Journal of Heavy Metal Toxicity and Diseases
ISSN: 2473-6457

Open Access Research Article

The Concentration of Heavy Metals in *Teucrium polium* Leaves: A Native Medicinal Plant of Kohgiluyeh Region

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ABSTRACT

Excessive accumulation of heavy metals in soils, not only leads to environmental pollution but also increases the absorption of heavy metals by plants. The main pathway of human exposure is food consumption. The purpose of this study was to determine the concentration of some heavy metals in *Teucrium polium*, a native medical plant of the kohgiluyeh region. Samples were prepared by dry digestion method and Cadmium (Cd), Lead (Pb) and Copper (Cu) concentrations were determined using Atomic Absorption Spectrometry (AAS). Our results showed a lower average concentration of Cd than other elements (0.17 ppb). About Cu, the maximum, the minimum, and the mean values were 5 ppm, 1.02 ppb, and 2.75 ppb, respectively. Furthermore, analysis of Pb in *T. polium* showed the results were appropriate in the range (4.63 ppb). Consequently, it is suggested these tolerant and native plant species could be used as tools for an effective traditional plant from the point of view of any heavy metal contaminated.

Keywords: Atomic Absorption Spectroscopy (AAS); Heavy metals; Traditional medicines; Pollutants; Environmental pollution

INTRODUCTION

Since the available chemical and synthetic drugs exert a wide range of side effects, the search for new therapeutic compounds with minimized probabilities of side effects has been a priority of pharmacologists and pharmaceutical industries [1]. Some plants are being emphasized to be an important source of new chemical substances with therapy effects [2]. Thus, the study of plant species which is traditionally have been used as homegrown herbal remedies should be seen as a logical research strategy [3]. The *Teucrium polium* L. is a wild growing flowering plant belonging to the family Labiate and is found abundantly in Iran [4]. *Teucrium* species have been used as medicinal herbs as a diuretic, diaphoretic, anorexic, antipyretic, antihypertensive, anti-inflammatory and many of them are used in folk medicine [5]. Aerial parts of *T. polium* L. are used widely in the daily diet and for medicinal purposes. The leaves and flowers of the plant are consumed as a refreshing beverage, as well [6]. Heavy metals are conventionally defined as elements with metallic properties and an atomic number >20 which is they have a specific gravity of more than 5 g/cm^3 [7]. These metallic elements are released by industrial activities, agricultural activities, combustion of fossil fuels and automobiles and pollute air, soils, and waters. Generally, this pollution is an important environmental problem since can

Received:	08-May-2021	**Manuscript No:**	IPJHMCT-22-8427
Editor assigned:	11-May-2021	**PreQC No:**	IPJHMCT-22-8427 (PQ)
Reviewed:	25-May-2021	**QC No:**	IPJHMCT-22-8427
Revised:	23-December-2022	**Manuscript No:**	IPJHMCT-22-8427 (R)
Published:	04-January-2023	**DOI:**	10.21767/2473-6457.23.8.1.06

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Citation Tehrani ES, Peikara A (2023) The Concentration of Heavy Metals in *Teucrium polium* Leaves: A Native Medicinal Plant of Kohgiluyeh Region. J Heavy Met Toxicity Dis. 8:06.

affect human health [8]. Plants require many elements to complete their life cycles, including the heavy metals Copper (Cu), Zinc (Zn) and Nickel (Ni). Conversely, sometimes accumulating nonessential metals such as Lead (Pb) and Cadmium (Cd) in plant tissues, when they are present in the environment and could have a negative influence on the physiological activities of plants (e.g. photosynthesis and nutrient absorption), determining the reductions in plant growth and dry matter accumulation. Most metals contained within plant tissues are acquired from the soil solution, and plants have evolved elaborate rooting systems and transport mechanisms to mediate this process [9]. The traces of heavy metals in low concentrations on plants or animals are not toxic. But Pb, Cd and Mercury (Hg) are exceptions; they are toxic even in very low concentrations and long half-life in humans and other animals [10]. Cu and Zn are essential for plant growth and development because these metals are structural components of many enzymes and protein [11]. Pb not only affects the microbial activity of the soil and the loss of soil fertility but also induces changes in physiological parameters, resulting in inhibition of the plant growth and ultimately reducing their performance of these physiological parameters [12]. It is a widely accepted concept that plants with exceptional metal accumulating capacity are known as hyper accumulator plants. The main problem with heavy metals is that the emissions of inorganic heavy metals, unlike organic pollutants, are not biodegradable. In point of fact, heavy metals and environmental pollutants have comprised one of the most dangerous groups [13]. The toxic heavy metals are required as alternative salts and minerals in the body. The material begins to deposition in the vessel tissue, muscles, bones, and joints, over time [14]. Heavy metal toxicity can lead to complications, such as neurological disorders, cancer, nutrient deficiency, hormone imbalance, miscarriage, respiratory disorders, cardiovascular disease, and damage to the liver, kidneys, brain, allergies, anorexia, premature ageing, memory loss, hair loss, osteoporosis, insomnia, weakened immune system, anemia, genetic damage, and even death. Heavy metals can also increase the acidity of the blood as a result of the body maintaining proper blood pH in the body; as a result, calcium is taken out of the bones [15]. The World Health Organization (WHO) has declared the maximum permissible rates of Cd, Arsenic (Ar), and Pb for medicinal plants to be 1, 0/3, and 10 mg/kg, respectively. Even some elements, including Cu, Zn, manganese, molybdenum, and Ni can be toxic at high levels; However, WHO to date has not imposed restrictions for on these elements in medicinal herbs [16,17]. This study aimed to determine the Pb, Cd and Cu concentrations of the leaves of *T. polium* from the wild around Kohgiluyeh region; the leaves of the plant are used in the treatment, prevention and management of different illness. Also, it was assessed whether residual metal levels are within or above maximum allowable regulation limits.

MATERIALS AND METHODS

Sample Collections

Samples of *T. polium* leaves were taken from a shrub growing in urban areas of are Kohgiluyeh. Each leaf was picked on the side of the crowns facing heavy traffic streets. The samples were collected as random with 3 repetitions in form of 3 samples composed of each section in clean cellulose bags separately and were brought to the laboratory on the same day. Pharmacognostic features of the medicinal herb under study are mentioned in **Table 1**.

Table 1: Pharmacognostic features of tested medicinal herb.

Plant species	Family	Part used	Medicinal importance
Teucrium Polium	Labiatae	Leaves	Antioxidant activities, anti-mutagenic activity, cytotoxic and anticancer activities, anti-inflammatory activity, antibacterial, antiviral and antifungal activities.

Instruments and Reagents

Heavy metals determination in the *T. polium* leaves was done using varian atomic absorption spectroscopy AA240FS (Analytik Jena AG, Jena, Germany). Standard operating parameters were set and given in **Table 2**. The hollow cathode lamps for Cu, Pb and Cd (Shimadzu) were used as a radiation source and fuel was pure argon (99.999%). All the samples and standard were run in duplicate. All containers and glassware were cleaned by soaking in the 3 mol/L HNO_3 for at least 24 hours and rinsed three times with deionized water before use. A TE313S electronic balance (Sartorius, Gottingen, Germany) was used to weigh the samples. An ETHOS, a microwave digestion system (Milestone, Milano, Italy) was used for the digestion of the samples.

Table 2: Working parameters of atomic absorption spectrophotometer.

S. No	Element	Wavelength (nm)	Slit width
1	Cd	228.802 nm	0.5 nm

2	Cu	324.754 nm	0.5 nm
3	Pb	283.306 nm	0.5 nm

Sample Preparation

After drying to a constant weight, the *T. polium* leaves samples were ground into powder. Approximately 0.40 g of dried leaves powder was weighed and added into the Polytetrafluoroethylene (PTFE) digestion vessel with 3 mL of concentrated HNO_3 and 1 mL of hydrogen peroxide (H_2O_2). Subsequently, the samples were digested using a two step temperature program. During the first step, the temperature was linearly increased to 190°C for 10 minutes; the maximum power of the rotating magnetron was 1000 W. During the second step, the temperature was maintained at 190°C for 25 minutes. After digestion and cooling, each solution was evaporated to ~2 mL and diluted with deionized water in a 50 mL volumetric flask for the AAS analysis. The results were reported as the average of three repeated measurements, and all digestions were conducted in duplicate.

Standard Preparation

The standard solutions for all the heavy metals under study were prepared in three to five different concentrations to obtain a calibration curve by diluting stock standard solution of concentration 1000 ppm. These solutions were prepared daily using appropriately diluted dilutions of the stock standard solutions. Next, 15.0 µL of the sample solution or the standard solution was transferred to AAS together with the modifiers.

RESULTS AND DISCUSSION

Heavy metals are classified as an environmental pollutant category due to their toxic effects on plants, human and food and they are introduced into the environment through so many sources which include, decomposition of fossil fuels, smelting, and the density of traffic [18]. Since have been reported heavy metals like Pb and Cd have no known bio importance in human biochemistry and physiology hence their consumption even at very low concentrations can be toxic. Rain and dust, according to Srinivas, deposit atmospheric metals on plant surfaces. Airborne pollutant could cling to leaf surfaces, and some elements could enter through the stomata and accumulate in leaf tissues REF 20. Heavy metals concentrations in methanol extracts of a medicinal herb from Kohgiluyeh were determined using atomic AAS and results obtained were tabulated in **Table 3**. The Heavy metals content of *T. polium* as an herbal drug is mentioned in **Figure 1**.

Table 3: Heavy metal contents (ppm ± SD) in Teucrium polium.

S. No	Heavy metals	Concentration	Maximum	Minimum
1	Cd	0.99 ± 0.44	0.17	0.01
2	Cu	2.75 ± 1.28	5	1.02
3	Pb	4.63 ± 1.56	8.02	2.67

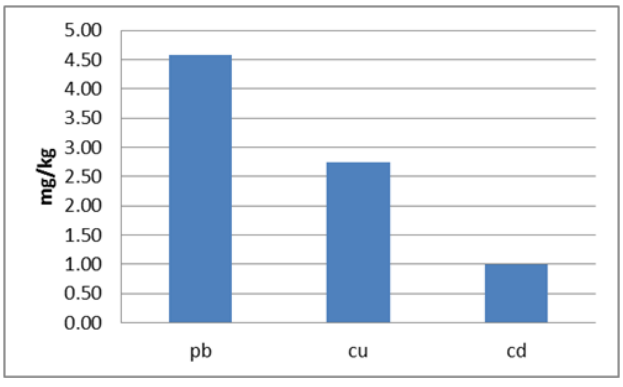

Figure 1: The content of heavy metals Pb, Cu, and Cd in *Teucrium polium* samples.

Optimization of the Atomic Absorption Spectra

AAS detection was carried out on positive ionization mode because this mode gave sharp and sensitive signals. It was optimized by using a standard linear calibration curve for various concentrations 0.5000 ppm, 1.0000 ppm, and 1.5000 ppm (3 points). In our research, the calibration curves were constructed by plotting the response against the concentration. Therefore, a linear relationship was obtained for each compound. The heavy metals (Cd, Pb, and Cu) were analyzed at their particular wavelength, and the ion with the uppermost intensity was selected as the basic ion. The maximum and the minimum concentrations of Cd in *Teucrium polium* were detected at 0.17 ppb and 0.01 ppb. Our findings for Cd in the leaves samples showed lower levels. The samples have lower Cd content than critical levels for plants (0.99 ppm). However, the comparison of concentrations of all metals showed that the Pb one had the highest concentration of all (4.63 ppm). The content of Pb in the analyzed samples of *T. polium* ranged from less than 2.67 ppb to 8.02 ppb as the minimum and the maximum concentrations of Pb, respectively. The FAO/WHO maximum permissible limit of Pb in the medicinal herbs consumed is 10 mg/kg. The environmental pollution

of Pb greatly influences the concentrations of this metal in plants. Pb is known as a deadly and cumulative poison even when consumed in small quantities and is capable of deadening nerve receptors in human. Many studies, have reported the relationship between Pb concentrations and traffic intensity. Recently, Pb concentrations in vegetation grown in industrial and urban areas have increased owing to human activities, road traffic and other indoor or outdoor sources REF. One of the highly toxic environmental pollutants is known Pb. It can combine with various biomolecules and can adversely affect their functions. Exposure to Pb may have a harmful effect on the blood, nervous, immune, renal and cardiovascular systems, causing gastrointestinal symptoms, brain and kidneys damage, hearing and vision impairments. According to our results, fortunately, the obtained concentration of Pb in plant under our research was observed in the standard range. Cu is an important component for many enzymes, which catalyze oxidation and reduction reactions. The main sources of Cu are home tools production, metal manipulating, road traffic and ashes reported that the normal content of Cu in plants ranges to be 2-20 ppm, but in most cases it is in a narrower range of 4-12 ppm. According to these values, the Cu concentrations found in this study are near the normal limits (2.75 ppm). The content of copper (cu) in the analyzed samples of *T. polium* ranged from less than 1/02 ppb to 5 ppb as the minimum and the maximum concentrations of copper, respectively. The regulatory limits of the WHO/FAO have not yet been established for the copper in herbal medicines. China and Singapore have set limits for copper in medicinal plants at 20 and 150 mg/kg, respectively. Cu is an essential component of many enzymes, thus playing a significant role in a wide range of physiological processes, including iron utilization, elimination of free radicals, development of bone and connective tissues, melanin production. Nevertheless, excessive intake of cu can cause dermatitis, irritation of the upper respiratory tract, abdominal pain, nausea, diarrhea, vomiting, and liver damage. The bioavailability of metals is influenced by several factors, among which are the soil pH, the metal levels in the soil, the oxidation reduction potential of the soil, and other chemical and physical factors. In general, herbs can be contaminated during growing, harvesting, and processing. The sources of heavy metal contamination in herbs could be linked to the water used in irrigation, polluted soils, fertilizers and pesticides, industrial emissions, transportation, and harvesting and storage processes. Due to metal contamination, the health risk generally, depends on the average daily dietary intake.

CONCLUSION

In this study, the contents of Pb, Cd and Cu in *T. polium* leaves in kohgiluyeh region were analyzed using AAS. The Pb, Cu, and Cd levels in *T. polium* were under the prescribed limits; The results of data analyses speculated, *T. polium* as a native plant species could be used as tools for an effective herb native plant from the point of view of any heavy metal contaminated and it will serve as a good plant with beneficial therapeutic properties. However, further studies to identify the active components and further verify their relevant pharmacological activities are warranted.

CONFLICT OF INTEREST

None declared

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