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Bioaccumulation of heavy metals in some medicinal plants from South Gujarat, India

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Abstract The different heavy metals such as Cu, Cr, Ni, Zn and Pb were estimated in medicinal plants *Azadirachta indica, Emblica officinalis, Gymnema sylvestre* and *Withania somnifera* from Surat district, Gujarat, India. The observations revealed that *A. indica* had the level of metals in the range Cu>Cr>Zn>Ni, *E. officinalis* had range viz. Cu>Zn>Cr>Ni, *G. sylvestre* and *W. somnifera* had concentrations as Zn>Cu>Ni>Cr and Zn>Cr>Cu>Ni respectively. It was also observed that Pb was not detected in any of the plant samples.

Keywords: Air pollution, Heavy metals, Herbal medicines, Medicinal plants, Water and soil contamination

Introduction

Heavy metals are among the contaminants in the environment and natural activities as well as human activities contribute in the pollution of heavy metals. The main cause behind this includes geogenic, industrial, agricultural, pharmaceutical, domestic effluents and atmospheric sources (He *et al.*, 2005). Some of the other industrial sources are: metal processing in refineries, coal burning in power plants, petroleum combustion, nuclear power stations, high tension lines, plastics, textiles, microelectronics etc. (Pacyna, 1996; Arruti *et al.*, 2010). Rapid industrialization altered the normal geochemical cycle of metals which in turn have accelerated their amount in soil horizons (Tak *et al.*, 2013). Any non-biologically degradable metal or metalloids that cause an environmental problem are considered as 'heavy metals' (Herrera-Estrella and Guevara-Garcia, 2009). They are also considered as trace elements because of their presence in trace concentrations (ppb range to less than 10 ppm) in various environmental matrices (Kabata-Pendiaand Pendias, 2001). Although they are natural components of the Earth's crust, their concentrations in soil and surface waters has significantly increased since last two decades (Larison *et al.*, 2000).

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Thus, increased bioaccumulation of heavy metals showed negative impact on the food chain and hence is now being perceived as an imminent threat to the ecosystem and environment (Saha *et al.*, 2017; Singh and Kumar, 2017) and has gained global attention due to the toxicological risks posed by such metals to human health (Ayodeji and Olorunsola, 2011).

Medicinal plants play an important role from the ancient period and till date in developing countries like India, traditional medicines are still used for ailments like cold, malaria, arthritis, ulcers, hepatitis, diabetes etc. (Sarma and Sarma, 2008). Medicinal plants are mainly collected from wild and delivered to market without knowing their botanical identity, origins, purity, safety and efficacy. Many curative effects of medicinal herbs used are due to the presence of very minute quantities of trace elements (micro-nutrients), and their inadequate supply results in a variety of deficiency or syndromes (WHO, 1996). Dietary elements required in amounts greater than 100 mg/day are called ‘minerals’, and those that are required in amounts less than 100 mg/day are called ‘trace elements’. These elements are iron, copper, cobalt, nickel, zinc, magnesium, manganese, molybdenum, chromium, vanadium, lithium, selenium, fluorine and iodine (Shirin *et al.,* 2010). But their higher concentrations have toxic effects to humans (Elekes *et al.,* 2010) and it also affects quality and efficacy of herbal medicines (Blicharska *et al.,* 2010). WHO and the USFDA have standardized safe limits for the occurrence of certain metals (e.g. As, Hg, Pb and Cd); however WHO has not yet decided the permissible limits in medicinal plants. When a root absorbs water or nutrients from the soil, other ions and metals move toward different organs either via mass flow or by diffusion (Robinson, 1990). The metals accumulation in plants is influenced by the physicochemical properties of the soil in which the plant is growing. Such properties are: bioavailability of the metallic element, characteristics of soil or sediments, pH level, exposure period, dispersion range and presence or absence of other elements. It has been observed that the heavy metals, especially at higher concentrations adversely affected the growth and biochemical content of many plants (Vyas 2017, 2022). Chibuike and Obiora (2014) also documented that heavy metals like copper (Cu), chromium (Cr), nickel (Ni), zinc (Zn) and lead (Pb) not only reduces the biomass and seed production, but also reduces plant nutrient content. Contamination of herbal raw materials with afflotoxins cause carcinogenic effects if absorbed even in small amounts and therefore it is necessary to measure the levels of metallic elements in medicinal plants (Schilcher *et al.,* 1987).

Azadirachta indica A. Juss. (Meliaceae) is commonly known as neem tree and is routinely usedas anticancer, antimalarial, antihypertensive, analgesic, anti-inflammatory, antiulcerogenic, orodental protection diabetes

(Subapriya and Nagini, 2005). *Emblica officinalis* Gaertn. (Euphorbeaceae) known as amla have fruits which are used as traditional medicine. The plant also possesses diuretic, hepatoprotective, hypocholestrolemia, antioxidant, antiulcerogenic, anti-inflammatory and analgesic properties (Sabahat and Perween, 2007; Khan, 2009). *Gymnema sylvestre* (Retz.) R.Br. ex Schult. (Apocynaceae) commonly known as gurmar and is considered as one of the major plant to treat diabetes in the Ayurvedic system of medicine and also called as anti-diabetic plant (Singh *et al.*, 2008). Leaves of this plant have cardiotonic, digestive, diuretic, laxative, stimulant, stomachic and uterine tonic properties (Mathew, 2004). Lastly, *Withania somnifera* (L.) Dunal (Solanaceae) is widely used medicinal plant, commonly known as ashwagandha, and being used to treat constipation, senile debility, rheumatism (Kirtikar and Basu, 1991) as well as for properties such as anti-inflammatory (Markert, 1995) andantimicrobial (Arora *et al.*, 2004).

Thus, aim of the present study was to evaluate the concentration of various metals elements e.g. Cu, Cr, Ni, Zn and Pb in common medicinal plants *A. indica, E. officinalis, G. sylvestre* and *W. somnifera* collected from South Gujarat region.

Materials and methods

Plant materials

The leaves of four medicinal plants were collected from the plants widely grown in the Surat district (21°10'12" N, 72°49'51" E), India. They were collected in a sterile cloth bag and transferred to the laboratory. The samples were thoroughly washed with running tap water to eliminate dust, dirt and parasites for 30 min followed by rinsing with distilled water. The plant materials were dried in shade at room temperature, air dried samples were powdered using mortar-pestle and finally stored in airtight bottles.

Sample preparation

1 g of the powdered sample into 100 ml flasks followed by the addition of HCl and HNO_3 in a ratio of 3:1 and allowed to stand for 24 h under a fume hood. The mixture was then heated at 40 °C for 40 min and then, the temperature was increased to 100 °C until the solution became clear with disappearance of white fumes which indicates the completion of the digestion process (Audu and Lawal, 2005). The digest was diluted with 10 mL of distilled water and boiled for 15 min. The resulting solution after cooling was

filtered into a 100 mL flask using a Whatman filter paper and diluted to the mark with distilled water. This was then stored in screw capped polyethylene bottle until analysis.

Calibration curve of heavy metals and sample analysis

Stock solutions of 1000 ppm was prepared for each metal by dissolving weighed quantities of appropriate dried analytical grade salts (SRL, Mumbai, India) in distilled water. For preparation of calibration curve, standards in 1-5 ppm concentration range were obtained by appropriate dilution of stock solutions for each metal. The contents of heavy metals in different samples were measured by using flame atomic absorption spectrometry under the optimum operating conditions with air acetylene flame.

Statistical analysis

All the experiments were repeated twice and five replicates were maintained for each experiment. The values are given as mean and standard error (SE). All the means were analyzed using one-way analysis of variance (ANOVA, $\alpha = 0.05$) followed by Tukey's test using GraphPad Prism 6.01.

Results

In the present study, leaves of *A. indica*, *E. officinalis*, *G. sylvestre* and *W. somnifera* growing in wild in Surat district, Gujarat were evaluated for the amount of different heavy metals like Cu, Cr, Ni, Zn and Pb. The study revealed that all the metals were accumulated to greater or lesser extents except Pb which was not detected in any of the selected species. In *A. indica* the highest level found was for Cu which was 21.51 ± 0.76 µg/g, followed by concentration of Cr (3.80 ± 0.29 µg/g), Zn (2.50 ± 0.33 µg/g) and Ni (1.00 ± 0.09 µg/g) (Figure 1). Similarly when *E. officinalis* was analyzed, similar to *A. indica*, the maximum content recorded was for Cu metal and it was 30.70 ± 0.99 µg/g, however its amount was higher as compared to *A. indica*. Similarly, accumulation of Zn was also higher (21.90 ± 0.79 µg/g) as compared to previous plant.Whereas Cr content recorded was less i.e. 2.00 ± 0.15 µg/g and Ni was almost similar to *A. indica* (1.80 ± 0.18 µg/g) (Figure 2). Further evaluating *G. sylvestre* for different metals revealed that maximum content was for Zn i.e. 50.13 ± 0.89 µg/g. This was followed by Cu and Ni which were 27.30 ± 0.77 µg/g and 7.67 ± 0.96 µg/g respectively. The least content recorded was for Cr (0.53 ± 0.09 µg/g) which was lowest amongst all the other studied plants (Figure 3). At last, assessment of *W. somnifera* leaves confirmed that amount of Zn was maximum 45.89 ± 1.78 µg/g, however it was less than *G.sylvestre*. This was followed by other metals which were significantly lower than Zn e.g. Cr (4.49 ± 0.18 µg/g), Cu (3.52 ± 0.27 µg/g) and Ni (2.25 ± 0.23 µg/g) (Figure 4).

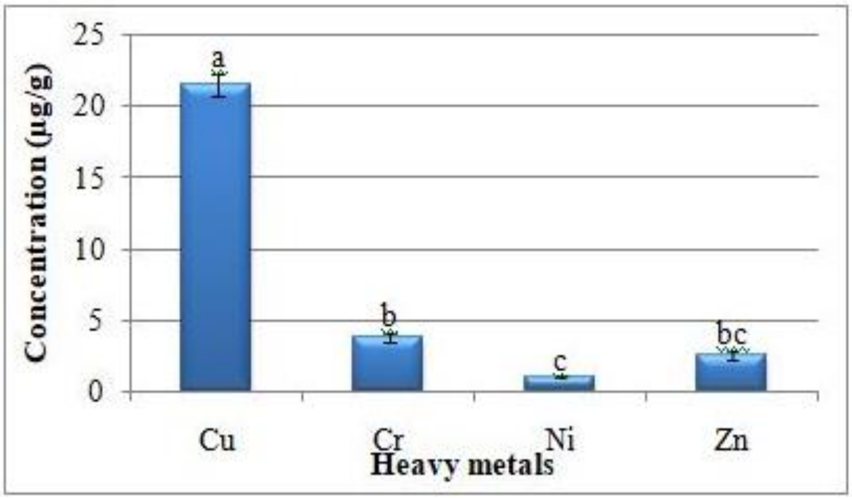

Figure 1. Concentration of different heavy metals in *A. indica*. Each bar shows the mean values (n = 5) and error bar as standard error. Bars having same letters are not significantly different ($p \leq 0.05$) according to Tukey's test

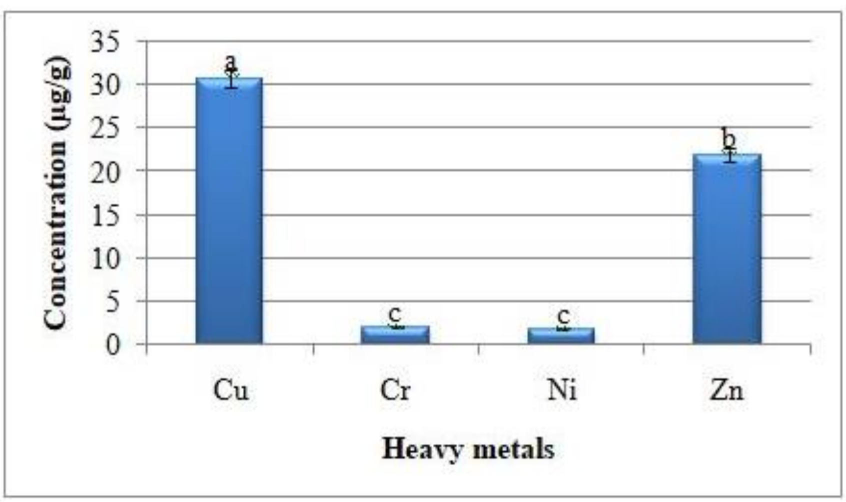

Figure 2. Concentration of different heavy metals in *E. officinalis*. Each bar shows the mean values (n = 5) and error bar as standard error. Bars having same letters are not significantly different ($p \leq 0.05$) according to Tukey's test

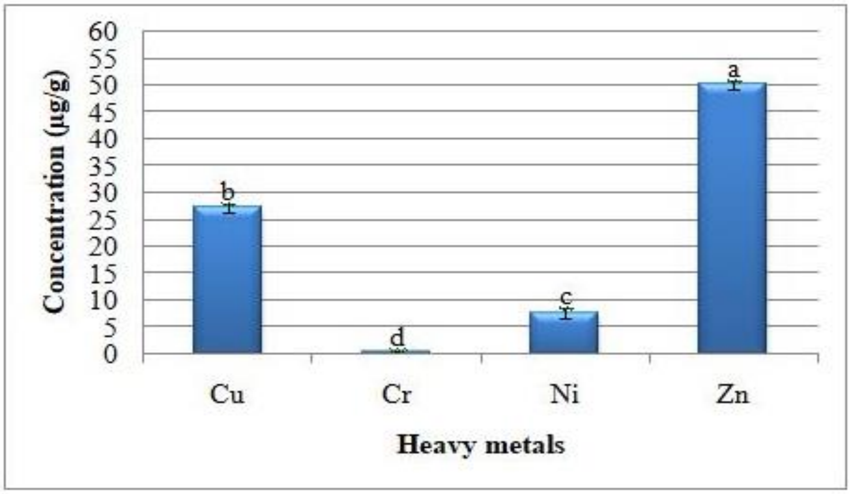

Figure 3. Concentration of different heavy metals in *G. sylvestre*. Each bar shows the mean values (n = 5) and error bar as standard error. Bars having same letters are not significantly different ($p \leq 0.05$) according to Tukey's test

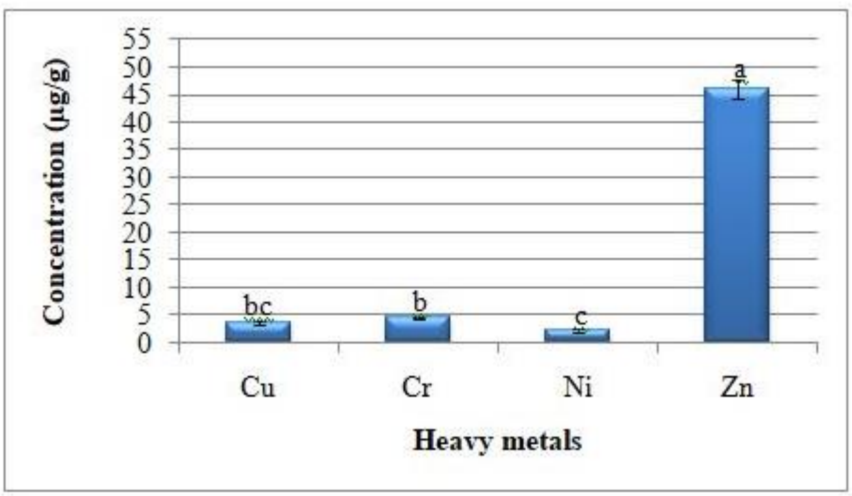

Figure 4. Concentration of different heavy metals in *W. somnifera*. Each bar shows the mean values (n = 5) and error bar as standard error. Bars having same letters are not significantly different ($p \leq 0.05$) according to Tukey's test

Thus it was inferred that accumulation of Cu was highest in *E. officinalis* followed by *G. sylvestre, A. indica* and *W. somnifera*. The Cr accumulation was maximum in *W. somnifera*, followed by *A. indica*, *E. officinalis* and *G.*

sylvestre. Whereas Zn and Ni both were accumulated maximum in *G. sylvestre*, followed by *W. somnifera*, *E. officinalis* and least in *A. indica*.

Discussion

Plants need certain elements in trace amount as growth regulators or cofactors in cellular and biochemical reactions as well as are also part of various biomolecules (Soetan *et al.*, 2010; Hasanuzzaman *et al.*, 2013). For this plants have evolved sophisticated mechanisms for metal uptake, storage and their detoxification to neutralize the toxic effect of heavy metals (Hossain *et al.*, 2012). When leaves of *A. indica* were evaluated for concentration of different metals, it was found in range Cu>Cr>Zn>Ni. In previous report on same plant only two metals i.e. Zn and Cu were evaluated and their presence in leaves has been reported (Ajas *et al.*, 2004). Later on Garg *et al.* (2007) studied the same plant and observed that maximum 34.8 µg/g Zn has been recorded which is followed by Cu (6.49 µg/g) and Cr (1.47 µg/g), whereas Gajalakshmi *et al.* (2012) reported the concentration of metals are in range Cu (1.655 ppm)>Zn (0.294 ppm)>Cr (0.145 ppm) in Vellor district, Tamilnadu. In both these studies Ni is not reported, which has been detected at less concentration in the present study, but absence of Pb in their results are in corroboration with present result. Similarly, absence of Ni and Pb and other metals in small quantities has been documented in range Cu>Zn>Cr in this plant (Verma *et al.*, 2013). On the contrary, some studies reported presence of Pb in leaves of this plant e.g. Lawal *et al.* (2011) documented that metal concentrations are in range Zn>Cu>Pb>Ni>Cr in Nigeria, whereas Patel *et al.* (2015) reported that the amount of metals in Chhattisgarh district in India is in range Cu>Zn>Cr>Pb. Similarly, accumulation of different metals in samples from Guayaquil, Ecuador and reported in range of Zn>Cu>Ni>Cr whereas Pb is in very trace amount and hence not detectable (Morales-Estupiñan *et al.*, 2020).

Analyzing *E. officinalis* leaves revealed that similar to previous plant concentration of Cu was maximum and the range for different metals were like Cu>Zn>Cr>Ni. In another study on same plant by Singh *et al.* (2010) documented maximum Zn (23.3 µg/g) content and it is almost similar to present investigation, whereas the content of Ni is higher (7 µg/g) but the amount of Cu (5.9 µg/g) is less than present study. Further they have not detected Cr and Ni in their report. The maximum accumulation of Cu followed by Cr and Zn, and absence of Ni and Pb was reported in same plant by Gajalakshmi *et al.* (2012). Verma *et al.* (2013) reported that the range of metals are Cu>Cr>Zn, but amounts of all the metals are less as compared to present investigation; whereas Ni and Pb are not detected in their study. Later on Gupta and Gupta (2013) analyzed samples from two different sites and observed variation in contents of metals, of which the amount of Cu and Zn are lower than present study whereas Cr, Ni and Pb are higher in their report.

In *G. sylvestre* the metals detected in range Zn>Cu>Ni>Cr which is contrary to previous results reported for same plant by Annan *et al.* (2010) as they have quantified maximum Cu (86.5 µg/g) followed by Zn (65.5 µg/g) and Ni (25 µg/g) and all of these are higher than present study. However they have not reported Cr which was present in less quantity in this study but absence of Pb is in line with present findings. In another study, only Cu and Zn are quantified which are 32.8 and 5.7 mg/kg respectively in *G. sylvestre* (Jothivel *et al.*, 2011). Later on Pingale *et al.* (2017) reported highest Zn (41.9 ppm) in leaves of *G.sylvestre* followed by Cu (20.6 ppm), Ni (6.9 ppm), Cr (5.5 ppm) and Pb (4.8 ppm), of which the content of Zn, Cu and Ni are less as compared to present report whereas the Cr and Pb are higher than present study. Lastly assessing leaves of *W. somnifera*, it was noted that the metal concentrations were in following order: Zn>Cr>Cu>Ni. In line with present study, maximum content has been recorded for Zn (43.6 µg/g) in previous report (Garg *et al.*, 2007), and the concentration of Cr (1.86) is lower and level of Cu (9.5 µg/g) is higher in their study. In present investigation Ni was detected but not Pb, whereas Garg *et al.* (2007) have not detected both Ni and Pb. Further Maharia *et al.* (2010) taken up samples from two different sites and reported variation in content of different metals, which are in range Cu>Zn>Ni>Pb>Cr and Zn>Cu>Ni>Pb>Cr for Khetri and Haridwar sites respectively. Whereas Jothivel *et al.* (2011) analyzed the amount of only two metals i.e. Cu (31.1 mg/kg) and Zn (1.3 mg/kg) in this plant. The concentration of Cr detected in present study was higher (4.49 ± 0.18 µg/g) as compared to limit defined by WHO (2.0 ppm). Similarly, higher concentration of Cr (4.93 ± 0.0185 ppm) is also observed in *W. somnifera* by Kulhari *et al.* (2013), as well as metal concentrations in the range Cr>Zn>Pb>Ni from Rajasthan district in India. From present investigation it can be found that the pattern of metal accumulation varied significantly for different metal from plant to plant which is also reported by Rawat *et al.* (2019).

It can be concluded that the heavy metal polluted soils leads to reduction in growth due to changes in physiological and biochemical activities. Considering the market demand for herbal drugs will increase worldwide, it is essential to refine procedures used for premarket drug testing to ensure the quality, efficacy and safety of such natural remedies. One of the consequences of heavy metal for plant is that they are known to inhibit various metabolisms like biomass production or other biochemical parameters. Whereas consumption of raw herbal drugs from the medicinal plants, grown in polluted sites can cause serious consequences on human health. Higher level of these elements are harmful as they affect central nervous system (Pb), cause kidney damage and liver dysfunction (Pb and Cu), toxic to skin, bones and teeth (Ni, Cu and Cr) and have adverse effects on memory and reproductive system. Hence regular assessments of raw material are must to check the levels of these pollutants in the plant parts and extracts before using them. Further evaluation of heavy metals in medicinal plants will pave the way for excluding extensively polluted environmental sites for collection of raw materials required for preparation of herbal drugs. Another inference can be derived that the toxicity of heavy metals can be removed by phytoremediation using plants generally not part of food chain and upon sufficient growth of plant, it can be harvested and in turn help in removal of metals from the site.

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