Determination of heavy metal contents in herbal plant leaves collected from different locations of Chennai city

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Abstract

Herbal medicines mainly based on plants enjoy a respective position today, especially in the developing countries, where modern health services are limited. Popularity of herbal drugs is increasing all over the world because of lesser side effects as compared to synthetic drugs besides it cost effectiveness and easy availability to poor people particularly in developing countries. The present study aimed to estimation of four heavy metals namely Iron, Cadmium, Lead, and Mercury *in Vitex negundo* leaves. In study we observed the road side sample contain more heavy metals compared to two other site samples. Lead, cadmium, mercury and iron accumulation 6.05, 0.29, 0.07 & 4.75 mg/kg, was found in road side sample S3 respectively. All the sample heavy metals concentrations are below the World Health Organization (WHO) and Ayurvedic Pharmacopoeia of India (API) permissible limits. The estimation of heavy metals is highly essential for raw drugs used for the preparation of herbal products. The periodic assessment is essential for quality control/ quality assurance and safer use of herbal products.

Keywords: Vitex negundo, Leaves, heavy metals, Quality control, Herbal products, Contamination.

Introduction

The important toxic metals include Cd, Hg, Pb and As (Meena et al. 2008; Sharma et al. 2014). All heavy metals, both essential iron, zinc, manganese, copper, nickel and non-essential Arsenic, cadmium, chromium, mercury and lead can cause toxic effects to plants and humans if found in high concentration (Das et al. 1997; An 2004). There are a number of factors which contribute to heavy metal contamination on agricultural soils and water bodies, climate, atmospheric deposition from town wastes, roadsides or highways which might be receiving deposits of metals from vehicle emissions, (Anyanwu et al. 2004; Khairiah et al. 2004; Shakya et al. 2013) including fertilizers, pesticides, industrial emissions, metal production, batteries, pigments, stabilizer, alloys paints, metallurgical, tannery chemical manufacturing, mining, pulp and paper, oil refining, electrical, rubber processing, household, electric power generators and dust storm (Meena et al. 2005&2008; Rao et al. 2010). Heavy metals are non-biodegradable and industrious natural contaminants which get saved on the surface and afterward retained into the tissues of plants/ herbs.

Herbal plants are consumed worldwide for the treatment of several diseases and are important raw materials for the Ayurveda, Siddha and Unani (ASU) pharmaceutical industry for the production of ASU drugs and herbal products as dietary supplements. In recent decades, the use of ASU drugs has increased worldwide, for several reasons, among them, that side-effects are often lower than those presented when synthetic drugs are employed, as well as due to the higher costs of many conventional pharmaceutical formulations.

WHO recommends that medicinal plants which form the raw materials for the finished products may be checked for the presence of heavy metals; further, it regulates maximum permissible limits of toxic metals like cadmium and lead, which amount to 0.3 and 10 ppm, respectively (WHO 1989, 1998). Permissible limits of heavy metals in Ayurvedic formulations as per Ayurvedic Pharmacopoeia of India (API) are arsenic (3ppm), mercury (1ppm), cadmium (0.3ppm) and lead (10ppm) (API, 2017).

Medicinal herbs are contaminated during growth, after collection and preparation of herbal drugs, the heavy metals which are present in plants finally enter the human body then accumulate in different organs. More content of toxic heavy metal may distract the normal functions of organs like central nervous system, liver, lungs, heart, kidney, brain and produce serious health hazards such as kidney and liver damage, renal failure, (Iwu et al. 1999). Higher levels, toxic heavy metals can cause different types of cancers, skin eruptions, intestinal ulcers, vomiting, diarrhea, musculo skeletal, cardiac infraction, ocular and reproductive effects (Agrawal et al. 2011). For these reasons, it is essential to control the level of toxic heavy metal contamination in herbal raw materials used in pharma industries (Meena et al. 2005& 2008; Rao et al. 2010).

Therefore, in the present study, attempts have been made to check the variation in toxic heavy metals Hg, Cd, Pb & Fe contents in plant materials collected from three different areas like public park area, botanical garden area and traffic congested road side area of the same city.

Materials and Methods

Sample Collection and Processing

Three samples of *Vitex negundo*, commonly known as the Chinese chaste tree, *Nirgundi*; leaves were collected from three different locations i.e. Public Park (S1), Botanical Garden (S2) and traffic congested road side (S3) of Chennai city. The samples were dried in shade and stored in the plastic food grade containers kept at room temperature until analysis.

Preparation of Sample Solution (Lead, Cadmium and Iron)

Heavy metals analysis was done according to AOAC guidelines (2016) for non-volatile heavy metal. Take 5 g of the sample in a pre-weighed silica dish and keep it in a muffle furnace with initial temperature not higher than 100ºC. Increase the temperature slowly to a maximum of 450ºC. Allow the dish to stand at least 8 hrs. or overnight. Wet ash with 1-3 ml water and evaporate on hot plate or water bath. Place the crucible in muffle furnace at not more than 200ºC and raise the temperature to 450ºC. Proceed with ashing at 450ºC for 1-2 hrs. or longer. Repeat procedure until the sample is completely ashed. Add 5 ml of 6 M Hydrochloric acid to crucible ensuring that all ash comes into contact with acid. Evaporate acid on water bath or hot plate. Dissolve residue in 10-30 ml of 0.1 M Nitric acid. Cover with watch glass and let stand for 1-2hrs. Then stir solution in dish thoroughly with glass rod and filter the solution in a 100 ml volumetric flask and make up the solution upto the mark with deionised water.

Analysis was done using atomic absorption spectrophotometer (GBC Avanta). The standard reference material of all the metals (E. Merck) was used for calibration and quality assurance for each analytical batch. Three replicates were analysed to assess precision of the analytical techniques, and results were averaged.

Preparation of Sample Solution (Mercury)

5-10 g of the plant material was taken in a 100 ml Round bottom flask of the bethge apparatus add 3 to 4 glass beads, 10-12 ml of concentrated Nitric acid and 2-5 ml of concentrated Sulphuric acid connect the flask to the condensate receiver and reflux condenser and Put the flask in the cold condition for about 1.5 hrs. Once, remove cold condition heat the flask and collect the nitric acid in the condensate receiver continue heating till the sulphuric acid starts. Fuming and chars the sample. Remove the burner, wait for a few minute and carefully allow the nitric acid to drain into the flask. Repeat this operation to all the sample solution becomes just pale yellow colour. Cool and then remove it from the condensate receiver. Collect all the condensate in 50 ml standard flask and make the solution with deionised water. Analysis was done by using Mercury analyser MA 5840. Three replicates were analysed to assess precision of the analytical techniques, and results were averaged.

Results and Discussion

Vitex negundo Linn (Five-leaved chaste tree or Nirgundi), is an important ASU medicinal plant found throughout India. All parts of this plant especially the leaf and root are used in various formulations in ASU medicines. Leaves of *Vitex negundo* were collected from three different areas like public park (S1), botanical garden (S2) and traffic congested road side (S3) of the same city. Presence of heavy metals, namely, lead, cadmium, iron and mercury were estimated in the samples, and the results are tabulated in Table 1. Heavy metals analysis was carried out according to AOAC (2016).

Table 1: Heavy metals concentration (mg/kg) in *Vitex negundo* leaves collected from three different locations

S. No.	Heavy Metals	Results (mg/kg)		
		S1	**S2**	**S3**
1.	Lead (Pb)	5.01	4.10	6.05
2.	Cadmium (Cd)	0.2	0.16	0.29
3.	Mercury (Hg)	0.02	0.04	0.07
4.	Iron (Fe)	2.39	1.66	4.75

Toxic heavy metal content in the plants depends on the factors such as the area of collection of samples for study. In the present study, road side sample contain more heavy metals compared to two other place samples. Lead content was higher (6.05 mg/kg) in road side sample S3 compared to botanical garden sample S2 (4.10 mg/kg). Cadmium content of all samples differed in a minor range. The highest mean level of cadmium 0.29 mg/kg was found in the sample S3 and the lowest mean level of cadmium 0.16 mg/kg was observed in sample S2. Mercury concentration of the plant samples ranged between 0.02 to 0.07 mg/kg, with the highest values in sample S3 and lowest in sample S1. The highest mean level of Iron 4.75 mg/kg was found in the sample S3 and the lowest mean level of Iron 1.66 mg/kg was observed in sample S2. All the samples concentrations are below the WHO and API permissible limits.

Conclusions

Excessive heavy metals contamination may be related to the source of these herbal materials, if they are grown under contaminated environment or during collection of these plant materials. Some of these environment related factors can be controlled by implementing standard operating procedures leading to Good Agricultural Practice, Good collection & Storage Practice and Good Manufacturing Practice for producing these medicinal plant material from herbal or natural sources.

Heavy metal content in the plant depends on the factors such as the area of collection. In the present study, road side sample S3 contains more heavy metals compared to two other site samples. Lead, cadmium, mercury and iron accumulation 6.05, 0.29, 0.07& 4.75 mg/kg, was found in road side sample S3 respectively. The samples contained safe levels of the heavy metals concentrations and all are below the WHO and API permissible limits. The estimation of heavy metals is highly essential for raw drugs used for the preparation of herbal products. The periodic assessment is essential for quality control/ quality assurance and safer use of herbal products.

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Conflict of Interest: None.

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