fortune Journals

Research Article

JOURNAL OF ANALYTICAL TECHNIQUES AND RESEARCH

ISSN: 2687-8038

Application of Atomic Absorption Spectroscopy to determine Mineral and Heavy Metal distribution level of Medicinal Plants

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Abstract

This study was done to apply the technique of atomic absorption spectrophotometery in the field of pharmacy to ensure the quality and purity of herbal plant material. In this regard samples of *Justicia adhatoda* were collected, dried, weighted, digested and analyzed using Atomic Absorption Spectrophotometer for their mineral contents (Copper, Chromium, Zinc, Cobalt, and Iron) as well as toxic heavy metal (Lead and Cadmium) contamination. A comparative study of two different sample preparation techniques i.e. dry ashing and wet digestion protocols was carried out in order to observe the effects of sample pre treatment on the qualitative and quantitative analysis prior to atomic absorption spectroscopic analysis. After spectroscopic analysis statistical evaluation of the data was performed using. On the basis of statistical evaluation using t-test ($p<0.05$), it was concluded that wet digestion method was found more significant for the analysis of Pb, Cu, Zn, Ni, and Fe. Moreover another comparison was also performed among 3 different samples collected from three various sites of Karachi city to detect changes in metallic composition. Application of one way ANOVA followed by Duncan test indicated no significant difference in Pb, Cd, and Zn concentrations collected from all three zones($p<0.05$). It can be concluded that atomic absorption spectroscopy can successfully utilized to monitor toxic metal contamination as well as to determine essential metal profile in herbs.

Keywords: medicinal plants; heavy metal toxicity; quality control; atomic absorption spectrophotometer.

Introduction

A variety of classical and advanced analytical techniques are available for the determination of heavy metals in medicinal plants [1]. Among all available techniques Atomic Absorption Spectroscopy (AAS) is one of the most advanced, sophisticated, reliable, and sensitive technique for the elemental analysis of medicinal plants. Flame atomic absorption spectroscopy (FAAS), Graphite furnace atomic absorption spectroscopy (GF-AAS) also known as Electro thermal AAS, Mercury Hydride system atomic absorption spectroscopy (MHS-AAS) are the various types of AAS to carry out both qualitative as well as quantitative analysis [2]. Prior to atomic absorption spectroscopy, matrix degradation of plant samples is very important step towards the accurate elemental analysis. Purpose of sample digestion and matrix degradation is to remove all organic matter leaving behind the metallic contents. Dry Ashing and wet digestion methods are the two important sample digestion protocols used for elemental analysis [3]. It is a common misperception that being natural in origin herbs and related herbal formulations

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Citation: Bushra Hina, Ghazala –H- Rizwani, Uzma Naseeb, Ambreen Huma, Zeeshan Hyder. Application of Atomic Absorption Spectroscopy to determine Mineral and Heavy Metal distribution level of Medicinal Plant. Journal of Analytical Techniques and Research 5 (2023): 26-32.

Received: December 01, 2023
Accepted: December 11, 2023
Published: December 28, 2023

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DOI:10.26502/jatr.38

is safe and can be taken as self-medication without consulting herbal specialist. But the idea is dangerously false because the green pharmacy has been originated from nature, so any disturbance in environment will ultimately affect the quality of phytopharmaceuticals made from it. Heavy metal contamination of herbal drugs is one of the most burning issues as far as the quality and safety of herbal medicaments is concerned [4-6]. It is very important to check the heavy metal contamination especially Pb, Cd, As, and Hg in crude drugs as well as in medicinal plants that are going to be used as raw material for herbal formulations [7]. *Justicia adhatoda* is very useful plant from Acanthaceae family that is widely utilized as sedative, expectorant, and antispasmodic. It is commonly known as Aroosa in Pakistan and its leaves are used to treat various respiratory tract diseases including cold, cough, bronchitis, chest diseases, pneumonia, asthma, tuberculosis, chronic bronchitis. Leaves are also effective in diarrhea, dysentery, and malaria fever. Major phytochemicals found in this medicinal plants are alkaloid vasicine, vasicol, adhatonine,vasicinone, and vasicinol responsible for its therapeutic activities [8-10]. Purpose of this study was to compare the effect of wet and dry digestion protocols for sample preparation/matrix degradation of *J. adathoda* leaves for its atomic absorption spectroscopy.

Material and Methods

Chemicals and reagents

Certified Atomic Absorption stock standard solutions 1000 ± 5 ppm were procured from May and Baker Rhone. All chemicals utilized in research were analytical grade purchased from Sigma Aldrich. De ionized water was used for making serial dilutions.

Collectionand Treatment of medicinal plants

Samples of *Justicia adhatoda* (100 g) were collected from local herbal market of Karachi city coded as region 1, region 2, and region 3. Sample was identified and authenticated by Prof. Dr. Ghazal H Rizwani. After collection samples were washed, dried, and grinded, labeled and stored in air tight amber colored jars till further use.

Digestion of plant samples for matrix degradation

Samples of *J. adhatoda* were prepared for atomic absorption spectrophotometric analysis using two different protocols i.e. Dry ashing and Wet digestion protocols following the procedure mentioned in [3].

Wet digestion

1 g sample was weighted and reflex heated with 10 ml of concentrated HNO3 until the formation of red nitrous oxide fumes has been ceased. After cooling at room temperature 4 ml 70% HCLO4 has been added and heated again till evaporation to a small volume. Solution was filtered and volume was made 50 ml with deionized water.

DryAshing

Ash of 1 g sample was prepared in a porcelain crucible by heating the sample at 500 °c overnight in a muffle furnace. After cooling ash was dissolved in 5 ml 20% HCL, warming the solution, then filtered and Volume was made up 50 ml with deionized water.

Instrumentation

Atomic absorption spectrophotometer [3] with flame atomization (FAAS) was used for elemental analysis. All recommended operating parameters for the successful execution of analysis were followed summarized in Table 1.

All measurements were run in triplicate for the samples and standard solutions and the average of these three absorption signals (ppm) were used for subsequent calculations. Calibration curves for all tested metals were obtained after appropriate serial dilution of "certified Atomic Absorption stock standard solutions 1000 ± 5 ppm". In order to maintain quality control and avoidance of any kind of degradation fresh Standard solutions for each metal was prepared.

Data Analysis

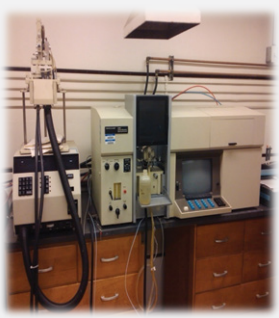
Results observed from atomic absorption spectroscopy were recorded as ppm. This data was used to calculate the concentrations of heavy metals in mg present in per Kg sample of *J. adhatoda*. Statistical evaluation was performed using t-test at significance level of $p<0.05$ in order to find differences between wet digestion and Dry ashing protocols. While to observe statistically significant differences in heavy metals content among samples obtained from regions 1, 2, and 3 ANOVA test was applied following post hoc test.

Results

Concentration of toxic heavy metals (Pb, and Cd) as well as trace elements (Cu, Co, Ni, Zn, Fe, and Cr) in *J. adhatoda* samples was determined by two methods i.e. dry ashing and

Table 1: Instrument setting of atomic absorption spectrophotometer (Perkin Emmer 3030 B)

Elements	Weave Length (nm)	Slit width (nm)	Lamp current (A)
Cu	324.8	0.7	10
Co	240.7	0.2	30
Ni	232	0.2	30
Cr	352.9	0.7	12
Zn	213.9	0.7	10
Pb	217	0.7	10
Cd	228.8	0.7	8
Fe	258.3	0.2	30



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DOI:10.26502/jatr.38

wet digestion. Graphical representation of results obtained from both protocols are mentioned in Figure1a, 1b, 1c, 1d, 1e, 1f, 1g, and 1h.

In order to find significant differences between the two digestion protocols opted for atomic absorption Spectrophotometric analysis, t test was used. Results of statistically significant comparison are given in Table 2.

Table 03 represents the statistical comparison of heavy metal contents of *J.adhatoda* collected from three different regions of Karachi city using One Way ANOVA followed by Duncan test at $p< 0.05$.

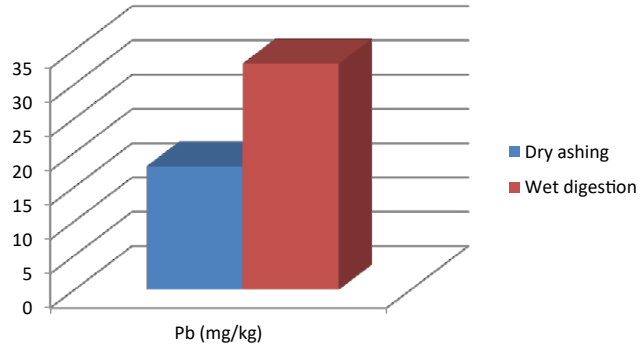

Figure 1a: Concentration of Pb in *J.adhatoda* after wet digestion and dry ashing

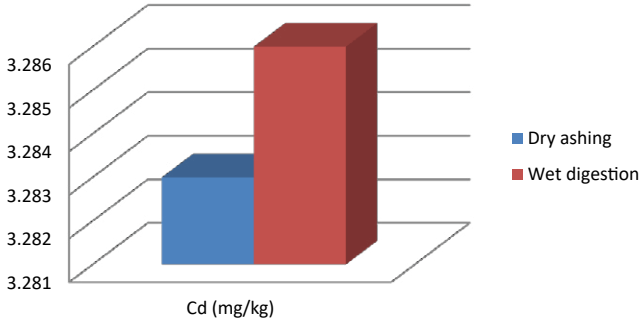

Figure 1b: Concentration of Cd in *J.adhatoda* after wet digestion and dry ashing

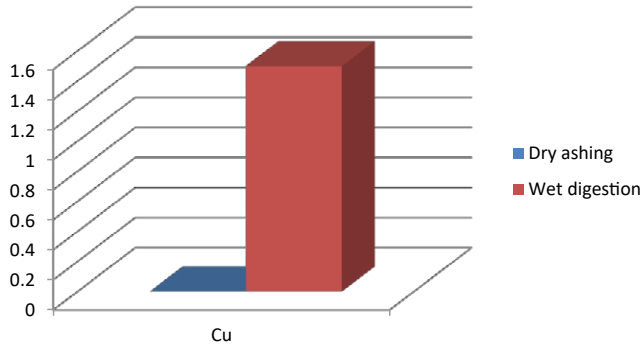

Figure 1c: Concentration of Cu (mg/Kg) in *J.adhatoda* after wet digestion and dry ashing

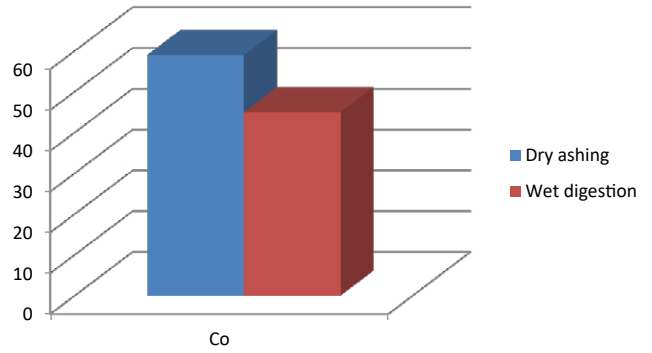

Figure 1d: Concentration of Co (mg/Kg) in *J.adhatoda* after wet digestion and dry ashing

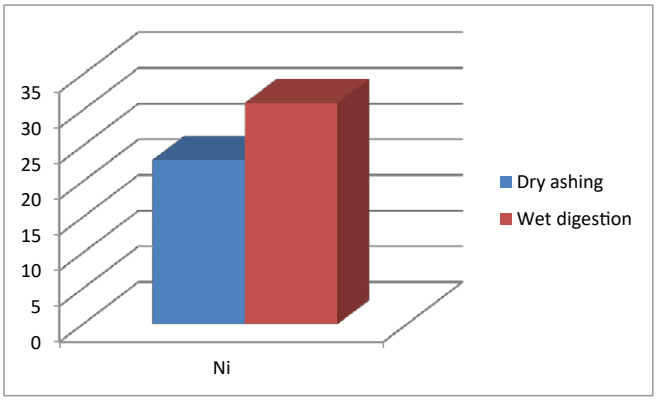

Figure 1e: Concentration of Ni (mg/Kg) in *J.adhatoda* after wet digestion and dry ashing

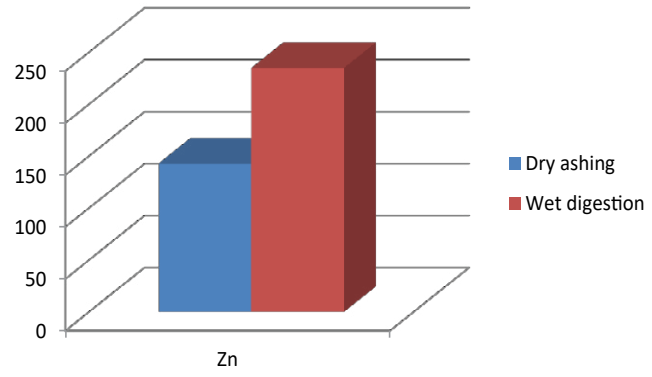

Figure 1f: Concentration of Zn (mg/Kg) *in J.adhatoda* after wet digestion and dry ashing

The average concentrations of toxic metals Pb and Cd detected were compared by different allowable limits provided by regulatory authorities and scientific contributors mentioned in Fig 2a and 2b. On the other hand fig 3a-3f represent the theoretical intakes of essential metals (Cu, Zn, Cr, Ni, Co, and Fe) if taken as 1-50 g sample.

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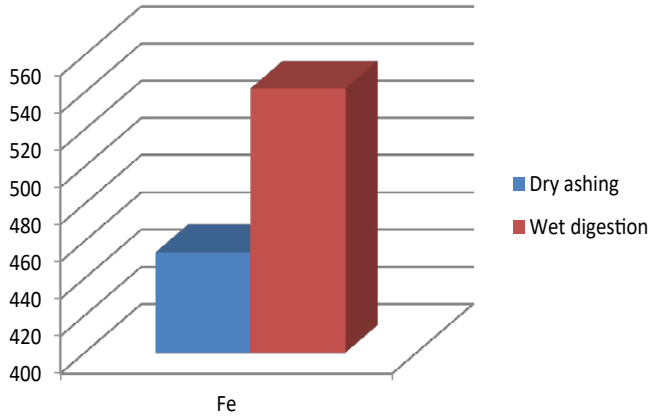

Figure 1g: Concentration of Fe (mg/Kg) in *J.adhatoda* after wet digestion and dry ashing

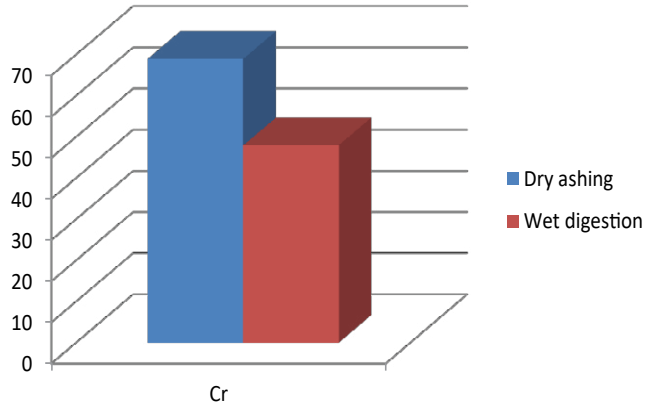

Figure 1h: Concentration of Cr (mg/Kg) in *J.adhatoda* after wet digestion and dry ashing

Table 02: comparison of Heavy Metal contents (mg/kg) in *Justica adhatoda* followed by two different sample preparation methods opted for AAS

Heavy metals		Concentration after Dry Ashing (mg/Kg)	Concentration after Wet Digestion (mg/Kg)
Toxic metals	Pb	18	33*
	Cd	3.283**	3.286
Essential Metals	Cu	0	1.5*
	Co	59**	45
	Ni	23	31*
	Zn	143	235*
	Fe	454	542*
	Cr**	69	48

* Each value represents mean of three replicates. Results are significant at p< 0.05 using t-test.

** No significant difference is noted using t- test at p< 0.05

Table 03: comparison of heavy metal contents (mg/kg) of *J.adhatoda* collected from different zones of Karachi city

Collection Zones	Heavy Metals* (mg/kg)		Trace elements* (mg/kg)					
	Lead	Cadmium	Copper	Zinc	Chromium	Nickel	Cobalt	Iron
Region 1	32	3.28	1.14^{b}	235	47.65^{b}	31.22^{b}	45.33^{b}	542.34^{a}
Region 2	17	0	0.00^{a}	228.3	0.00^{a}	57.09^{c}	53.66^{b}	1508^{b}
Region 3	29	0.003	1.98^{c}	234.7	.003^{a}	2.81^{a}	0.00^{a}	529.09^{a}

*each value represents the mean of three observations. Mean followed by same letter in a column represent no significant difference One Way ANOVA following Duncan test (p<0.05)

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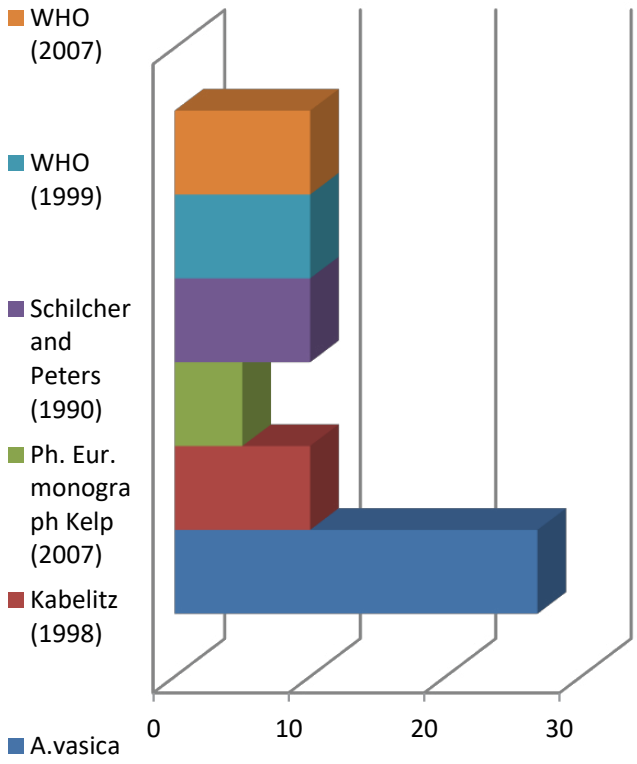

Figure 2a: Comparison of Pb (mg/kg) content of *J. adhatoda* with allowable limits

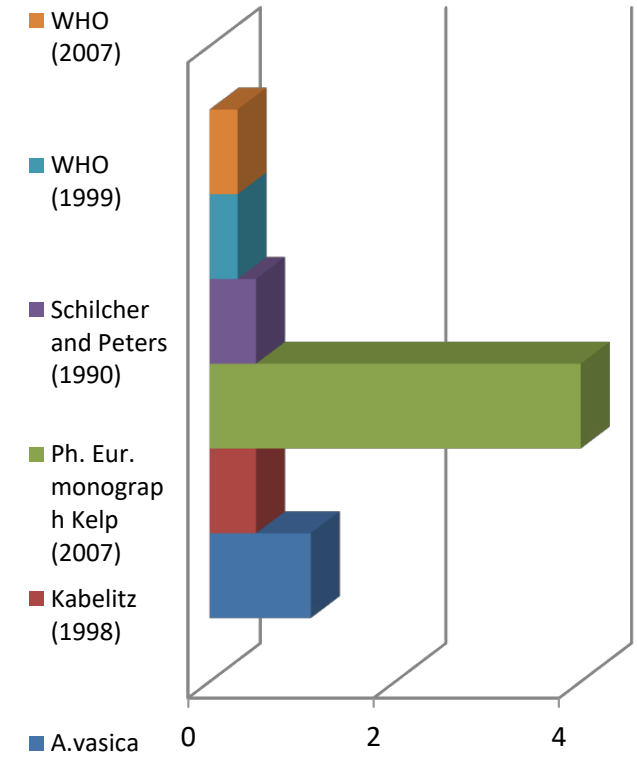

Figure 2b: Comparison of Cd (mg/kg) content of *J. adhatoda* with allowable limits

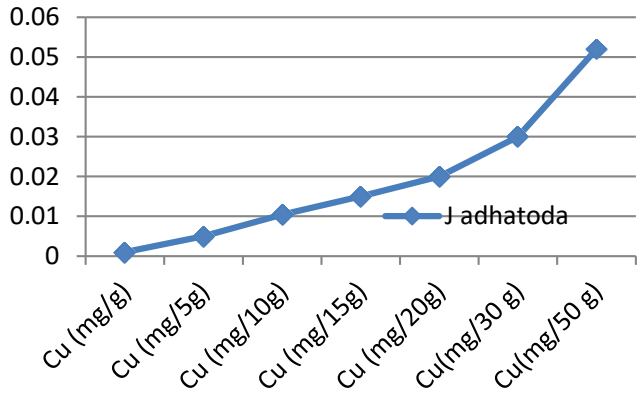

Figure 3a: Theoretical intake of Cu from 1-50g *J.adhatoda*

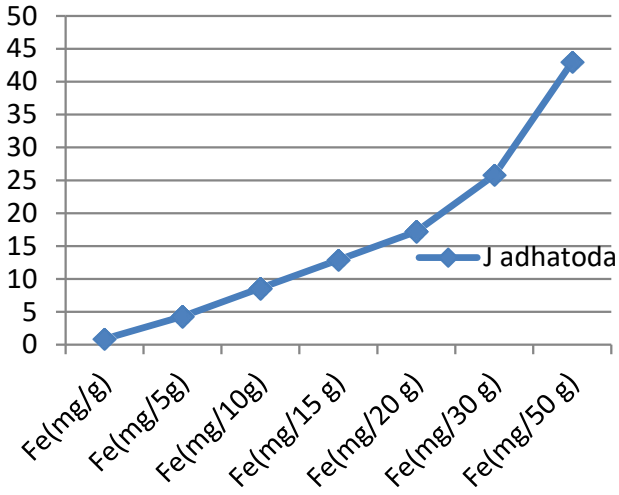

Figure 3b: Theoretical intake of Fe from 1-50g *J.adhatoda*

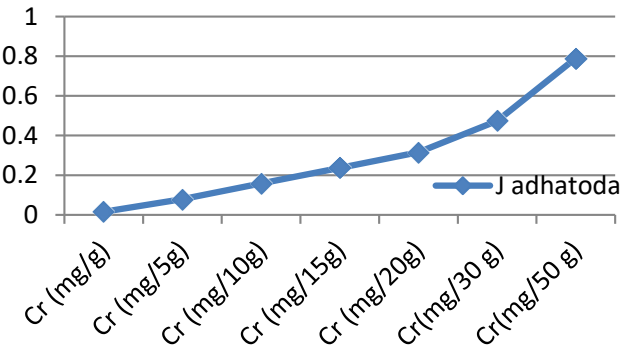

Figure 3c: Theoretical intake of Cr from 1-50g *J.adhatoda*

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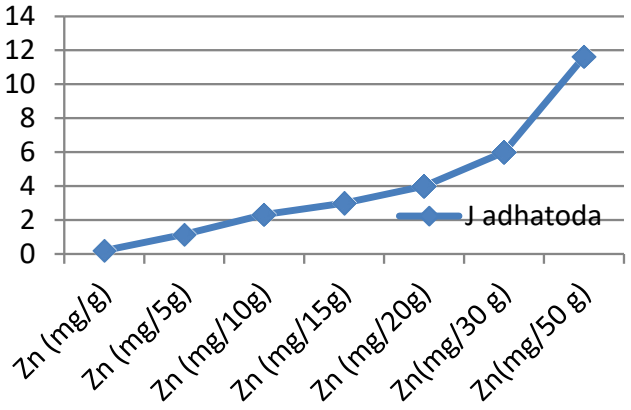

Figure 3d: Theoretical intake of Zn from 1-50 g *J. adhatoda*

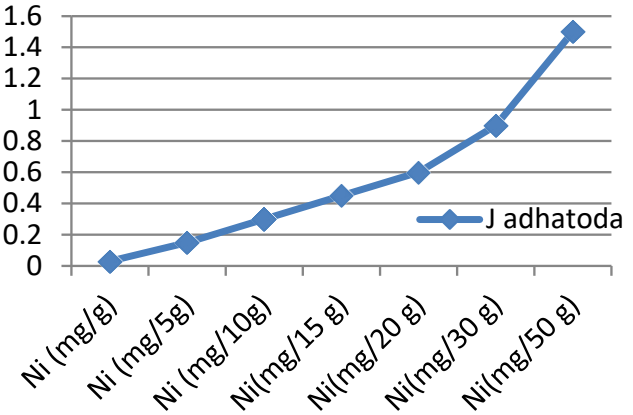

Figure 3e: Theoretical intake of Ni from 1-50 g *J. adhatoda*

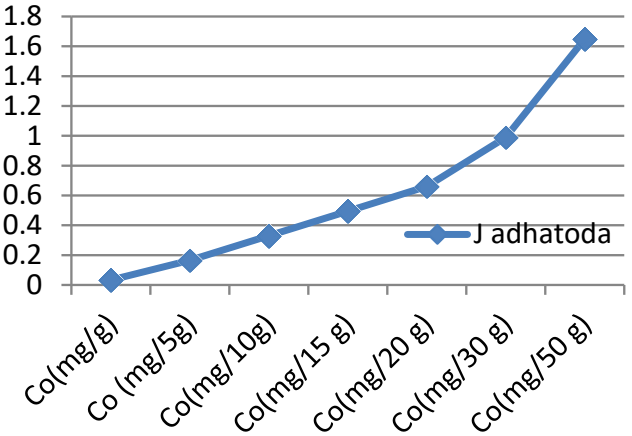

Figure 3f: Theoretical intake of Co from1-50g *J.adhatoda*

Discussion

Justicia adhatoda is widely used both as crude drug in complementary and alternative system of medicines and as raw material for herbal formulations by herbal industries especially in respiratory tract diseases. Being natural in origin there are chances of contamination of toxic heavy metals like Pd, Cd, As, and Hg and other environmental pollutants. Moreover medicinal plants contain some essential metals also that are not only necessary for plant life and metabolism but also beneficial for human consumption provided that their concentrations remain in safety limit [11, 12]. So it is very important to detect any kind of toxic metal contamination as well as concentration of essential metals for the safety of consumers. Technique of AAS is successfully utilized in the qualitative and quantitative analysis of heavy metals in phytopharmaceuticals. However accuracy of analytical results is mainly dependent on complete matrix degradation of organic matter of plant samples. This paper describes the comparison of two different sample preparation techniques i.e. wet digestion and dry ashing protocols for the atomic absorption spectroscopy. Results indicates the presence of all trace elements in the samples treated with wet and dry digestion except Cu that was not detected using dry ashing procedure (Figures1a-1g). Although both methods can be successfully utilized for the detection of elemental composition but the wet digestion was found statistically significant for the analysis of lead, copper, zinc, nickel, and iron (Table 02). Karachi is counted as one of the most polluted city in the world. Due to this reasons all specimens of *J adhatoda* sampled from regions 1, 2, and 3 was not only found contaminated with Pb and Cd, but trace element levels were also towards higher side (Table 3, Figures 2a and 2b). Pb and Cd are the two major toxic metals that are not required by the body and can produce deleterious effects on various body organs and systems. The average contents of Pb and Cd were compared with different allowable limits given by different regulatory and scientific contributors [13, 14] that are mentioned in Figs. 2a and 2b. Unfortunately mean concentrations of Pb and Cd were found beyond the allowable limits.

Essential metals (Cu, Zn, Cr, Ni, Co, and Fe) are required in trace quantities to regulate general metabolism and physiological activities of human beings. However reference daily intakes are recommended for the safe consumption of theses trace elements 15-18]. Figures 3a-3f presents graphical comparisons of theoretical elemental intake upon consumption of *J. adhatoda* in the dose range of 1-50 g. I t can be concluded that higher the dose of medicinal plant, higher will be the amount of essential metals reach to the body. The herb is found as a good source of essential metals but quantities should not exceed the safety limits.

Conclusion

Atomic absorption spectroscopy can be successfully employed in toxic heavy metal analysis as well as elemental profile using both wet digestion and dry ashing protocols in medicinal plants. However wet digestion method was found more superior then the dry ashing method in this regard. This analytical technique not only helps in maintaining the quality and purity of medicinal plants but also to monitor contaminants. AAS can be utilized in herbal industries quality control and research organizations working on the efficacy and safety of herbal medicines.

Study limitations

Since the heavy metal uptake depends on various factors as well as environmental pollution, so their concentration may vary from batch to batch.

Acknowledgements

Authors are very thankful to Fuel Research Centre (FRC, PCSIR) Karachi, Pakistan to provide technical support for this study.

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