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QUALITY ASSESSMENT FOR THE ACCUMULATION OF HEAVY METALS IN FLOWERS OF AVARAM (*Senna auriculata*) GROWN ON NATURAL AND POLLUTED AREAS

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Abstract
Medicinal plants are still taken from their natural habitats for personal use and the creation of plant-based pharmaceuticals. Detecting highly hazardous heavy metals in plant extracts is crucial for food safety and quality assurance. The objective of this study was to assess the heavy metals in Senna auriculata flowers from both natural and polluted sources. We selected two hills areas as natural areas and two roadsides near industrial areas as polluted areas. The samples were prepared by the wet digestion method. The concentration of heavy metals such as lead (Pb), chromium (Cr), cadmium (Cd), zinc (Zn), copper (Cu), and iron (Fe) was detected using Atomic Absorption Spectroscopy. Analytical results show that samples A and B (natural sources) had heavy metal contents below the permissible level, except for lead and cadmium, which were not detected in sample B, whereas samples from polluted sources had heavy metal contents above the permissible level, with the exception of cadmium and copper. The amounts of lead and zinc in floral extract sample C, as well as iron and chromium in sample D, are above the standard limit. As a result of this finding, it is plausible to assume that heavy metal pollution has an impact on plants cultivated in contaminated environments. This study found that medicinal plants used for human consumption and standardized extracts should be collected from an unpolluted natural environment.

Keywords: Heavy metals, quality assurance, senna flower, polluted sources

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INTRODUCTION

Herbal medicines have been used all over the world since the ancient period and play an important role in international trade. Herbal medicines' clinical, pharmacological, and economic value is increasing, however, it differs greatly from country to country. Scientists and pharmacologists are desperately trying to find novel pharmaceutical sources and are increasingly turning to traditional medicine. The discovery of numerous natural and synthetic drugs is a significant achievement in the area of medicine, owing to scientific and technological advancements (Preethi et al., 2010). Many natural products have arrived in everyone as a result of traditional scientific research into treatments, with the majority of them derived from plants (Uniyal et al., 2006). One of the difficulties to the widespread acceptance of herbal products is the lack of quality control standards. Traditional medicine quality control includes the quality of both raw materials, mostly plants, but also animals, metals, and minerals, as well as final goods. Detecting highly hazardous heavy metals in plant extracts is crucial for food safety and quality assurance. This is because pharmaceuticals manufactured in the wild are harder to verify for all potential sources of contamination. The probability of hazardous pollutant deposition on plants, as predicted, cannot be ignored. Little is known about the toxic metal status of these herbal medicinal plants produced in polluted environments. The risk of exposing humans and animals to environmental contaminants if plants grown in contaminated areas are used. Heavy metal detection in plants is essential. The consumption of harmful trace elements by humans can induce organ failure and chronic toxicity even at low levels over a long period.

Heavy metals are very dangerous due to their non-biodegradability, extended biological half-lives, and ability to collect in many bodily areas. Heavy metals are absorbed faster than they can be metabolized or eliminated. Even heavy metals that are thought to be needed might become poisonous if present in high concentrations. (Turkdogan et al., 2003). There are two types of metals found naturally. One of these categories is micronutrients, which plants require for normal growth, while the other is not required for plant growth. Heavy metals such as Zn, Cu, and Fe are required for human metabolism and play a vital part in chemical, biological, biochemical, and enzymatic processes in the cells of plants, animals, and humans (Singh et al., 2011). Heavy metals such as Cd, Pb, Mg, As, and Sb have no biological role and are harmful to living organisms (Chove et al., 2006). These heavy metals are absorbed by both the aerial and underground organs of plants.

Medicinal plants are still taken from their natural habitats for personal use and the creation of plant-based pharmaceuticals (Sarma et al., 2011). It is also critical to underline that the safety and advantages of plant-based products are closely tied to the quality of the raw materials, including the presence of heavy metals within acceptable levels (Salgueiro et al., 2010). To use plants as field monitors of metal pollution, it is vital to distinguish between uptake from natural metal sources and uptake from polluting sources. Metals from both natural and polluted sources have the potential to be absorbed by the plant via root absorption mechanisms. Numerous investigations on heavy metal accumulation plants have been performed (Delia, 2015; Turkyilmaz et al., 2018; Sevik et al., 2019). However, no attempt has been made in Senna auriculata (L.) Roxb. to do so. Senna flower is a natural antioxidant source. The floral extract has been shown to have anti-diabetic properties. Consumption of avaram flowers as tea reduces blood sugar levels. It functions as a laxative, preventing constipation. It aids in the loss of extra fat and the maintenance of healthy body weight. Fresh flower extracts resist microorganisms that cause typhoid, cholera, and urinary tract infections, such as Staphylococcus aureus, Enterococcus faecalis, Salmonella typhi, Escherichia coli, Vibrio cholera, and Shigella dysentrae (Kainsa et al., 2012). As a result, it was crucial to assess the heavy metals (Pb, Cr, Cd, Zn, Fe, and Cu) in Senna auriculata flowers from both natural and polluted sources.

MATERIAL AND METHODS

Collection and preparation

Flowers of *Senna auriculata* were collected in June 2021 from four distinct research locations, including two natural areas (site 1 pachaimalai hills and site 2 kolli hills) and two roadsides in polluted areas (site 3 avur road, and site 4 samayapuram road). The obtained flower samples were air-dried at room temperature for 5 -7 days before being pulverized into a fine powder. For further investigation, the powdered samples were placed in airtight polythene bags.

Wet digestion for heavy metal analysis

The most efficient digesting procedure for herbal product samples was the combination of nitric-hydrochloric acids HNO_3 – HCl in a 1:3 ratio (Uddin et al., 2016). A total of 2.0 g of each sample was weighed and placed in a conical flask. The conical flask was then filled with a 9 ml combination of % HNO_3 and 37 % HCl. The mixture was then gently heated over a water bath at 95 °C for 4–5 hours, or until the sample was completely dissolved. The digest was allowed to cool at room temperature before being filtered through a Whatman No. 42 filter paper and diluted with deionized water to a final volume of 50 mL.

Standard preparation

In deionized water, stock standard solutions with a concentration of 1000 ppm for each metal were prepared (Urmila, 2016).

Lead: Dissolved 1.598 g of Lead nitrate ($Pb(NO_3)_2$) in 100mL of deionised water. After complete dissolution, it is made to 1 liter with deionized water.

Chromium: Dissolve 1.923 g of chromium trioxide, CrO_3, in reagent water, acidify (to pH = 2) with redistilled HNO_3 (conc.), and dilute to 1 L with reagent water.

Cadmium: Dissolved 2.036 g of Cadmium chloride ($CdCl_2$) in 250 mL deionized water. This solution was further made to 1 liter in a volumetric flask.

Table 1: Instrumental condition of AAS for heavy metal analysis

Metals	AAS Parameters			
	Wavelength (nm)	Slit width (nm)	Current (A)	Flame
Pb	283.3	0.7	10	Air-acetylene
Cr	357.9	0.7	12	Air-acetylene
Cd	228.8	0.7	3.5	Air-acetylene
Zn	213.9	0.7	5.0	Air-acetylene
Cu	324.8	0.7	15.0	Air-acetylene
Fe	248.3	0.2	30	Air-acetylene

Zinc: Dissolved 1.245 g of Zinc oxide (ZnO) in 5mL of deionised water followed by 25mL of 5M hydrochloric acid. The solution was made to one liter in a volumetric flask with deionized water.
Copper: Dissolved 3.798g of ($Cu(NO3)_2.3H_2O$) Copper nitrate in 250 ml deionized water. Volume was made 1 liter with deionized water.
Iron: Dissolved 1.000 g of iron wire in 50 mL of (1:1) HCl and 5 ml of concentrated HNO_3. This solution was dilute to 1 liter with deionized water.

Analytical procedure

The digested samples were utilized to quantify heavy metals such as lead (Pb), chromium (Cr), cadmium (Cd), zinc (Zn), copper (Cu), and iron (Fe) using Atomic Absorption Spectroscopy (THERMO SCIENTIFIC- ice 3000) at the National College Instrumentation Facility (NCIF), National College, Trichy. The instrumental conditions used during heavy metal analysis were listed in Table 1. All measurements on samples and standard solutions are done in triplicate.

Calculation

The metal concentration which we get from AAS is in mg/L of the solution. This can be converted into mg per Kg of the dry weight of the plant by the following formula.

$$\textit{Concentration of the metal in sample} = \frac{\text{Measured concentration in AAS (mg) x Volume (L)}}{\text{Weight of the sample (kg)}}$$

Statistical analysis

The mean and standard deviation of the mean for the heavy metal data from three replicates were calculated using Microsoft Excel 2013. SPSS 16.0 was used to perform an ANOVA to see whether there is a significant difference between the mean concentrations of heavy metals in samples obtained from natural and polluted areas.

RESULTS AND DISCUSSION

Heavy metals such as Pb, Cr, Cd, Zn, Cu, and Fe were observed in the flowers of *Senna auriculata* (L.) Roxb. Obtained from both natural and polluted sources. The contents of these elements in each sample as a means of triplicate with their standard deviation. Table 2 shows the mean heavy metal concentrations in *Senna auriculata* flowers, as well as the acceptable limits for heavy metal concentrations in plants.

Lead (Pb)

The concentrations of lead in samples from the study sites ranged from 1.02 to 12.78 mg/kg, compared to the WHO-recommended tolerable limit for plants of 10 mg/kg. This criterion was satisfied by the determined Pb contents in the natural source samples. However, the Pb concentration in sample C (site-3) polluted source was significantly greater than the allowable limit. Pb is a non-essential hazardous

Table 2: Heavy metal concentrations in *Senna auriculata* flowers

Elements	Natural sources		Polluted sources		Permissible limits
	Sample - A	Sample - B	Sample - C	Sample - D	
Pb	1.02±0.01	ND	12.78±0.03	7.38±0.37	10
Cr	0.42±0.01	0.91±0.26	1.65±0.04	3.06±0.02	2
Cd	0.03±0.01	ND	0.27±0.03	0.19±0.02	0.3
Zn	0.87±0.02	1.38±0.04	56.12±0.65	38.96±0.03	50
Cu	1.46±0.02	3.35±0.01	9.46 ±0.02	5.73±0.01	10
Fe	3.84±0.43	5.07±0.02	10.72±0.01	18.02±0.24	15

All data are the mean ± SD of three replicates (mg/kg). SD – Standard deviation; ND – not detected. Permissible limits in plants sources: (WHO, 1996; 2005; 2007)

heavy metal that is widely used and has a wide range of harmful effects on living organisms. Pb causes cancer, affects the respiratory and digestive systems, and suppresses the immune system. This metal is particularly hazardous to children since it impairs their intelligence and neurological systems (*Borges et al*., 2003). Pb exposure can induce urinary tract and cardiovascular disorders via an immunomodulatory, oxidative, and inflammatory pathway.

Chromium (Cr)

Cr concentrations in flowers ranged from 0.42 to 3.06 mg/kg on the mean. Sample D had the highest Cr concentration (3.06 mg/kg), which was higher than the allowable limit. Cr is required for metabolic processes in the body. The hexavalent form, on the other hand, is harmful to humans (*Khurshid and Iqbal,* 1984). It is extensively used in sectors such as metal surface plating, leather tanning, glassware cleaning, textile manufacture, and so on due to its anti-corrosive qualities. Nausea, vomiting, acute renal failure, irritation, contact dermatitis, eczema, allergies, contact dermatitis, and reproductive damage are all symptoms of acute chromium overdose.

Cadmium (Cd)

The mean concentrations of Cd in flowers ranged from 0.03 to 0.27 mg/kg. According to the WHO, the maximum permissible concentration of Cd in plants is 0.3 mg/kg. As a result, Cd contents in all samples from natural and polluted sources were determined to be below the standard limit. Cadmium is not yet recognized to have any biological purpose; rather, it is considered a non-essential element that is very toxic. Cadmium toxicity has been seen in the liver, brain, kidneys, lungs, placenta, and bones. It produces muscle weakness, nausea, vomiting, and abdominal discomfort (*Davis et al.*, 2006; *Rezende et al*., 2011). The Itai-Itai disorders in Japan drew attention to the hazards of cadmium.

Zinc (Zn)

Zinc concentrations in flower samples ranged from 0.87 mg/kg to 56.12 mg/kg. The highest Zn level was discovered in sample C, which was above the allowable limit (50 mg/kg). Zn is a common element found in the earth's crust. Cofactor in many enzymes involved in macronutrient metabolism and cell reproduction. Excessive consumption can cause arteriosclerosis, stomach cramps, pancreatic damage, and caustic effects on the skin, as well as liver failure, renal failure, and anaemia (*Duruibe et al*., 2007).

Copper (Cu)

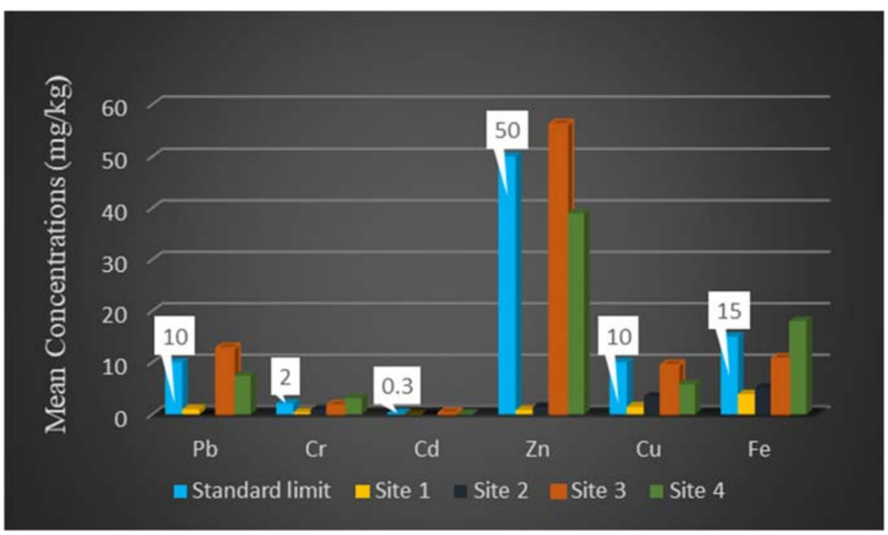
The Cu concentrations of S. *auriculata* flowers varied from 1.46 to 9.46 mg/kg, which was less than the WHO permissible limit of 10 mg/kg. Copper is essential for human health, but excessive consumption can harm the body's organs and systems, resulting in severe symptoms such as anaemia, headache, abdominal pain, dizziness, nausea, vomiting, diarrhoea, hemolytic jaundice, liver damage, kidney failure, and central nervous system depression (*Hashem et al*., 2011). Long-term copper exposure can irritate the nose, mouth, and eyes. Overexposure to copper has been linked to a higher risk of health consequences in people with Wilson's disease. Intentionally consuming too much copper can cause liver and kidney damage, while also death.

Iron (Fe)

Fe concentrations in flowers varied from 3.84 to 18.02 mg/kg on the mean. According to the WHO, the maximum allowable level of Fe in plants is 15 mg/kg. Sample D had higher Fe values that are over the allowable limit. Fe is a necessary element. It is an essential component of haemoglobin. Siderosis, a kind of pneumoconiosis, can be caused by chronic inhalation of high levels of iron oxide fumes or dust particles. It has the potential to induce conjunctivitis, choroiditis, and retinitis. Excessive inhalation of iron oxide can increase the risk of lung cancer. Iron, in general, destroys heart and liver cells, resulting in serious side effects such as coma, metabolic acidosis, shock, liver failure, coagulopathy, adult respiratory distress syndrome, organ destruction, and even death.

Analytical results show that samples A and B (natural sources) had heavy metal contents below the permissible level, except for lead and cadmium, which were not detected in sample B, whereas samples from polluted sources had heavy metal contents above the permissible level, except for cadmium and copper. The amounts of lead and zinc in floral extract sample C, as well as iron and chromium in sample D, are above the standard limit shown in Figure 1. This could be due to the presence of paint, battery, cement, electrical, chemical, and steel manufacturing companies on site 3 and site 4, and also the samples were collected from roadsides. This result was by the earlier studies of Hussain and Khan (2010) found that *Taraxacum Officinale* grown in polluted areas had higher levels of heavy metals such as Pb, Cd, Cr, Mn, Fe, Co, Ni, and Cu than plants grown in unpolluted areas. Juan et al. (2020) discovered in *Jatropha curcas L.* translocated high amounts of metals to its aerial parts, in contaminated soil. Automobile transportation contributes significantly to heavy metal pollution. Different branches of industry, as well as road traffic, have a significant impact on environmental pollution with heavy metals such as chromium from metallurgical, paint, and tanning industries, cadmium from metal smelters, and grease used in motor vehicles. Anthropogenic activities such as coal mining, garbage combustion, and steel production are key contributors to increased zinc levels. The iron and steel industries, sewage, iron mining dust, iron sulphate fertilizer, and herbicides are all anthropogenic sources of iron.

Figure 1: Heavy metal concentrations in *Senna auriculata* flowers obtained from natural and polluted sources



Natural areas: sites 1&2,
Polluted areas: sites 3&4

Analysis of Variance

Two-way ANOVA was performed different study sites Vs Concentrations of heavy metals and types of heavy metals. The ANOVA result was presented in Table 3.

There are two null hypothesis: one for the rows and the other for the columns. H_0: Heavy metal concentrations between the study sites are the same. Since in flowers, the p-value for the, rows = 0.0187< .05 = α (or F = 3.28 > 2.75 = F-crit). We reject the null hypothesis, and so we conclude that there is a significant difference in the heavy metal concentrations in flowers of S. auriculata between the four study sites. The null hypotheses for the columns is H_0: there is no significant difference in concentrations between the heavy metal types. Since in flowers, the p-value for the, columns = 0.0425 > .05 = α (or F = 2.90> 2.80 = F-crit). We reject the null hypothesis, and so we conclude there is a significant difference in the concentrations for the six types of heavy metals. From two-way ANOVA results, there seemed to be wide variation in the heavy metal concentrations of S. auriculata collected from natural and polluted sources.

Statistically, the plant collected from site 3 had higher concentrations of heavy metals followed by site 4 than site 1 & site 2 (natural sources). Heavy metal pollution from industrial sources navigates its way to plants via soil and water.

Though all the six heavy metal content were found statistically significant in *S. auriculata*. Zn was found significantly greater than the remaining metals.

Table 3: Two way ANOVA for different study sites Vs Concentrations of heavy metals and types of heavy metals in *S. auriculata* flowers

Site wise Demographical statistics						
Samples	Count	Sum	Average	Variance		
A	6	7.64	1.273333	1.824707		
B	6	10.71	1.785	4.10851		
C	6	91	15.16667	427.9643		
D	6	73.34	12.22333	208.5679		
Element wise Demographical statistics						
Pb	4	21.18	5.295	35.5617		
Cr	4	6.04	1.51	1.3234		
Cd	4	0.49	0.1225	0.016625		
Zn	4	97.33	24.3325	767.2384		
Cu	4	20	5	11.89287		
Fe	4	37.65	9.4125	41.90289		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sites	914.82	3	304.94	3.287382	0.018783	2.757164
Heavy metals	1553.34	5	310.668	2.901295	0.042521	2.808954
Error	1658.987	15	110.5992			
Total	4127.147	23				

SS sum of square, df degree of freedom, MS mean square, *significant at p<0.05 level.

Fe and Pb are moderately varied, Cr, Cd, and Cu were showed low variance. These variations in heavy metal concentrations across the samples analyzed might be attributed to a variety of factors, including pH, temperature, redox potential, cation exchange capacity, and organic matter (*Gregor*, 2004). Heavy metal accumulation in plants is determined by plant species, and the effectiveness of various plants in absorbing metals is measured by either plant absorption or metal transfer factors from soil to plant (*Khan et al.*, 2015). Heavy metals from the soil may also be transferred to the plants. As a result, additional knowledge on plants that grow in metal-rich soils is needed to identify their potential for contaminated soil management.

CONCLUSION

When we compared the results of the heavy metal analysis, we discovered that the plant samples collected from natural sources had all of the heavy metal concentrations below the permissible limit, whereas the plants collected from polluted sources had concentrations of some heavy metals above the permissible limit. As a result of this finding, it is plausible to assume that heavy metal pollution has an impact on plants cultivated in contaminated environments. This study found that medicinal plants used for human consumption and standardized extracts should be collected from an unpolluted natural environment.

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