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Monitoring of inorganic impurities in dietary supplements used in Indian system of medicine

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Abstract:

Forty six samples of widely used dietary supplements were collected and analyzed by atomic absorption spectrometry in order to estimate contamination with Pb, Cd, Cu, Co, Hg, Cr, Ni and Zn due to their potential toxicity if present above the Maximum Allowable Levels (MAL). Many of the analyzed formulations had metal levels above the maximum allowable limits (Pb-21; Hg-6; Cr-3; Ni-2 and, Cu, Zn and Mn-1). The cumulative daily intakes (CDI) of metals from dietary supplements were estimated based on the recommended daily doses (RDD) of dietary supplements to find out harmfulness to human health. The estimated cumulative daily intakes of several metals from some dietary supplements were higher than the oral permitted daily exposures set by the USP advisory panel on metal impurities (Pb:17 samples; Zn-1 sample; Hg-4 samples). Such formulations present a significant additional source of metals in the human diet, and therefore could be harmful to human health**.**

Keywords: Heavy metals, dietary supplements, Indian system of medicine, FAAS

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1. Introduction

A dietary supplement is defined as a product that intends to supplement the diet, which contains vitamins, minerals, herbs, or other botanicals, amino acids, or any combination of the above ingredients. The human use of these dietary ingredients intends to increase the total daily intake [1,2], therefore it is essential to ensure the quality of dietary supplements and detect the presence of contaminants. Monitoring and controlling of impurities generally gives assurance of the quality and safety of a drug. Thus the analytical activities concerning impurities in drugs are among the most important issues in modern pharmaceutical analysis [3]. The inorganic impurities generally originate from various sources and phases, i.e., raw materials, reagents, solvents, electrodes, catalysts, reaction vessels, plumbing and other equipments used during the synthesis of pharmaceuticals. These are characteristic of the synthetic route of a manufacturing process. lead (Pb), cadmium (Cd), and mercury (Hg) are potential for toxicity and pose the risk of serious health hazards even at very low toxic at lower concentration [4,5]. Pb is known to induce renal tumors, reduce cognitive development, and increase blood pressure and cardiovascular disease in adults [6-13]. Cd may induce kidney dysfunction, osteomalacia and reproductive deficiencies[14-20]. Hg may cause neurological disorders and has toxic effect on the kidney [21-30]. The permissible levels of heavy metals in pharmaceuticals are usually defined by the regulatory agencies and controlled by limit tests. These tests ensure that no inorganic-based contaminants are introduced into the drugs at any of the steps during the manufacturing process. The United States Pharmacopoeia (USP), British Pharmacopoeia (BP), European Pharmacopoeia (EP) and Japanese Pharmacopoeia (JP) propose collective monitoring of total metal content in pharmaceutical products. The detrimental effects of some of the heavy metals usually found in medicinal products and the guidelines on specification limits for residues have been described by Europan Medicines Agency (EMEA)[31].

2. Literature survey

Several attempts were made on estimation of heavy metal contamination of herbal drugs and pharmaceutical products. E.*D. Caldas et al.*, reported the presence of undesired metals in some of the medicinal herbs in Brazil [32]. Cadmium, mercury and lead were primary concern due to their toxicity and potential contaminants in medicinal herbs. AAS was used to analyze the products for Cd, Hg, and Pb. Plant material were digested with 10% HNO_3 v/v,

and then analyzed with FAAS. For Hg analysis, the digested solution was analyzed by Cold Vapour AAS after reduction with $NABH_4$. The limits of quantification (LOQ) of the method were 0.01mg/g for Hg, 2mg/g for Pb and 0.2mg/g for Cd**.** The DCA (Drug Control Authority), [33] Malaysia has implemented the phase three registration of traditional medicines on 1 January 1992. As such, a total of 100 products in various pharmaceutical dosage forms of a herbal preparation found in Malaysia, containing tongkat Ali hitam, either single or combined preparations, were analyzed for the presence of a heavy toxic metal, mercury, using Cold–vapor atomic absorption spectrophotometer(CVAAS). CVAAS is the predominant technique for mercury analysis because of its high selectivity and sensitivity. This improved sensitivity is achieved through 100% sampling since all the mercury in the sample solution placed in the reaction flask in chemically atomized and transported to the sample cell for measurement. The samples were digested with aqua regia**.** H.H. Ang *et al.,* [34] reported the Pb concentration in various pharmaceutical dosage forms of a herbal preparation, containing *Eugenia dyeriana*, either single or combined preparations (more than one medicinal plant), were analyzed for the presence of Pb contamination, using GBC 906 AA model AAS. Samples bought from different commercial sources in Malaysia, containing *E.dyeriana* were digested using aquaregia. Results showed that 22% of the above products failed to comply with the quality requirement for traditional medicines in Malaysia. Although this study showed that 78% of the products fully complied with the quality requirement for traditional medicines in Malaysia pertaining to Pb, however, they cannot be assumed safe from lead contamination because of batch-to-batch inconsistency. The determination of trace elements in *Hypericum perforatum* leaves and flowers, their teas, tinctures and tablets was carried out by Electro thermal Atomic Absorption Spectrometry (ETAAS) and Ultrasonic Nebulization System coupled to Inductively Coupled Plasma Optical Emission Spectrometry (USN-ICP-OES)[35]. The measurements were performed with a Shimadzu Model AA6800 atomic absorption spectrometer (Tokyo-Japan) equipped with a deuterium background corrector and the measurements were based on peak height. Metals hollow-cathode lamps (Hamamatsu Photonics K.K., Japan) were employed as radiation source. A digestion method with an acid mixture (including HNO_3, $HClO_4$ and HF) was used to destroy the organic material. The tolerance limits for Ni^{+2}, Cu^{+2} and Pb^{+2} were 1 mg, 100mg and 100mg, respectively. A number of traditional South African herbal remedies which are associated with morbidity and mortality were analyzed for Se, Mg, Cu, Pb, Hg concentrations using Varain SpectrAA-640 GFAAS equipped with Zeeman background correction [36]. The precision of the GFAAS technique was determined using internal quality control specimens which were analyzed with each batch. With in -batch precision data were as follows- Pb:2.13% at a level of 0.54 µmol/l: Mn 1.92% at a level of 0.163 µmol/l: Se 3.35% at a level of 0.60µmol/l: Zn: 2.23% at 24.9 µmol/l; Cu 1.49% at 1.95µmol/l. between-batch precision for Cu was ±5.58% at 0.82µmol/l and for Zn ±4.24% at 27.9 µmol/l. E.M.M Flores *et al.,* (2002)[37] reported the concentration of Sb by batch hydride generation atomic absorption spectrometry(HG AAS) in commercial samples of inject able drugs, containing high concentrations of Sb(V). The procedure was based on the complexing effect for Sb of citric, oxalic and acetic acids as reaction media. Aquaregia was used for sample digestion prior to As determination by HG AAS. The following experimental conditions for the determination of total As, as As(V), were evaluated: the acid medium and its concentration, sodium tetra hydro borate concentration, purge time, and influence of the different oxidation states of As. The effect of the delay time after mixing of sample and acid solution was also studied. Optimized conditions were: 10 %(myv) citric acid, 1.5%(my v) sodium tetra hydro borate solution and 30 s for purge time. A delay time of 1 h was required after the digested sample had been mixed with citric acid, before As determination could be carried out. No interference on As(III) and As(V) signals was observed in the presence of up to 1 mg Sb(V). Recovery tests for As(III) and As(V) resulted in values between 97 and 101%. Characteristic mass and detection limit (3s), using the recommended conditions, were 0.52 and 0.8 ng, respectively, for total As. Jan Malik *et al.,* [38] determined the accumulated Al, B, Cu, Fe, Mn, P and Zn (using ICP-OES) and Ca, K and Mg (using AAS) in both the raw material and infusions from traditional plant stimulants (tea and coffee) and mate, rooibos, honey bush and chamomile. The results were discussed with respect to differences to the beverage quality and their role in the human diet. The levels of elements not significantly differ between tea types (black, green, oolong, white), and between Arabica and Robusta coffee. In comparison with tea, coffee was found to be a poor source of elements with the exception of Ca and Fe. High levels of B, Ca, Cu, Mn, Mg and Zn were found in mate (mainly green type) and of B, Ca, Cu, Fe and P in chamomile, whereas the amounts of all elements in rooibos and honey bush infusions were low

(except of Ca). Apart from tea, other stimulants appeared to not represent important sources of potentially harmful amounts of Al for the human diet. Leticia Garcıa-Rico *et a.,l*[3] investigated the presence of Cu, Zn, Cd, Pb, and Hg in 24 dietary supplements purchased in different health stores across the city of Hermosillo, located in the northwest of Mexico. Analysis of metals was done by microwave digestion and atomic absorption spectroscopy. The most abundant elements in dietary supplements were Cu (<0.19–137.85 µg/g) and Zn (<2.83–4785.71 µg/g), followed by Pb (<0.003–66.32 µg/g), Cd (<0.001–2.90 µg/g), and Hg (<0.24–0.85 µg/g). The estimated daily intakes of metals were below those recommended by WHO and the Institute of Medicine, showing that little risk from heavy metals is associated with the consumption of the dietary supplements analyzed. However, some products presented more than 10% of the tolerable daily intake of Pb, indicating that production processes should be improved. Ba, Cd, Cr, Cu, Fe, Ni, Pb and Zn were determined in birch leaves (Folium Betulae), dandelion roots (Radix Taraxacae), hawthorn blossom (Inflorescentia Crataegi) and their infusions by GFAAS after microwave digestion of plant samples. Infusions were made from herbs according to prescription for patients, provided by the producer of medicine on the package [39]. The results obtained were compared with daily requirements for each element. Results show high content of cadmium in the medicinal plants analyzed. The highest level in infusions was observed for Ni and Zn (over 90% of the total element concentration for Ni and in most cases over 50% for Zn), and the lowest for Cd and Pb. Seven herbal drugs and corresponding rhizosphere soil samples from the plantation near Belgrade were analyzed for mineral content. Eleven metals (Cu, Zn, Mn, Fe, Cr, Ni, Pb, Cd, Ca, K and Mg) were selected as chemical features/descriptors and analyzed by FAAS/FAES and ETAAS [40]. In a chemometrics evaluation of investigated plants–soil system, univariate, as well as multivariate statistics methods were applied. Principal component analysis (PCA) allowed considerable reduction in a number of variables and the detection of structure in the relationships between metals that give complementary information about the relations and elemental patterns within plants–soil system. The power of hemometrics was also used in exploring the potential natural and/or anthropogenic sources responsible for metal content in both medicinal plants and soil samples. Hierarchical cluster analysis (HCA) was used to explore herbal drugs grouping according to corresponding soil samples as additionalinformation to the output obtained by PCA. Thirty samples of widely used vitamins and herbal preparations distributed on the Croatian market were analyzed by AAS in order to estimate contamination with Pb, Cd, As, Hg, Cr, Ni and Zn due to their potential toxicity if present above the maximum allowable levels (MAL) [41]. The following concentration ranges were obtained (in µg/g): Pb 0.25–3.86; Cd 0.05–0.28; As 0.10–0.19; Hg 0.02–0.12; Cr 0.11–64.38; Ni 0.24–338.90; and Zn 1.00–95.3. Several analyzed formulations had metal levels above the maximum allowable limits. The estimated cumulative daily intakes of several metals from some dietary supplements were higher than the oral permitted daily exposures set by the USP Advisory Panel on metal impurities.

3. Aims and objectives

The objective of this study was to evaluate metal contamination levels in dietary supplements available in Indian market, manufactured by both domestic and foreign manufacturers. The levels of potentially toxic heavy metals Cd, Pb, Ni, Co, Mn, Cu, Cr, Zn and Hg were determined and compared with maximum allowable levels (MAL) [42]. Since metal bioaccumulation could cause toxic effects and ultimately result in health disorders or diseases if contaminated dietary supplements are used on a long-term basis, a further objective of the present study was to estimate metal cumulative daily intakes (CDI) from analyzed dietary supplements. These were compared with the oral permitted daily exposures (PDE) for dosage forms, as stated by the USP Advisory Panel on Metal Impurities [42], to deduce whether the analyzed products could present a potential threat to human health.

2. Experimental

2.1 Apparatus

Metal concentrations were determined by Atomic absorption spectrophotometer (Perkin Elmer Aanalyst 300, USA), Hollow cathode lamp was used for detection of Pb, Cd, Cu, Ni, Fe, Co, Cr, Mn and Zn. The instrument was calibrated with standard solutions using the concentration mode and, instrument conditions and limits of quantification (LOQ) for each metal were given in the Table.5.1. LOQs were based on the following criterion: standard deviations of 20 consecutive metal measurements in the blank sample were multiplied by 10, with subsequent addition of the metal concentration measured in the blank sample. Calibration curves were plotted using Pb, Cd, As, Hg, Cr, Ni and Zn stock standard solutions adequately diluted with Milli-Q water, prepared on the day of analysis. Measured metal concentrations in the digestion solutions were expressed as µg/ml. The final results (µg/g) were obtained by multiplying the measured metal concentrations

(µg/ml) with the dilution volume (mL), and dividing it with the sample weights (g). The standard reference materials of all the metals (E. Merck, Germany) were used to provide calibration and quality assurance for each analytical batch. Replicate (n = 3) analyses were conducted to assess precision of the analytical techniques.

2.2 Reagents

All reagents were of analytical grade. Sub-boiled water and conc. HNO_3 (69%) (Merk, India) were used in the preparation of samples. Stock standard solutions of Zn, Cu, Fe, Mn, Pb, Ni, Cr, Co, Cd and Hg containing 1000 ppm of each metal were prepared. Calibration standards of each element were obtained by appropriate dilution of the stock solutions. All drug samples were procured from market outlets.

2.3 Sample Preparation

The names of the drugs under investigation are given in the Table.5.2. To estimate the metals in the different types of drug samples, 1.0 g powdered drug was taken in 100 ml beaker, 5 ml conc. HNO_3 were added and kept overnight (16 h). The solution was digested on a hotplate at 80°C for 10 min and allowed to cool at room temperature. 20 ml of sub-boiled distilled water was added to the solution and filtered through Whatman filter paper No.42 into a standard flask. The final volume was made up to 100 ml with sub-boiled distilled water. Necessary precautions were adopted to avoid possible contamination of the samples.

Table.5.1. Wavelengths (nm) and limits of quantification (LOQ) for analyzed metals in dietary supplements. LOQs are expressed as µg of metal per gram of dietary supplement, taking into consideration the average sample weight (0.987±0.089) and sample dilution in the process of preparation.

Metal	Wavelength	Slit width (mm)	Air flow Liter. min^{-1}	Acetylene flow Liter. min^{-1}	Relative Noise	Read Time	Read Delay	Lamp current	LOQ
Cd	228.9	0.7	10	1	1.0	2	1	4	0.019
Cr	357.9	0.7	10	3	1.0	2	1	25	0.043
Hg	253.7	0.7	10	3	1.0	5	1	6	0.35
Ni	232	0.2	10	3	1.0	3	1	25	0.096
Pb	217.0	0.7	10	3	1.0	2	1	10	0.085
Cu	324.7	0.7	10	1	1.0	2	1	15	0.019
Fe	248.8	0.2	10	3	1.0	2	1	30	0.029
Co	240.7	0.2	10	1	1.0	3	1	30	0.053
Zn	213.9	0.7	12	2	1.0	2	1	15	0.012
Mn	279 .5	0.2	10	3	1.0	5	1	20	0.015

Table. 5.2. Classification, dosage forms and active ingredients of analyzed dietary supplements.

Classification	Product name	Sample No	Dosage form	Active Ingredients
Medicinal herb-based Products	Mruthyunjaya Ras	1	Pills	Sudha parab, gandhak, vassanabh, tankan, manshila, marich
	Habb-e Mubarak	2		
	Bryonia –200	3	Pills	Not given
	Shwaskuthar Ras	4	Pills	Sudha parab, gandhak, vassanabh, tankan, manshila, marich
	Qurs-e Nazla	5	*Tablet*	*pudina kushk, anardana, boora armani, aab leemun, sat pudina,*
	Habe-Surfa	6	*Tablet*	*Not given*
	Allium Cepa-200	7	*Pills*	*Not given*
	Habb-e-Qula	8	*Tablet*	*Not given*
	Jawarish Anarain	9	*Suspension*	*Not given*
	Jawarish-e-Kafoor Qawi	10	*Suspension*	*curcuma lenga, withania sominifera,*
	Sualin	11	Tablet	Rub-e-mulethi, rub-e-bansa, rub-e-tulsi, rogan badyan, rogan darchini
	Kasamrita	12	Syrup	Somlatha, kantakari, vasak mool, mulethi, pudina satva
	Adulsa	13	Syrup	Adhathoda vasica, glycyrrihiza glabra, ocimum sanctum, madhurica, sunthi
	Honitus	14	Syrup	Tulsi, mulethi, banaphsa, kantkari, pudina, shudhu madhu
	Himalaya pure herbs	15		
	Jawarish pudina wilaiti	16	Suspension	Agar hindhi, ilsichi kalan, berg suddab,

				pudina kushk, anardana, boora armani, aab leemun, sat pudina, maweez munaqqa
	Paenwin	17	Tablet	Boswellia serrata, curcuma lenga, withania sominifera, pluchea lanceolata
	Habb- E-Suranjan	18	Tablet	Elwa , thkhm saya, tukhm safaid, hubbel need, surnjan shirin, mastagi, roomi
	Fit	19	Capsules	Triphala, sankha bhasma, zinziber officinale
Allopathic drugs	Paracetamol	20	Tablet	
	Citrazine hydrochloride	21	Tablet	Citrazine hydrochloride
Vitamin supplements	Becosules Capsules	22	Capsules	vitamin B_1, B_2 , B_6, B_{12} and c, niacinamide ,calcium pantothenate , folic acid,
	Zincovit	23	Tablet	Niacinamide,Thiamine mononitrate Riboflavine , Calcium Pantothenate Pyridoxine Hydrochloride , Folic aicd Vitamin A ,D_3
	Cobadex Forte	24	Capsules	Calcium pantothenate, folic acid , nicotinamide , vitamin B1, B12, B2 , B6 ,C
	B complex forte	25		
	Becozyme C forte	26		Vitamin B, Vitamin C
	Formic –O	27	Tablet	Cifixine
	Formic-Injection	28	Injection	Ceferiaxone, sulbactum
	Augpod	29	Tablet	Cefrodoxine + calvunanic acid
	Tazolusti	30	Injection	Piceracillin + trazobactin
	Webcef	31	Tablet	Cifroxine axetel
	Webcef-inj	32	Injection	Cifroxine sodium
	Kefbactum	33	Injection	Cefoperazone + salbactum
	Tobranag	34	Injection	Tobramicin
	Pepamino	35	Tablet	Enzymatic protein hydrolyphase
	Tantum	36	Solution	Benzydamine hcl
	Tantum gel	37	Gel	Benzydamine hcl
	Repel	38	Tablet	Rabeprazole
	Repel-D	39	Capsule	Rabeprazole + domperidine
	Enzar forte	40	Tablet	Pancreatin + sodium taro glycolate
	Enzar liquid	41	Syrup	Fungal diastage + pepsin
	Hybor	42	Inject ion	Bemiparin sodium
	Baxativ	43	Tablet	Bemiparin sadium
	Chymoral plus	44	Tablet	Diclopinac + trypsine + chymotrypsin
	Chymoral forte	45	Tablet	Trypsine + chymotrypsin
	Flextra-D	46	Tablet	Glucosamine sulphate, diacerein

3. Results and discussion

The obtained levels of metals found in the analyzed samples, expressed as one gram of analyte per gram of dietary supplement, are summarized in Table.5.3. It may be stressed that the obtained results were compared to the MALs proposed by the experts in the USP Advisory Panel on Inorganic Impurities and Heavy Metals, since their Stimuli article (Pharmacopeial Forum, 2008) including the revised document ‘‘Draft Metals and Limits [42]. Provided all the necessary data (Table.5.3.) The low levels of Cd, Fe and Co found in the analyzed formulations, none above the MALs, were not similar to those reported by Maria R. Gomez *et al.*, [12] in Argentinian herbal medicines and the exposure to these contaminants through the tested dietary supplements is not expected to affect human health. Iron salts have an astringent action resulting in irritation of the gastrointestinal mucosa which gives rise to gastric discomfort, nausea, vomiting and diarrhoea or constipation [20]. With large oral doses of iron, the astringent action of iron salts damages the mucosal cells. Severe damage causes bleeding in the stomach or haematemesis. Necrosis of mucosal cells may also lead to perforation of the gut wall [43]. For Cd, the levels found in the present study were similar than those reported in other dietary supplements (Leticia Garcıa-Rico *et al.*, [44] and in medicinal[7], but higher than those reported by tumir *et al.*, [41]Khan *et al.*, and Dolan *et al.*, [2]. As

indicated previously these differences may due by the kind and number of ingredients presented in the study dietary supplements. For Cd almost 50% of the samples are below the LOQ limits and none of the sample is above MAL. The manifestations of cadmium nephrotoxicity, aminoaciduria, glycosuria and tubular necrosis have been detected at renal Cd concentration of less than 50µg/g tissue. The effect of cadmium on the kidney takes the form of renal tubular dysfunction and subsequent pathological changes.

Six samples (sample nos.1, 2, 5,6,10 and 11; Table.5.3) had Hg levels above the MAL and all of these were medicinal herb-based Ayurvedic and Unani Products with Hg as the active substance. The toxicity of the Hg depends on the oxidation state of metal present in the formulations. Compared to the values described in the literature for Hg, the levels found in this study were mostly lower or similar than those reported in other dietary supplements [45, 46,2], but higher than those values (0.0026–0.010lg/g) reported by Bin *et al.*. (2001)[47] in Chinese medicinal herbs.Furthermore, one analyzed product was found to be contaminated by Zn, Cu and Mn above the MAL. It is Dietary supplements with Zn, Mn and Cu as the active substance, and therefore could not be considered contaminated. On the contrary, a recent Nigerian study on dietary supplements reported higher Zn, Cu contamination levels [48].

Although Cr is an essential element, it is well known that it can also be toxic, depending on the level of contamination and its valent form [49-51]. This study indicates that three formulations had a Cr level above the MAL (sample no's 3, 21, 23 were having (128%), (350%) and (480%), respectively. Table.5.3). It can be hypothesized that the possible source of contamination of the tested formulations by Ni and Cr was the inappropriate maintenance of the production machines. Stainless steel used in food processing equipment may leach certain quantities of Ni, Cr and other metals, where the extent of the metal migration depends on various factors, such us pH, contact time, etc. [52]. However, this hypothesis should be further investigated.

Table.5.3. Metal levels measured in 46 samples of dietary supplements, expressed the range. Number of samples with metal levels below the level of quantification (<LOQ), and above the maximum allowable level (>MAL) and permitted daily exposure for dosage forms (>PDE) were given. The cumulative daily intakes (CDI) of metals (expressed as medians, with minimum and maximum values given in brackets) based on measured metal levels and the maximum recommended daily dose of dietary supplements.

Metal name	<[e]LOQ	Metal level(µg/g)	MAL(µg/g)	>[f]MAL	[a]CDI (µg/day)	[b]PDE (µg/g)	>[c] PDE
Cd	24	1.05-1.38	2.5	0	1.02(0.87-5.7)	25	0
Cr	20	0.36- 118.8	25.0	3	16.8(0.26-89.7)	250	0
Hg	27	2.4- 9752	1.5	6	1.7(0.56-6769)	15	4
Ni	8	0.5- 36.9	25.0	2	5.9(0.8-231)	250	0
Pb	6	4.1-185.2	1.0	21	12.9(0.6-43.7)	10	17
Cu	4	0.21-763.0	50	[g] 1	18.5(1.1-31.2)	2500	0
Fe	0	7.25-719.4	1500	0	23.5(3.8-213.4)	15,000	0
Co	22	0.44-5.4	100	0	3.2(0.7-89.0)	1000	0
Zn	8	0.1-15219.9	1500	[g] 1	7.9(0.6-753.4)	15000	[d]1
Mn	9	0.85-1275.8	700	[g] 1	6.5(1.1-702.9)	7000	0

- *[a]CDI was calculated for samples having metal level above the LOQ.*
- *[b]PDE for a person weighing 50 kg.*
- *[c]Number of samples for which ingestion would lead to CDI of analyzed metals higher than proposed PDE.*
- *[d]dietary supplement with estimated CDI of Zn higher than PDE contained Zn as the active substance.*
- *[e]Number of analyzed samples with metal level below the level of quantification.*
- *[f]Number of analyzed samples with metal level above the maximum allowable level.*
- *[g]A food supplements with Zn, Mn and Cu concentrations above MAL contained Zn, Mn and Cu as the active substance and therefore could not be considered contaminated.*

Compared to the values described in the literature for Pb, the levels found in this study were higher than those observed in *Eugenia dyeriana* herbal preparations [34] and lesser than E. Obi *et al.,* [48] in Nigerian herbal remedies and Hight *et al.,* [53], Au *et al.,* [45] and Dolan *et al.,* [2] in other dietary supplements and, the medicinal plants [7, 54]. In a similar study conducted by Garvey *et al.,* [55] with Asian herbal remedies, 60% of the medicaments tested gave a daily dose of lead in excess of 300 µg/day, whereas in this study, 40% of the remedies tested gave a daily dose of lead in excess of 10 µg/day. Compared to the values described in the literature for Pb and Zn, the levels found in this study were higher than those reported by tumir *et al.,* [41] in other Croatian dietary supplements, but higher than those observed in medicinal plants [7]. This difference may due by the number and kind of ingredients in dietary supplements. Naithani and Kakkar *et al.,*[56] reported lower levels of metals in herbal teas with fewer ingredients than in polyherbal teas.

For Ni, the levels found in the present study were lesser than those reported by tumir *et al.,* [41] and higher than those in Nigerian herbal remedies reported by E. Obi *et al.,* [48]. 18% of the sample were having the below the LOQ limits and 2 sample were above the MAL levels. Nickel is a widely used heavy metal, which exert a potent toxic effect on peripheral tissues as well as on the reproductive system [57]. The primary toxic effects of nickel sulfate are expressed in the myeloid system .Nickel causes dose-related decreases in bone marrow cellularity and in granulocyte macrophage and pluripotent stem cell proliferative responses.

In order to conclude whether the metal levels measured in this study could be considered harmful to human health, the cumulative daily intakes (CDI) of metals from dietary supplements, expressed as one gram per day, were estimated based on the recommended daily doses (RDD) of dietary supplements. The CDIs of each metal tested in this study were then compared with the oral permitted daily exposures for dosage forms (PDEs; Table.5.3.), as recommended by USP [42]. Based on the results of the study, the CDI of Hg for four samples (sample nos 1, 2, 5 and 6) were having 1000%, 30000%, 300%, 45126%, respectively. The CDI of Pb calculated for seventeen tested sample were in the range of 101%-18500% of the allowed PDE for Pb (Table.5.3.). The CDIs of other products included in the study were lower than permitted and may not be considered harmful.

4. Conclusions

The detection of heavy metal content was highly relevant for assessment of drug safety and quality. This study has provided a status of heavy metal concentration in various drugs readily available in the Indian market. There should be periodical assessment of heavy metal concentration in all drugs, in order to have quality assurance and safer use of drug products.

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