

Atomic absorption spectroscopic analysis of some Pakistani herbal medicinal products used in respiratory tract infections

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Abstract: Heavy metals are one of the major causes of widespread occurrence of respiratory tract infections (RTIs). Unani system of medicines utilizing plant extracts and herbal medicinal products (HMPs) are known to have remedial potential in different RTIs (flue, cough, bronchitis, allergy, asthma, nasal and chest congestion). Being natural in origin chances of metal contamination cannot be ignored in Phytopharmaceuticals, therefore toxic metal contents (Pb, Cd, As, and Hg) were determined in some Pakistani branded HMPs being used in various RTIs through atomic absorption spectrophotometer. Research findings showed detectable levels of metals in all these formulations. Daily metals intakes via these HMPs were calculated as: 0.86-277.44 (Pb), 1.442-10.986 (Cd), 0.16-5.83 (As), and 0.00-0.09 µg/ day (Hg). Prevalence of toxic metals in HMPs especially elevated levels of Pb and Cd, pose severe health risks to the consumers. Statistical interpretation of data using ANOVA followed by LSD ($p < 0.05$), revealed significantly different metal concentrations among HMPs. To identify the metal contaminant of HMPs and highlight their toxic manifestations is the basic aim of this research. The study is not a bias to the beneficial effects of the Phytopharmaceuticals, rather is done to improve their quality and safety parameter according to the international health standards.

Keywords: Arsenic, Cadmium, heavy metal toxicity, herbal formulations, lead, mercury, respiratory tract diseases, toxic metal contamination, Unani medicines.

INTRODUCTION

Herbalism through its deep roots in history, is the most popular and effective form of traditional medicine. In earlier times due to their low prices, easy availability, and affordability, these botanical preparations were the drug of choice in third world countries, but in the current scenario emerging trends towards natural health care system in developed countries have also been witnessed. According to WHO 2008, 80% African and Asian while 70-80% population in developed countries rely on traditional medicines for primary health care. Such tremendous global increase in the demand of natural Phytopharmaceuticals has drawn the attention towards safety and quality of these plant based medicaments (WHO 2007 and 2008).

Contamination of heavy metals in the crude medicinal plants as well as in herbal medicinal products (HMPs) pretense severe hidden health risks to the consumers. Plants raw materials are the basic and major source of metal contamination of these products, while leeching of metal contents from containers and chemicals during different stages of manufacturing, and intentionally addition of some heavy metals in Chinese and Indian origin traditional medicines for their therapeutic claims are the other sources (Ernest 2002; Saper *et al.*, 2004; WHO 2005 and 2007).

Several cases of subsequent metal poisoning (As, Hg, Pb, and Cd) after utilization of HMPs are reported. Arsenic

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poisoning has been noticed in children using Chinese herbal medicines in Singapore (Saper *et al.*, 2004). Similarly deleterious effects of Pb, Cd, As, and Hg poisoning associated with various Chinese and Indian traditional medicines from all over the world has been documented (Ernst 1998; Ernst 2002; Ernst 2003; Parmar 2005; Dargan *et al.*, 2008). WHO 2005 has warned consumers not to use some heavy metal contaminated Asian origin HMPs. Some Pakistan based herbal formulations were also included in that list.

According to WHO 2006, quarter of the global disease burden is related with environment risk factors (Inter ministerial conference, 2008). Credible scientific data proved that air borne particles and heavy metals (Pb, Cd, As, Hg, Ni, Co, and Cr) produce deleterious and damaging effects on respiratory organs and can give rise to flue, cough, asthma, bronchitis, congestive chest diseases, rhinitis, nasal allergy, lung cancer, and even may result in death (Fengyuan PIAO *et al.*, 2008; Sterubeck *et al.*, 2002; Verougstraete *et al.*, 2004). Like other polluted cities of the world, heavy metals are inflowing in local inhabitants of Karachi city through air, water, soil, and food. Elevated levels of heavy metals especially Pd was detected in water supply to Karachi through Damloti well, tube well, Indus and Hub water supply (Nargis *et al.*, 2000; Nargis *et al.*, 2010). Intolerable level of air and noise pollution in the Karachi city lead to the prevalence of respiratory tract infections (RTIs) and related disorders. Among them 70% patients suffering from chronic bronchitis, pulmonary emphysema, and pulmonary edema

were found the residues of heavily polluted areas of the city (Rehman and Ali, 1993).

Utilization of herbal drugs in Pakistan, is also gaining popularity due to their research findings. These are not only utilized in modern medicines, but also being utilized as crude medicinal plants, polyherbal formulations and herbal extracts in Unani, ayurvedic, and homeopathic systems of medicine. Herbal industry is flourishing at a high rate in this cosmopolitan city, but unfortunately pharmacovigilance of herbal drugs is rarely seen in resulting in the poor quality control and lack of safety measures of the use of herbal medicinal products (HMPs). These HMPs and crude drugs themselves become hazardous when are contaminated with toxic heavy metals mainly Pb, Cd, Hg, and As and turn out to be serious threat of metal poisoning to the patients. Hina *et al.*, 2011 published about the toxic levels of metals especially Pb and Cd in most of the crude drugs used as basic raw materials in various HMPs. Heavy metals are continuously inflowing in the local population of Karachi city through contaminated environments. Furthermore consumption of metal contaminated Phytopharmaceuticals can increase the toxic metal loads many fold higher resulting in more aggravated conditions. Detection of toxic metals and their comparison with USP allowable limits in some most common herbal medicinal products used in respiratory tract infections is the main purpose of our present research. Furthermore outcomes of this research study will be more beneficial for creating awareness not only to the lay person but also to herbal practitioners and physicians about the toxic manifestations of metal poisoning. This will be helpful to upgrade the quality and safety standards of herbal formulations by minimizing the health hazards related with their consumption.

MATERIALS AND METHODS

Materials

Ten most frequently used Unani herbal medicinal products (HMPs 01-10) were selected that are most effectively used in various respiratory tract diseases to detect concentration of lead, cadmium, arsenic, and mercury. Each herbal formulation was marked for the code No. assigned to the particular formulation. table 1 contained the code No., dosage form, compositions, dose as per label claim, and therapeutic uses of all HMPs.

Analytical grade Nitric acid HNO_3 supplied from Merck Germany was used as reagent 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. Standard solutions of each metal Pb, Cd, As, and Hg were prepared by diluting stock solutions of 1000 ± 5 ppb for each metal to obtain calibration curve.

Method

Wet digestion method using nitric acid was employed for the digestion of herbal medicinal products (HMP 01-10) following the procedure of Obi *et al.*, 2006 with some modifications. Briefly approximately 1 gm of each sample was digested by adding 5 ml of HNO_3 . Upon complete digestion, solutions were allowed to cool and then filtered. Volumes were finally made up to 50 ml by adding deionized water.

Perkin Elmer 700 Atomic Absorption Spectrophotometer was used for the heavy metal detection in HMPs. Results was obtained in ppb concentration for each metal. Table 2 contained the standard working parameters of AAS.

STATISTICAL ANALYSIS

Metal intakes (Pb, Cd, As, and Hg) for unit dose as per label claim of HMPs 01-10 were calculated and subjected to one way analysis of variance test (ANOVA) followed by least significant difference test (LSD) at $p < 0.05$ using SPSS V.17; results are summarized in table 3.

RESULTS

Metal concentration detected in HMPs, unit dose, and number of maximum dosages/day mentioned on label claim were used to determine daily metal intake ($\mu\text{g}/\text{day}$) to the patients of RTIs utilizing these HMPs and compared to the different established and proposed allowable limits that are mentioned in figs. 1-4 for Pb, Cd, As, and Hg respectively. Figs. 5 and 6 showed the increased metal load on the patients if these HMPs are consumed continuously for a week and month respectively. Table 3 contained the summary of statistical outcomes for heavy metals contents among HMPs present in unit dose.

Lead (Pb)

Maximum concentration of lead (Pb) was detected in HMP-02 ($277.44 \mu\text{g}/\text{day}$), while the minimum concentration of Pb ($0.862 \mu\text{g}/\text{day}$) was detected in HMP-06 (figure 1). Pb content of HMP- 07, 03, and 01 were found significantly greater than rest of the herbal medicinal products in the study (table 3).

Cadmium (Cd)

Based on maximum daily exposure, HMP-04 contained the highest concentration of $10.986 \mu\text{g}/\text{day}$. Minimum concentration of $1.442 \mu\text{g}/\text{day}$ was detected in HMP 09 (fig. 2). Statistically HMP-05 and 04 contained significantly greater amount of Cd in single dose than remaining herbal formulations (table 3).

Arsenic (As)

Maximum daily exposure of arsenic (As) was calculated for HMP-07 ($5.834 \mu\text{g}/\text{day}$), while the minimum

concentration was observed for HMP-06 (0.168 µg/ day) mentioned in fig. 3. Arsenic concentration in HMP-07 was found significantly greater than remaining herbal medicinal products (table 3).

Mercury (Hg)

Mercury (Hg) was not detected in any HMP except for HMP-06 (0.0921 µg/day).

DISCUSSION

Prevalence of respiratory tract infections RTIs (cold, productive and non productive cough, bronchitis, pharangitis, tonsillitis, asthma, allergy, chest and nasal congestion) are very common in Karachi Pakistan. Unani system of medicine is the most effective and commonly used form of traditional medicine to cure all kinds of ailments especially RTIs. Our research was designed to find out the prevalence of toxic metals lead, cadmium, arsenic, and mercury in some Pakistani branded herbal medicinal products (HMPs). These are available in oral dosage forms (syrup, pill, and freeze dried extracts of herbs) that are widely used to treat and prevent these pectoral diseases. Advocates of the herbal medicines generally claim for no side effects associated with their use in such ailments; but according to our research findings, consumption of these HMPs is not safe and risk free because of prevalence of high proportion of these

herbal formulations with detectable levels of toxic metals lead (100%), cadmium (90%), arsenic (50%), and mercury (10%). We compared the maximum daily metal intake with different established and proposed elemental impurities limit, if these HMPs are taken according to the manufacturer recommended daily dose. The American National Institute (ANSI) /National Sanitation Foundation (NSF) International Dietary Supplement Standard -173 gives the limits of 20 µg/d for lead, 6 µg/d for Cadmium, 20 µg/d for mercury, and 10 µg/d for arsenic (NSF/ANSI-173, 2003). The United States Pharmacopeia, in its proposed new general test chapter USP <232> “Elemental Impurities- Limit” recommends permitted daily exposure (PDE) of 10 µg/ day for Pb, 5 µg/ day for Cadmium, and 15 µg/ day for Arsenic and Mercury in pharmaceuticals (USP <232>, 2010). Detailed toxicological and regulatory information has also been published to provide basis for these proposed elemental impurities limits (De Stefano et al., 2010). Graphical representation in fig. 1 showed the presence of Pb beyond the NSF/ANSI-173 allowable limit of 20 µg/d and USP <232> proposed PDE of 10 µg/d in 80% and 90% HMPs respectively. 40% of HMPs crossed the allowable limit of 6 µg/d (NSF/ANSI-173, 2003) and USP <232> Proposed PDE of 5 µg/d Cd (Figure 2). Presence of these toxic metals in HMPs is a very alarming situation because Pb, Cd, As, and Hg are of major concern due to toxic manifestations on human health. Respiratory tract being involved in breathing is the

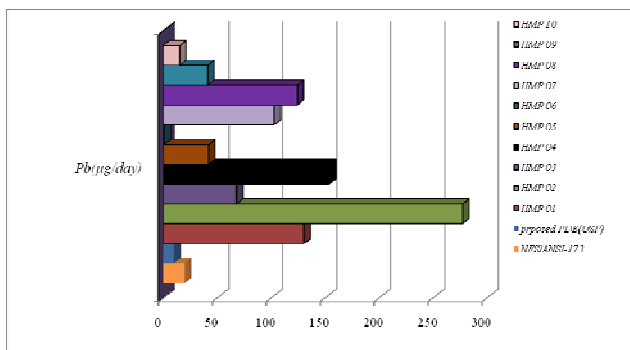


Fig. 1: Intake of Pb via HMPs as compared to allowable limits (µg/day)

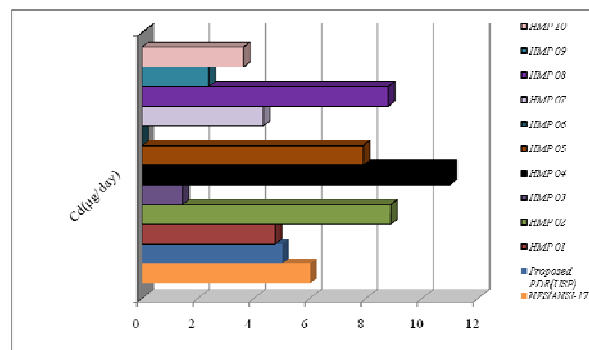


Fig. 2: Intake of Cd via HMPs as compared to allowable limits (µg/day)

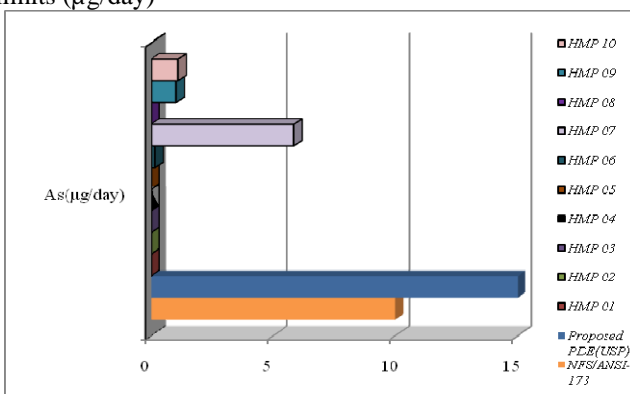


Fig. 3: Intake of As via HMPs as compared to allowable limits (µg/day)

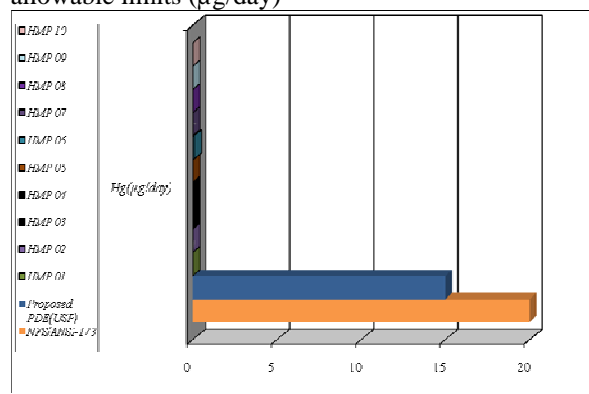


Fig. 4: Intake of Hg via HMPs as compared to allowable limits (µg/day)

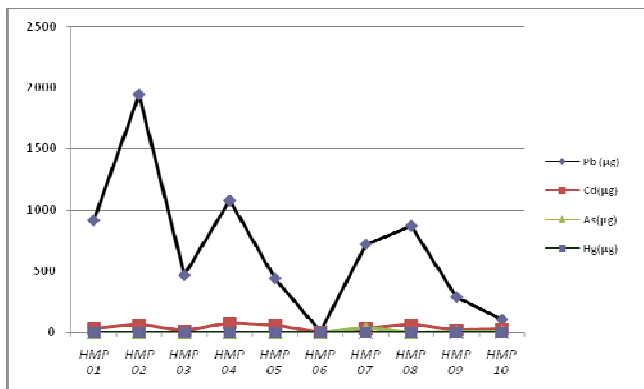


Fig. 5: Toxic metals accumulation ($\mu\text{g}/\text{week}$) from weekly consumption of HMPs (01-10)

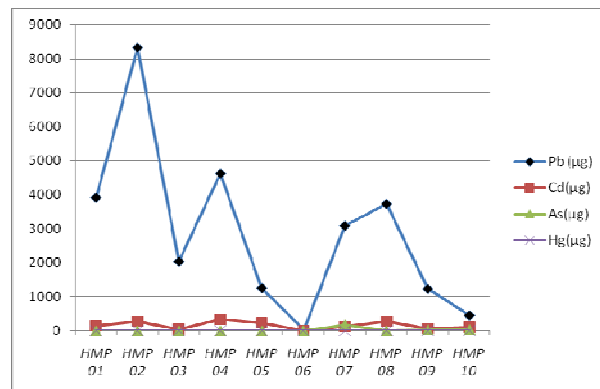


Fig. 6: Monthly accumulation ($\mu\text{g}/\text{month}$) of toxic metals from HMPs (01-10)

maximally exposed system to the contaminated air along with normal air which makes it more sensitive to the deleterious effects of toxic metals. Weak immune system, poor dietary status, nutritional deficiency, and presence of inter current diseases result in increased absorption of these metals leading to more complicated conditions (Friberg *et al.*, 1986). For these above mentioned reasons utilization of metal contaminated HMPs in respiratory tract diseases may lead to produce more critical effects and complications in such patients.

Hazard of metal toxicity associated with the use of these HMPs mainly depends on quantity of dose, No. of dosages per day, and duration of therapeutic use. In case of syrups selected in our study, frequency of repetition of doses per day is more than those recommended for solid dosage form (Table 1), resulting in increased daily metal intake. Most of the HMPs in syrup form have high risk of metal poisoning due to repetition of doses; therefore consumption of HMP-01, 02, 03, 04, 05, 07, and 08 offer greater risk of metal poisoning. The second important criterion for determining impact of toxic metals on health through these HMPs is the duration of therapy. Longer the therapeutic duration, higher will be the metal intake to the body. As pointed out in figure 5, concurrent use of these HMPs even for one week can increase the body burden of these toxic metals (Pb, Cd, As, and Hg) many fold higher due to gradual deposition. Furthermore, if the treatment protocol requires prolonged therapeutic durations (allergy, bronchitis, asthma, whooping cough, and congestive chest diseases) the picture becomes even worse. In such cases where monthly consumption of these HMPs are prescribed, tremendous increase in the heavy metal load can be noticed not only for Pb and Cd, but also for As and Hg accordingly as compared to their short term exposures. Apparently, these HMPs may seem to be effective in treating such conditions of diseases for which these are being taken, but simultaneously results in gradual increase in the toxic metal load in the body organs and systems leading to chronic toxicity.

Pb and Cd were found predominant in all selected herbal medicinal products used in respiratory tract infections. These metal contaminated HMPs can increase the blood Pb and Cd levels, and produce their toxic manifestations. Cd causes throat dryness, cough, headache, vomiting, chest pain, extreme restlessness and irritability, pneumonitis, bronchopneumonia, lung cancer, and can cause even death due to severe lung damage. Some biochemical disorders may also caused by Cd poisoning including the symptoms of calcium metabolism, osteoporosis, osteomalacia, proteinuria, nephrotoxicity, aminoaciduria, glycosuria, tubular necrosis. Deficiency of iron and other nutrients in food can increase the absorption rate of Cd as high as 20% (ATSDR, 2008; Friberg *et al.*, 1986; Obi *et al.*, 2006). Pb dust, fumes or vapors are more easily absorbed from the respiratory tract. The developing fetus and infants are far more sensitive than the adults. Toxic symptoms of Pb poisoning on various body organs and systems include hematopoietic (micro-normocytic anemia), nervous system (encephalopathy in children, mental deterioration, aggressive behavior, and sleeping difficulties), gastrointestinal tract (loss of appetite, upset stomach, diarrhea, and constipation), and kidney (nephropathy, renal tubular dysfunction, tubular atrophy, and dilatation) (De Stefano *et al.*, 2010; Friberg *et al.*, 1986). Several cases of Pb toxicity due to herbal formulations have been reported worldwide. Dargen *et al.*, 2008 found the increased blood Pb level of patients using ayurvedic medicines for diabetes. Severe symptoms of Pb toxicity including anemia and encephalopathy in children of Indian, Taipei, and Omani origin were reported using Indian and Chinese traditional medicines (Ernst, 2003).

Arsenic and mercury are also proved to be toxic for human health. These metals identified in our study were found within the allowable limits of $10 \mu\text{g}/\text{day}$ As and $20 \mu\text{g}/\text{day}$ Hg (ANSI-173, 2003) and USP <232> proposed permitted daily exposures of $15 \mu\text{g}/\text{day}$ for arsenic and mercury. However numerous cases of arsenic and mercury poisoning have been reported associated with the

Table 1: Description of Herbal Medicinal Products (HMPs) selected for study

Code No. *	Dosage Form	Compositions	Indications	Dose as per label claim
HMP -01	Syrup	<i>M. nigra</i>	Diphtheria, Hoarseness of Voice, Pharyngitis, Sore Throat	10 ml (2 teaspoonfuls) three or four times a day or as directed by the physician.
HMP -02	Syrup	<i>V.odorata, M.alba, A.vasica</i> , and other herbal medicines	Cough(all kinds), Cold, flue, whooping cough, sore throat, laryngitis, pharyngitis, congestion of lungs	1 teaspoon after every two hrs.
HMP -03	Syrup	<i>G. glabra, Z.vulgaris, O.bracteatum, C.latifolia, A.officinalis, Adiantum C.veeneris, L.usitatissimum, P.ajowan, F.vulgare, B.mori</i>	Catarrh, Cough, anti bronchial, and anti phlegmatic	10 ml mixed with one cup of hot water 2 times a day or as directed by the physician.
HMP -04	Syrup	<i>G. glabra, E.vulgaris, A. vasica, Z.vulgaris, H.officinalis, A.veneris, M. piperita, E.globulus</i>	Weakness of Lungs, Trauma, Cough, Difficulty in Breathing, Dryness and Roughness of Bronchial Tubes, Catarrh, Cold, Tuberculosis at its early stag, General Physical Weakness	Children:5 ml (a teaspoonful) every four hours Adults:10 ml (2 teaspoonfuls) every four hours or as directed by the physician.
HMP -05	Syrup	<i>H.officinalis, S.irrio, Z.vulgaris, O.basilium, P.longum, Z.officinalis, A.vasika, G. glabra</i>	Cough depressant, bronchodilator, expectorant, congestion of lungs, chronic and acute bronchitis, colds, catarrah, asthma, tubercular cough, whooping cough, and post influenza cough	2 teaspoon two times a day, if severe condition 5 ml after 4 hrs.
HMP -06	Pill	Not available	Cough, sore throat, flue	2 pills two times a day
HMP -07	Syrup	<i>E.vulgaris, A.chloridum, G. glabra, A.vasica</i>	Acute Asthma, Bronchitis, Chronic Asthma, Cold, Dyspnoea, Flu, Throat Inflammation	Two teaspoonfuls thrice a day or as directed by the physician.
HMP -08	Syrup	<i>A.aspera, E.vulgaris, K.musaffa, G.glabra, A.vasaka, R.succedanea, M.piperita</i>	Acute Bronchitis, Asthmatic Cough, Irritational Cough, Wooping Cough	10 milliliters (Two teaspoonfuls) every four hours or as directed by the physician.
HMP-09	Freeze dried extract	<i>A. vasaka, H.officinalis, E. globulus, T. sinensis, S. kalpa, M.piperita F.vulgare</i>	Cold and cough, flu, sore throat, fever	1 sachet three times a day
HMP-10	Freeze dried extract	<i>A.Officinalis, C.dichotoma, G.glabra, M.sylvester, B. officinalis, V.odorata, Z.vulgaris</i>	Cough, bronchitis, tonsillitis, catarrh, sore throat, headache, catarrhal fever, catarrhal pain	1 sachet 2-3 times a day

*Code number of herbal medicinal products.

use of traditional medicines (Ernst, 1998; Ernst, 2002; Ernst, 2003; Parmar 2005; Saper *et al.*, 2004). Arsenic poisoning is characterized by damage to upper respiratory tract, perforation of the nasal septum, skin changes, peripheral neuritis lung cancer, immunosuppression, hepato-renal damage, and fetotoxicity (ATSDR, 2007; De Stefano *et al.*, 2010; Friberg *et al.*, 1986).

Such high levels of Pb and Cd in these Pakistani branded Unani medicines can be explained as the result of contamination, because unlike rasa shastra medicines of ayurvedic origin metals are not deliberately added to these Unani formulations. Metal contaminated crude drugs used as raw material actually lead to the contamination to the final dosage form. As high concentrations of heavy metals are transferred into water during processing, this

contamination remains continued during different stages of manufacturing and packaging (Ernst, 2002; WHO, 2007; Abou-Arab and Donia, 2000).

In view of the fact that these HMPs are available in oral dosage forms i.e. (pills, syrups, and freeze dried extracts) symptoms of metal poisoning need prolonged durations to appear. Still advocates for herbal medicines claim for no side effects probably due to unrecognized, misdiagnosed, or subclinical metal intoxication (Saper *et al.*, 2008). Furthermore critical concentration for a metal to produce toxicity and nutritional status differs in individuals due to biological variations in sensitivity (Friberg *et al.*, 1986). Lack of awareness of the patients, local healers, and physicians about this critical issue, lead to continuous intake of these metals contaminated HMPs used against

Table 2: Working parameters of Atomic Absorption Spectrophotometer

S. No.	Heavy Metals	Application on AAS	Weave length (nm)	Slit width (nm)
01	Pb	Graphite burner	283.3	0.7
02	Cd	Graphite burner	228.8	0.7
03	As	Mercury hydride system (MHS)	193.7	0.7
04	Hg	Mercury hydride system (MHS)	253.7	0.7

Table 3: Toxic metal content ($\mu\text{g}/\text{dose}$) in herbal medicinal products

Code of HMPs	Pb	Cd	As	Hg
HMP 01	32.642 ^{fs}	1.188 ^{bc}	0.000 ^a	0.000 ^a
HMP 02	23.119 ^{de}	0.739 ^{ab}	0.000 ^a	0.000 ^a
HMP 03	33.768 ^t	0.720 ^{ab}	0.000 ^a	0.000 ^a
HMP 04	25.647 ^e	1.831 ^{cd}	0.000 ^a	0.000 ^a
HMP 05	20.977 ^d	2.633 ^d	0.000 ^a	0.000 ^a
HMP 06	0.431 ^a	0.000 ^a	0.084 ^a	0.046 ^b
HMP 07	34.293 ^t	1.444 ^{bc}	1.963 ^b	0.000 ^a
HMP 08	20.705 ^d	1.464 ^{bc}	0.000 ^a	0.000 ^a
HMP 09	13.727 ^c	0.792 ^{ab}	0.345 ^a	0.000 ^a
HMP 10	6.040 ^b	1.208 ^{bc}	0.369 ^a	0.000 ^a

Note: Statistical comparisons are made between herbal medicinal products (HMPs) for each heavy metal.

*Each value represents the mean heavy metal content of 3 observations, mean followed by same letter (s) in a column stand for no significant difference using ANOVA ($p < 0.05$) followed by multiple comparison of mean by least significant difference (LSD) test.

respiratory tract infections resulting in the bioaccumulation of these toxic metals in human organs at a high rate that can aggravate patient's condition.

CONCLUSION

Most of the Pakistani herbal medicinal products widely used to treat and prevent various respiratory tract ailments contained detectable levels of Pb, Cd, As, and Hg. The elevated levels of Pb and Cd in these HMPs, make these formulations a potential source of toxic metal poisoning to the patients utilizing these herbal drugs. Prevalence of these toxic metals in Phytopharmaceuticals not only can produced deleterious effects on the respiratory tract but also effect other body organs and systems. In spite of being useful in different RTIs (productive and nonproductive cough, cold, flue, asthma, allergy, bronchitis, nasal irritation, congestive chest diseases, and other related disorders), these Unani medicines pose severe health risks because of increased load of these non essential metals to the body, leading to more complicated situations. However metal hazards related to the use of these HMPs can be eradicated by minimizing the metal contamination to these medicines following the good agricultural practices, good keeping practices, and current good manufacturing practices (CGMP) by herbal industries. Raw materials, excipients, and packaging components should also be checked for their heavy metal contents prior to be used in the formulation. Awareness

should be made to the general public, local healers, and the physicians about the toxicities of metals to the human health when treating the patients of RTIs.

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