
06	Ni	232.0	0.2	30
07	Co	240.7	0.2	30
08	Fe	258.3	0.2	30

Table 3: Heavy metal content of herbal drugs ($\mu\text{g/g}$)

	<i>G. glabra</i>	<i>O. bracteatum</i>	<i>V. odorata</i>	<i>F. vulgare</i>	<i>C. cyminum</i>	<i>C. sativum</i>	<i>Z. officinalis</i>
Pb	18.98 ^{ab}	18.86 ^{ab}	16.77 ^{ab}	13.49 ^a	19.27 ^{ab}	21.71 ^b	17.30 ^{ab}
Cd	1.08 ^{ab}	1.63 ^c	0.54 ^{ab}	1.63 ^{bc}	0.00 ^a	0.00 ^a	0.54 ^{ab}
Cu	104.65 ^c	2.05 ^b	0.21 ^a	0.00 ^a	0.91 ^{ab}	2.22 ^b	0.00 ^a
Zn	418.64 ^e	201.87 ^c	207.97 ^c	212.56 ^{cd}	133.72 ^b	223.0 ^d	105.53 ^a
Cr	5.95 ^a	4.37 ^a	108.01 ^b	0.53 ^a	0.00 ^a	0.00 ^a	0.00 ^a
Ni	17.29 ^c	40.58 ^e	24.67 ^d	9.00 ^b	3.57 ^{ab}	0.91 ^a	0.32 ^a
Co	28.99 ^{ab}	51.1 ^d	48.15 ^{cd}	16.64 ^a	38.96 ^{bcd}	28.17 ^{ab}	32.14 ^{abc}
Fe	958.43 ^f	370.02 ^c	268.3 ^b	451.02 ^d	763.83 ^e	938.72 ^f	163.7 ^a

*Each value represents the mean heavy metal content of 3 samples of each herbal drug collected from three zones, mean followed by same letter (s) in a raw stand for no significant difference using ANOVA ($p < 0.05$) followed by multiple comparison of mean by least significant difference (LSD) test

Note: Statistical comparisons are made between herbal drugs for each heavy metal.

measurements were run in triplicate for the samples and standard solutions.

STATISTICAL ANALYSIS

Heavy metal contents among the herbal drugs and the three different collection zones were analyzed by two way analysis of variance test (ANOVA) using a probability factor of $p < 0.05$, by using software SPSS V.17, followed by multiple comparison of mean using least significant difference (LSD) test to locate the significantly different pairs of mean.

RESULTS

Heavy metal concentrations of herbal drugs collected from southern, eastern, and western zones of Karachi city are mentioned in figs. 1-3. Fe content of herbal drugs is represented in fig. 4. Statistical outcomes of heavy metal concentration among herbal drugs as well as their collection zones using two way ANOVA followed by LSD test are mentioned in tables 3-4.

Lead (Pb)

O. bracteatum (eastern zone) contained the maximum concentration of Pb 30.46 $\mu\text{g/g}$, while *F. vulgare* (western zone) showed the minimum contamination of Pb 3.26 $\mu\text{g/g}$. Pb content of herbal drugs was found in the range of 9.72-26.11, 4.83-30.46, 9.59-21.11, 3.26-29.05, 0-28.92, 17.85-24.32, and 13.13-22.63 $\mu\text{g/g}$ representing *G.*

glabra, *O. bracteatum*, *V. odorata*, *F. vulgare*, *C. cyminum*, *C. sativum*, and *Z. officinalis* respectively.

Cadmium (Cd)

O. bracteatum and *F. vulgare* procured from southern zone showed the maximum level of 4.91 and 4.89 $\mu\text{g/g}$ Cd. Almost same concentration of Cd i.e. 1.63 $\mu\text{g/g}$ was detected in *G. glabra* (southern and western zones), *V. odorata* (southern zone), and *Z. officinalis* (eastern zone). Cd was not detected in any sample of *C. cyminum* and *C. sativum*.

Copper (Cu)

Highest concentration of Cu 120.21 $\mu\text{g/g}$ was detected in *G. glabra* (western zone) while the minimum concentration of 0.653 $\mu\text{g/g}$ was present in *V. odorata* (southern zone). Cu was not detected in any sample of *F. vulgare* and *Z. officinalis*.

Zinc (Zn)

G. glabra (southern zone) showed the maximum concentration of Zn 433.76 $\mu\text{g/g}$ while *Z. officinalis* (western zone) contained the minimum concentration of Zn 83.74 $\mu\text{g/g}$.

Chromium (Cr)

V. odorata contained the maximum concentration of Cr 186.75 $\mu\text{g/g}$ purchased from eastern zone while the minimum concentration of Cr 1.61 $\mu\text{g/g}$ was detected in *F. vulgare* (western zone). It was not detected in any sample of *C. cyminum*, *C. sativum*, and *Z. officinalis*.

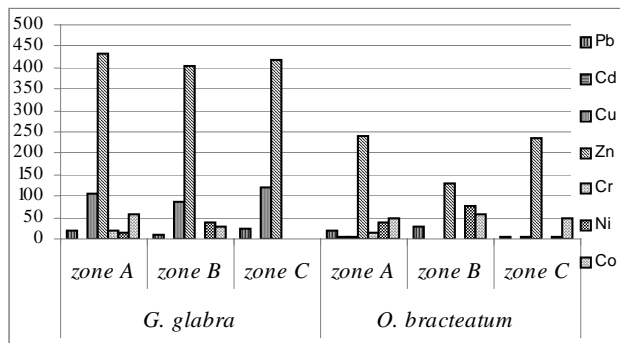


Fig. 1: Concentration of heavy metals (µg/g) in *Glycyrrhiza glabra* L. and *Onosma bracteatum* wall.

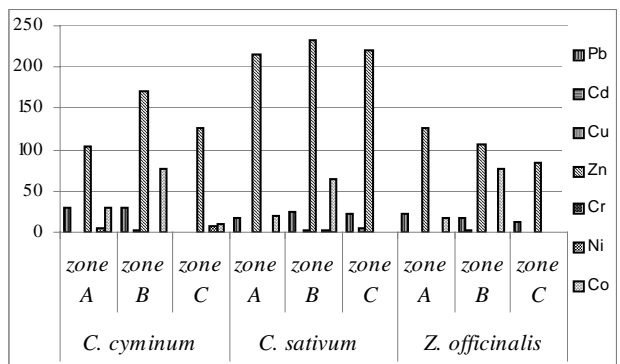


Fig. 3: Concentration of heavy metals (µg/g) in *Cuminum cyminum* L., *Coriandrum sativum* L., and *Zingibar officinalis* Roscoe.

Nickel (Ni)

O. bracteatum (eastern zone) showed the maximum level of Ni 76.97 µg/g, while the minimum concentration of Ni 0.48 µg/g was observed in *C. cyminum* procured from eastern zone.

Cobalt (Co)

Z. officinalis and *C. cyminum* from eastern zone showed the maximum concentration of Co 77.97 µg/g and 77.44 µg/g respectively, while *F. vulgare* (western zone) contained the minimum concentration of Co 5.54 µg/g.

Iron (Fe)

G. glabra (eastern zone) showed the maximum Fe content Of 1652.89 µg/g, while *Z. officinalis* (western zone) showed the minimum level of Fe 65.68 µg/g.

Cu, Zn, and Fe content were found statistically significant in *G. glabra* than remaining drugs under study. Ni (40.58 µg/g) and Co (51.1 µg/g) were found significantly greater in *O. bracteatum* than reaming herbal drugs. *V. odorata* contained the highest amount of Cr (108.01 µg/g) than other herbal drugs under study. *C. sativum* contained significantly highest concentration of Pb (21.71 µg/g) (table 3).

Statistically plant drugs sold at herbal markets situated at eastern (22.81 µg/g) and southern zones (19.93µg/g) of Karachi city were more contaminated with Pb then those

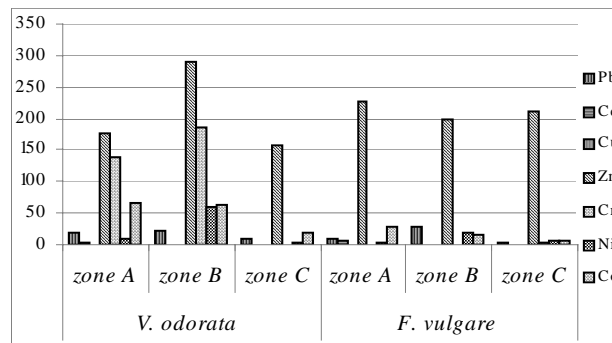


Fig. 2: Concentration of heavy metals (µg/g) in *Viola odorata* L. and *Foeniculum vulgare* Mill

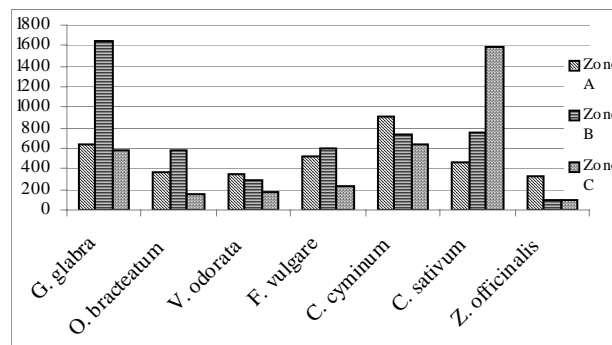


Fig. 4: Fe content of herbal drugs (µg/g)

obtained from western zone (11.41µg/g). Cd is the major pollutant of herbal drugs from southern zone (1.86µg/g). Table 4 represents the multiple comparisons for each heavy metal among herbal markets of different zones of Karachi city.

DISCUSSION

Glycyrrhiza glabra L. is a drug of choice for inflammation, expectorant, cough, detoxification of liver, and hormonal and laxative effects (Bown, 1995-2002, WHO, 1999). Rhizome of *G. glabra* has such anatomical features that support the absorption of heavy metal from the soil, consequently exposure to polluted environment increases risk of heavy metal contamination. After absorption through root hair, metals are stored in the collenchymas, epidermis, and vascular bundle of the cell. Diffusion and cation exchange are the probable mechanisms of metal absorption through root system (Brooks, 1972). *G. glabra* sold at southern and western zones of Karachi city pose serious health risks to the local public because of Pb, Cd, and Cu contamination, crossing the safety limit of 10 mg/kg Pb, 0.3 mg/kg Cd (WHO 1998), 4-20 µg/g Cu by Stevensen 1986 (Haider et al., 2004) respectively. However heavy metal contents of *G. glabra* collected from the eastern zone were found within the allowable limits (fig. 1). *G. glabra* can give rise to damage of respiratory organs, nerve cells, and

hemoglobin due to Pb and Cd intoxication (ASTDR 2008, Divrikli *et al.*, 2006, Friberg *et al.*, 1986, Obi *et al.*, 2006, Khan *et al.*, 2008). Concentrations of essential metals like Fe, Zn, Ni, Co, and Cr were found below their recommended daily allowances that is 18 mg/day for Fe, and 15 mg/day for Zn given by National Research Council 1989, and average daily intake of 0.3 mg / day Ni given by WHO 1996 (Divrikli *et al.*, 2006), 0.02- 1.0 mg/kg body weight for Co, (Abu- Darwish *et al.*, 2009), and 120 µg for Cr given by FDA 1999 (Haider *et al.*, 2004) (fig. 1) making this drug a good source of essential metals.

***Onosma bracteatum* Wall.** Flower is widely used in Unani system of medicine against fever, bronchial infections (including pleurisy and tuberculosis), mouth and throat infection, dry skin condition, and cirrhosis (Monograph on Unani Medicines, 2003). Heavy metal uptake in the aerial parts of plant drug is followed by the mechanism of foliar absorption and metal transportation from root to shoot through xylem vessels. Heavy metal concentration in the aerial parts of plants is restricted by exclusion mechanism, irrespective of the metal concentration in the soil (Brooks, 1972). *O. bracteatum* procured from southern zone of Karachi city pose serious health risks because of toxic contamination of Pb (21.3 µg/g) and Cd (4.91 µg/g). Sample obtained from eastern zone was also unsafe for human consumption due to elevated level of Pb (30.46µg/g) (fig. 1). Cd contamination in the drug can lead to throat dryness, cough, headache, pneumonitis, bronchopneumonia, lung damage, lung cancer, fibrosing alveolitis, and chest pain (Divrikli *et al.*, 2006; Friberg *et al.*, 1986). However *O. bracteatum* was rich Cu, Co, Cr, Ni, Zn, and Fe.

***Viola odorata* L.** Flowers are used in lung troubles, asthma, cough, cold, and cancer of breast, lung, stomach and digestive tract (Bown, 1995-2002). Violet flowers collected from southern and eastern zones can lead to Pb, Cd, and Cr intoxication to the local public (fig. 2). FDA 1999 has given RDI of 120 µg Cr (Haider *et al.*, 2004). Toxic level of Cr can cause irritation of skin, damage to kidneys, liver, circulatory and nerve tissues, respiratory problems and nose bleeds (Friberg *et al.*, 1986; Khan *et al.*, 2008). Concentrations of Cu, Zn, Ni, Co, and Fe were found within safe limit.

***Foeniculum vulgare* Mill** is a drug of choice for its analgesic, antipyretic, antiseptic, carminative, digestive, expectorant, and stimulant activities (Duke *et al.*, 2002). Its samples procured from eastern and southern zones can produce risk of metal toxicity to patients due to Pb (29.05 µg/g) and Cd (4.89 µg/g) respectively. However samples sold at other herbal markets were free from toxic concentrations of heavy metals and can be used safely in Phytopharmaceuticals.

***Cuminum cyminum* L.** is effective in common cold and stomach diseases (Youngken, 2004). *C. cyminum* from

southern and eastern zones can lead to Pb toxicity. Fortunately all samples of *C. cyminum* were free from Cd and Cr contamination. Cu, Zn, Co, Ni, and Fe levels were found safe for consumption (fig. 3).

***Coriandrum sativum* L.** has been used as a folk medicine for the relief of anxiety and insomnia, anxiolytic, diuretic, carminative, digestive aid, cold and cough (Youngken, 2004). Cd was not detected in any analyzed sample. Like other drugs under study, Cr, Zn, Cu, Co, Ni, and Fe content of *C. sativum* was also found safe (fig. 3).

***Zingibar officinalis* Roscoe.** Rhizome is used in motion sickness, nausea, morning sickness, cold, cough, influenza, shock, and digestive disturbances such as antiemetic, flatulence, stomach complaints (Bown, 1995-2002; WHO, 1999). Dangerous amount of Pb was detected in its all tested samples, while toxic concentration of Cd 1.62 µg/g was found in the sample that obtained from eastern zone (fig. 3). Cu and Cr were not detected in any sample, while safe amounts of Zn, Ni, Co, and Fe were determined.

Table 4: Heavy metal content of zones of collection of herbal drugs (µg/g).

Heavy metals	Southern zone	Eastern zone	Western zone
Pb	19.93 ^b	22.81 ^b	11.41 ^a
Cd	1.86 ^b	0.23 ^a	0.23 ^a
Cu	15.73 ^b	13.28 ^a	18.19 ^c
Zn	217.82 ^b	218.71 ^b	208.00 ^a
Cr	24.03 ^b	26.67 ^b	0.23 ^a
Ni	9.74 ^b	28.42 ^c	3.12 ^a
Co	37.87 ^b	55.30 ^c	11.48 ^a
Fe	509.03 ^a	657.80 ^b	493.06 ^a

*Each value represents the mean heavy metal content of herbal drug (1-7) collected from each zones, mean followed by same letter (s) in a raw stand for no significant difference using ANOVA (p<0.05) followed by multiple comparison of mean by least significant difference (LSD) test

Note: Statistical comparisons are made between collection zones of herbal drugs for each heavy metal.

CONCLUSION

Most of the herbal drugs that are being sold in the local herbal markets of Karachi city were found toxic and unsafe for human consumption due to elevated levels of heavy metals especially Pb and Cd. Comparative studies on the heavy metal contents of same species of herbal drug procured from three different herbal markets revealed wide variation in the results. Pb was found as a major pollutant in crude drugs procured from all three zones of karachi city selected for study. Cd contamination of herbal drugs was predominant in southern zone. Handling of herbal drugs in such zones needs especial care due to potential risk of heavy metal toxicity. Pharmacovigilance must be done to improve the quality, safety, and efficacy of herbal drugs not only during their

growth, but also during their storage at herbal shops in order to avoid heavy metal contamination. Concentrations of essential metals (Cu, Zn, Cr, Ni, Co, and Fe) were found safe in most of the herbal drugs.

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