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Determination of heavy metals contents in individual Ayurvedic raw materials and a comparison with a same type of homeopathic products

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Abstract

It is very essential to know the heavy metal content in each every herbal or ayurvedic product used for human treatment or day to day uses, as constituent of water and soil is continuously getting changed due to pollution. Therefore it is essential to know the status of these heavy metal using modern instrumental techniques. Essential and non-essential heavy metals like Lead (Pb), Cadmium (Cd), Cobalt (Co), Mercury (Hg) and Zinc (Zn) were quantified in selected 13 medicinal plant parts by using Atomic Absorption Spectrophotometer and Mercury Analyzer. The main purpose of this study was to document evidence of essential and non-essential heavy metals in these medicinal plant parts, which are extensively used in the preparation of herbal products, and by the common people of our country in their day to day purpose and in standardized extracts and homeopathic tinctures. From the experiment it was found that the heavy metal As was far below the detectable limit of the instrument for all the samples of raw ayurvedic herbs. It was found that the samples were contaminated with Lead, Cadmium, Cobalt, and Mercury but all were within the permissible limits of WHO/FDA except in Methi fruit Cd was found in much higher concentration i.e. 9.25 mg/kg and therefore the use should be avoided. Homeopathic tinctures of the same herbs were also collected from company A and quantified for heavy metals. In this the limit of As, Hg, Cd and Pb were within the limit and in most cases it was far below the detectable limit of the instrument.

Keywords : Heavy metals, herbs, homeopathic tinctures.

1. Introduction

A WHO report showed that about 70 – 80% of world population relies on non conventional medicine which predominantly of plant sources in their primary health care. (1) Heavy metal ranks highest among the chief contaminants of harmless herbs and herbal remedies. Herbs and herbal remedies are known to take up metals by absorbing them from contaminated soils as

well as from deposits on different parts of the herbs and herbal remedies exposed to air from polluted environments. (2) It's 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 heavy metals which pose a great health risks to all living organisms upon long term exposures. (3) The term "Heavy Metals" defined as commonly held for those metals, which have specific weights more than $5g/cm^3$. (4) Heavy metals are kept under environmental pollutant category due to their toxic effects in plants, human and food. Heavy metals pose a number of hazards to humans. They are potent carcinogenic and mutagenic agents and they are known to be "cumulative poisons". Heavy metals are classified into four major groups,

I) Essential: Copper (Cu), Zinc (Zn), Cobalt (Co), Chromium (Cr), Manganese (Mn), and Iron (Fe).

II) Non-essential: Barium(Ba), Aluminium (Al), Lithium (Li) and Zirconium (Zr).

III) Less toxic: Tin (Sn) and Aluminium (Al).

IV) Highly toxic: Mercury (Hg), Cadmium (Cd) and Lead (Pb). (5)

The raw materials of herbal plants are always tested for the maximum limits of heavy metals as specified by WHO (World Health Organization). Taking this into consideration, thirteen raw Ayurvedic herbs were obtained from the polished market, namely Tulsi (a sample of leaves of a herb *Ocimum sanctum*), Thankuni (a sample of leaves of a herb *Centella asiatica)*, Vasaka (a sample of leaves of a herb *Adatodha vasica)*, Ashwagandha (a sample of a root of a herb *Withania somnifera*), Sarpagandha (a sample of a root of a herb *Rauwolfia serpentine)*, Satavari (a sample of root of a herb *Asparagus racemosus)*, Amala (a sample of a fruit of a herb *Emblica officinalis)*, Bael (a sample of a fruit of a herb *Aegle marmelos)*, Methi (a sample of a fruit of a herb *Trigonella foenumgraceum)*, Neem (a sample of a bark of a herb *Azadirachta indica*), Asoka (a sample of bark of a herb *Saraca asoca)*, Turmeric (a sample of rhizome of a herb *Curcuma longa)*, Ginger (a sample of rhizome of a herb *Picrorrhiza curroa)* was analyzed by using atomic absorption spectrophotometer for six heavy metals arsenic (As), cadmium (Cd), cobalt (Co), lead (Pb), mercury (Hg) and zinc (Zn) to generate scientific preliminary data on their toxicity and are compared with a sample of the same type of homeopathic tincture products. The results will help to create awareness among the public health regarding its safe use at collection areas and their adverse health effect.

2.Material and Method

The experimental material consisted of 13 raw ayurvedic herbs: Tulsi (*Ocimum sanctum*), Brahmi (*Centella asiatica*), Vasak (*Adatodha vasica*), Sarpagandha (*Rauwolfia serpentine*), Ashwagandha (*Winthania somnifera*), Satavari (*Asparagus racemosus*), Amala (*Emblica officinalis*), Bael (*Aegle marmelos*), Methi (*Trigonella foenum-graecum*), Neem (*Azadirachta indica*), Asoka (*Saraca indica*), Turmeric (*Curcuma longa*) and Ginger (*Zingiber officinale*). Samples were purchased directly from polished market of Kolkata, West Bengal, India. Homeopathic tinctures of the same herbs were also collected from company A from Kolkata and quantified for heavy metals.

The content of heavy metals was determined according to the procedure advised by the Indian Pharmacopoeia. Samples were weighed (5-10 g) in quartz crucibles, dried at 105°C for 24 hours and subsequently burned in the muffle furnace at 450°C. Ash was dissolved in a mixture of 5ml conc. nitric acid (AR, ISO, Merck) and 1 ml conc. sulphuric acid and the solution was filtered through Whatman filter paper No. 1 and volume was made up to 50 ml with distilled water free of heavy metals. The above solution was used to measure the content of heavy metals of Pb, Cd, Co and Zn by using the instrument Atomic absorption spectroscopy AA140 (VARIAN) at 283.3 nm, 228.5 nm, 240.7 nm and 213.9 nm wavelength respectively. For estimation of As heavy metal by using Atomic absorption spectroscopy, vapor generation method was used. To the above solution 20% potassium iodide was added and the content of As was measured at 193.7 nm wavelength. For estimation of Hg by using the instrument Mercury Analyzer MA 5840. It is a cold vapor atomic absorption spectroscopic method. To the digested solution potassium permanganate and conc. Hydrocholric acid was added and boiled, cooled and filtered. To this solution 20% stannous chloride in HCl was added and the content of Hg was measured at 253.7 nm wavelength.

The accuracy and precision of the method was assured by simultaneous analysis of the reference material. A calibration graph was performed for each of the heavy metal determined (Pb, Cd, Co, Hg, As and Zn). The prepared concentration of heavy metal standards used for the plot varied between 0.00 ppm to 10.0 ppm depending on the absorbance of the metal. The coefficient of correlation (r) for standard calibration graph for the heavy metals ranged between 0.998 to 0.999.

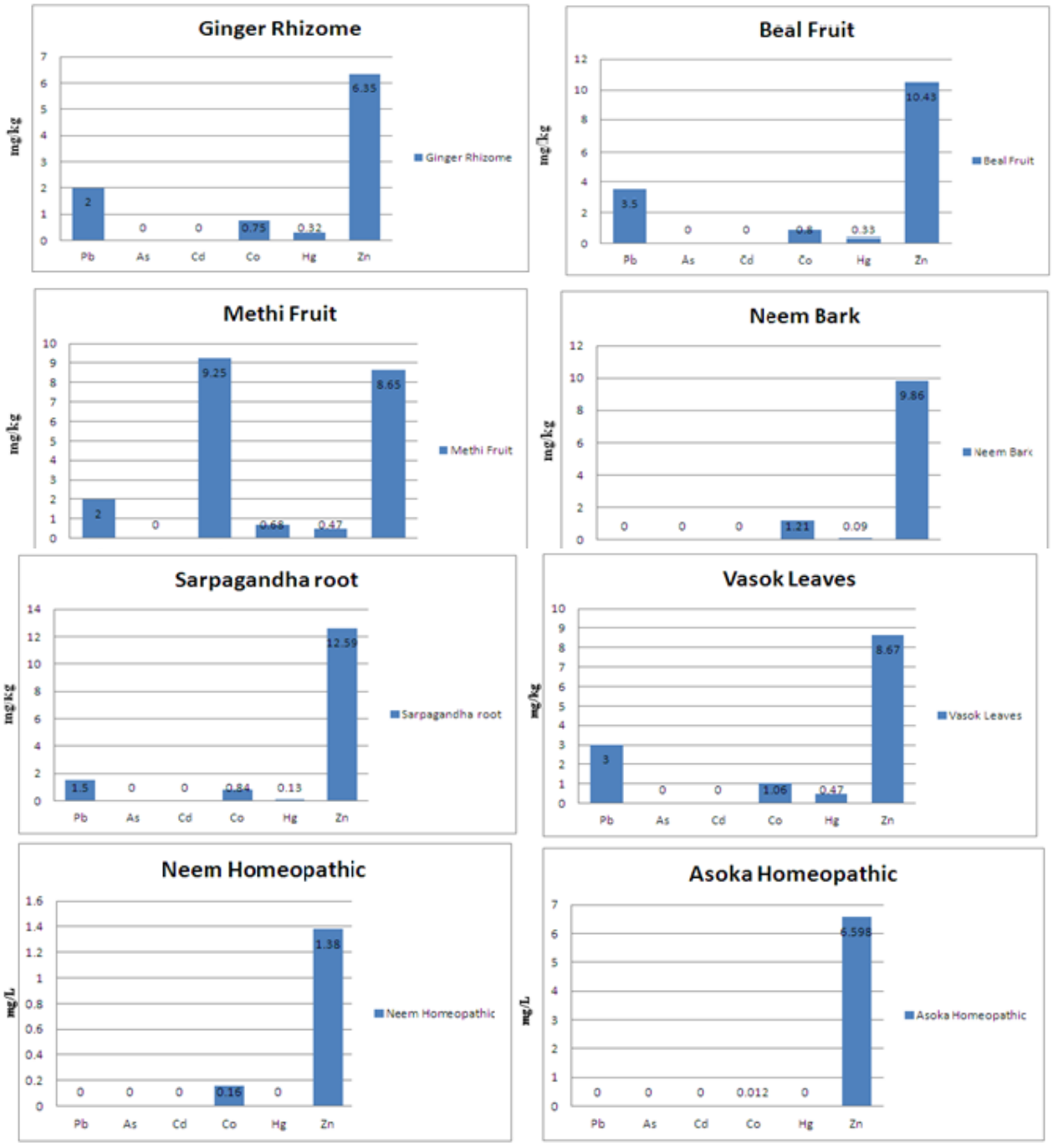

Fig 1. Graphs for heavy metal content in Raw herbs and Homeopathic tincture by AAS.

3.Results and Discussion

The Government of India, Department of Ayush, Ministry of Health and Family Welfare has issued new safety standards for the ayurvedic drugs. The permissible limits of the heavy metals

in ayurvedic drugs with herbal ingredients as per WHO (World Health Organization) and FDA (Federal Drug Administration) are shown in the Table No. 1

Table No. 1 Heavy metal permissible limit

Heavy Metal	Maximum Permissible Limit
Arsenic (As)	10 mg/kg
Cadmium (Cd)	0.30 mg/kg
Lead (Pb)	1.0 mg/kg
Cobalt (Co)	Not specified
Mercury (Hg)	1.0 mg/kg
Zinc (Zn)	Not specified

The above heavy metals are essential for performing several biological functions in human body. But when these metals are present in high concentrations in blood stream, then they can accumulate in vital organs (like liver, kidney heart etc.) and can cause various toxic effects (such as high BP, kidney damage, blindness, mental retardness).

The concentration of selected heavy metals in thirteen (13) different medicinal plant parts were measured. All the results are summarized in Table No. 2 and 3 below. The results obtained were compared with the permissible limit for each medicinal plant part, whichever is available as per WHO and FDA. Arsenic concentration was below the detectable level (BDL) of the instrument in all the samples.

Table No. 2. Mean concentration of heavy metals (Pb, Cd, Co, As, Hg and Zn) of thirteen different raw medicinal plant parts are as follows:

Serial No.	Name of Medicinal plant part	Lead (Pb) in mg/kg	Arsenic (As) in mg/kg	Cadmium (Cd) in mg/kg	Cobalt (Co) in mg/kg	Mercury (Hg) in mg/kg	Zinc (Zn) in mg/kg
1.	Bael fruit (Aegele marmelos)	3.5	BDL	BDL	0.8	0.33	10.43
2.	Methi fruit (Trigonella	2	BDL	9.25	0.68	0.47	8.65
3.	Amla fruit (Emblica officinalis)	BDL	BDL	BDL	0.62	0.25	3.94
4.	Ginger rhizhome (Zingiber officinale)	2	BDL	BDL	0.75	0.32	6.35

5.	Turmeric rhizome (Curcuma longa)	2	BDL	BDL	0.94	0.27	3.81
6.	Vasok leaves (Adatodha vasica)	3	BDL	BDL	1.06	0.47	8.67
7.	Brahmi leaves (Centella asiatica)	BDL	BDL	BDL	0.92	BDL	9.37
8.	Tulsi leaves (Ocimum sanctum)	BDL	BDL	BDL	0.77	0.15	8.62
9.	Satavari root (Asparagus racemosus)	1.5	BDL	BDL	0.80	0.11	11.50
10.	Sarpagandha root (Rauwolfia serpentine)	1.5	BDL	BDL	0.84	0.13	12.59
11.	Ashwagandha root (Winthania somnifera)	BDL	BDL	BDL	1.18	BDL	10.30
12.	Neem bark (Azadirachta indica)	BDL	BDL	BDL	1.21	0.09	7.86
13.	Asoka bark (Saraca indica)	BDL	BDL	BDL	1.08	0.15	4.87

BDL: Below detectable limit

Table No. 3. Mean concentration of heavy metals (Pb, Cd, Co, As, Hg and Zn) of ten different Homeopathic product (mother tincture) of same raw medicinal plant parts are as follows:

Serial No.	Name of Homeopathic mother tincture	Lead (Pb) in mg/L	Arsenic (As) in mg/L	Cadmium (Cd) in mg/L	Cobalt (Co) in mg/L	Mercury (Hg) in mg/L	Zinc (Zn) in mg/L
1.	Bael mother tincture	BDL	BDL	BDL	0.015	BDL	2.13
2.	Amla mother tincture	BDL	BDL	BDL	0.01	BDL	0.59
3.	Ginger mother tincture	BDL	BDL	BDL	0.009	BDL	4.79

4.	Vasok mother tincture	BDL	BDL	BDL	0.002	BDL	2.55
5.	Brahmi mother tincture	BDL	BDL	BDL	0.011	BDL	2.59
6.	Tulsi mother tincture	BDL	BDL	BDL	0.011	BDL	2.36
7.	Sarpagandha mother tincture	BDL	BDL	BDL	0.014	BDL	1.73
8.	Ashwagandha mother tincture	BDL	BDL	BDL	0.011	BDL	1.43
9.	Neem mothet tincture	BDL	BDL	BDL	0.016	BDL	1.38
10.	Asoka mother tincture	BDL	BDL	BDL	0.012	BDL	6.598

Lead: Lead is regarded as very hazardous for plants and humans. The sources of lead pollution of agricultural soils and plants are lead mines, fuel combustion, sewage sludge and farmyard manure. Among all the raw herb samples the high Lead concentration was found in Bael fruit i.e 3.5 mg/kg and it was below detectable level in Amla fruit, Brahmi leaves, Tulsi leaves, Ashwagandha root, Neem root and Asoka root. The concentration Lead in Homeopathic mother tincture product of company A for all the samples was found below the level of detection by the instrument.

Cadmium: Cadmium is a toxic metal having functions neither in human body nor in animals or plants. Once accumulated in the kidney then it stays there resulting in high blood pressure and kidney diseases and it is difficult to remove by excretion. Cadmium directly damages nerve cells. It inhibits the release of acetylcholine and activates cholinesterase enzyme, resulting in a tendency for hyperactivity of the nervous system. (7) The high Cadmium concentration was found in Methi fruit i.e 9.25 mg/kg and Cd concentration was below detectable label in the rest of the crude drugs. The concentrations of Cd in Homeopathic mother tincture products of company A for all the samples were found below the level of detection by the instrument.

Cobalt: Cobalt is necessary for normal growth of plants and animal. Although cobalt is toxic at elevated concentration however, the body needs only in trace amount. Cobalt in the form of vitamin B12 is in its physiologically active form. It is very essential to provide 3.0 µg (microgram) per day in the form of vitamin B12 for a diabetic individual (8). Among all the raw herbs, the high Cobalt concentration was found in Neem bark i.e 1.21 mg/kg. The concentration of Cobalt in homeopathic mother tincture products of company A was high in Neem i.e. 0.016 mg/L and lowest concentration in Vasak i.e 0.002 mg/L.

Arsenic: Chronic or lower levels of exposure can lead to progressive peripheral and central nervous changes, such as sensory changes, numbness and tingling, and muscle tenderness. The concentrations of Arsenic in all raw herb samples were below the detection level of the instrument. The concentration of Arsenic in homeopathic mother tincture product of company A for all the samples was found below the level of detection by the instrument.

Mercury: Chronic exposure to mercury may result in permanent damage to the central nervous system and kidneys. Mercury can also cross the placenta from the mother's body to the fetus (levels in the fetus are often double than those in the mother) and accumulate, resulting in mental retardation, brain damage, cerebral palsy, blindness, seizures, and inability to speak. Among all the raw herb samples, the high Cobalt concentration was found in Methi fruit and Vasak leaves i.e 0.47 mg/kg. The concentrations of Mercury in Homeopathic mother tincture product of company A, for all the samples, were found below the level of detection by the instrument.

Zinc: Zinc is a cofactor of over 200 enzymes involved in metabolic pathways but its high levels in human body can be toxic due to its interference with copper metabolism. Therefore, dietary intake of Zinc should be appropriate. Among all the raw herb samples, the high Cobalt concentration was found in Sarpagandha root i.e 12.59 mg/kg. The concentrations Zinc in Homeopathic mother tincture product of company A for the samples , were high in Asoka i.e. 6.59 mg/L and lowest concentration in Amla i.e 0.59 mg/L.

4. Conclusion

This study from a toxicological point of view showed that all the raw ayurvedic herbs are safe as the heavy metals determined are well below the critical limit except for Methi fruit for which

Cadmium concentration was very high i.e.9.25 mg/kg but as per WHO / FDA permissible limit is 0.3 mg/kg. So it is highly toxic and should be avoided for human consumption. Also for neem bark Cobalt was found to be high i.e 1.21 mg/kg, though WHO /FDA has not prescribed any limit. So care should be taken and must be checked for cobalt heavy metal before using as high concentration of cobalt can harm the human. Maximum concentration of Pb (3.5 mg/kg) was found in Bael fruit, Hg (0.47 mg/kg) in Methi fruit and Basak leaves. According to ATSDR (2001), the minimal risk levels for hazardous Lead, Cadmium and Cobalt through oral route having acute effect are 0.0002 mg/kg/day, 0.0002 mg/kg/day and 0.01 mg/kg/day respectively. Maximum concentration of Zn (12.59 mg/kg) was found in sarpagandha. Zinc is an essential trace element for normal growth, brain development, behavioural response, bone formation and wound healing. Zinc deficient diabetics fail to improve their power of perception. It also causes loss of sense of touch and smell. The dietary limit of Zn is 100 ppm. Concentration of Zn was found below the detection limit for all the ayurvedic samples.

In case of Homeopathic mother tincture samples of the same type raw ayurvedic herbs, the heavy metals Pb, Cd, Hg and As for all was below the detection limits. So in order to prevent the presence of heavy metals in medicinal herbs it is better to go for alcoholic extraction of the active constituent.

Hence, the implication of the findings may be taken into consideration whilst using the herbs for human consumption. The results suggest that medicinal plants used for human consumption or for preparation of herbal products and standardized extracts should be collected from an unpolluted natural habitat. Heavy metal contents vary with sources of herbs, so it is essential for every producer of herbal products who supply herbs must know the area wise heavy metal content of particular herbs. Hence it is an important area of research for supply of documented evidences.

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