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Research Article

The Influence of the Sensitivity of the ICP-MS and AAS Method for the Determination of Cadmium, Lead and Arsenic in Lettuce (*Lactuca sativa* L.) on the Results of the Analysis

Amra Bratovcic[1]*, Amna Dautovic[2] and Edita Saric[3]

[1] *University of Tuzla, Faculty of Technology, Urfeta Vejzagica, Bosnia and Herzegovina, Europe*

[2] *Multilab, Plane bb, Bosnia and Herzegovina, Europe*

[3] *Federal Institute for Agriculture in Sarajevo, Ilidza, Bosnia and Herzegovina, Europe*

***Corresponding Author:** Amra Bratovcic, University of Tuzla, Faculty of Technology, Urfeta Vejzagica, Bosnia and Herzegovina, Europe.

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Abstract

In this paper the concentrations of heavy metals such as lead (Pb), cadmium (Cd) and arsenic (As) were determined in dried lettuce leaves under greenhouse cultivation. They were determined by two methods: using inductively coupled plasma mass spectrometry (ICP-MS) and atomic absorption spectrometer (AAS). The percentage of humidity was 10.7 %. Concentrations of heavy metals determined by the ICP-MS method were slightly higher than by AAS indicating higher sensitivity of the ICP-MS method. The highest concentration was found for cadmium (1.2 mg/kg), then for arsenic (0.186 mg/kg), and finally for lead (0.04 mg/kg). The obtained results indicate that lettuce cultivated in soil irrigated with tap water containing dissolved mineral fertilizers under commercial name starter 1, 2, and 3 can elevate the concentration of heavy metals in edible parts of the crop, affecting food safety and public health worldwide.

Keywords: Lettuce, Cadmium, Arsenic, Lead, ICP-MS method, AAS method, Food contamination

Abbreviations

ICP-MS: Inductively Coupled Plasma Mass Spectrometry; AAS: Atomic Absorption Spectrometer

Introduction

A wide variety of phytochemicals with antioxidant properties are present in fruits and vegetables that may contribute to health-promoting benefits [1]. Suggesting that phytochemicals can prevent a number of chronic and degenerative diseases [2] many dietary guidelines recommend fruits and vegetables as part of a daily diet [3]. Lettuce (*Lactuca sativa L.*) is known to contain phytochemicals, including vitamins, carotenoids, and other antioxidants [4,5]. Lettuce is one of the leafy vegetables most consumed in raw form for its good taste, low price, and high nutritive value [6]. Tuberous and leafy vegetables tend to accumulate higher concentration of heavy metals than fruits and grains [7].

Heavy metals are non-biodegradable and persistent and are known to cause deleterious effects on animal and human health. Owing to industrialization, heavy metal pollution of aquatic ecosystems has become topic of concern worldwide [8].

The essential mineral elements are classified as macronutrients (N, K, Ca, Mg, P, and S) and micronutrients (Cl, Fe, Mn, B, Zn, Cu, Mo, and Ni). Six elements (C, H, O, N, P, and S) are constituents of organic compounds, such as protein, sugar and nucleic acid. Micronutrients (Fe, B, Mn, Cu, Zn, Mo, Cl, and Ni) are found in the lowest concentrations in plant tissues. However, those in plant tissues must be present at certain concentrations, not only to achieve high plant productivity, but also to survive.

While some metals are essential for human health at trace concentrations due to their role as coenzymes (Fe and Cu), others are toxic at any concentration level (Pb and Cd). Chromium (Cr VI), cadmium (Cd), lead (Pb), and arsenic (As) have been recognized as water, soil, and sediment pollutants in many places in the world [9].

Non-essential metals like cadmium (Cd) and lead (Pb) are released to the surrounding environment through various ways including natural as well as anthropogenic sources. They are even at low concentrations toxic and can cause serious disorders and health problems in plants, animals and human beings because of their non-biodegradablity, higher bioaccumulation rate and biotoxicity. Toxic metals like Cd can cause serious health hazards on exposure, as they are cytotoxic and cause cancer and mutation in humans [10].

Recently, the concentration of some important biogenic elements such as Mg, Fe, Cu and Zn which are known cofactors in enzymes were determined in chia seed [11]. The same authors discussed the role of each biogenic element in many biological processes and enenzymes.

The less toxic heavy metals such as Fe and Zn at normal background concentrations are essential nutrients for plant growth may pose reduced risk to human health. However, at elevated concentrations, these metals deplete some essential nutrients, retard intrauterine growth, cause immunological weakness, organ damage, and reproductive dysfunction in human [12]. More toxic metals such as Cd, Hg, Ni, and Cr even at low concentrations contribute to upper gastrointestinal cancer, lung cancers, postmenopausal cancer, renal tubular dysfunction and anaemia in human [13]. Furthermore, these metals are persistent in the environment and bioaccumulate in human tissues [14].

By the the development of various antioxidant defense systems, plant cells have been able to alleviate potentially harmful effects and damage that may be caused by free radicals [15]. A various antioxidant defense systems such as superoxide dismutase (SOD), catalase, (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), and glutathione reductase (GR) and nonenzymatic antioxidants which includes ascorbate (AsA), glutathione (GSH), carotenoids, alkaloids, tocopherols, proline, and phenolic compounds (flavonoids, tannins, and lignin), serve as free radical scavengers [16,17].

Lead (Pb)

Lead is the second most toxic metal on Earth and is toxic to humans. In plants, Pb commonly inhibits growth when it is at a concentration in the soil of 30 mg/kg or more. The research carried out by [18] has shown a progressive increase in enzymatic activities of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), and glutathione reductase (GR) due to Pb treatment. The results of this study suggest that *T. qataranse* is a Pb hyperaccumulator. Increased antioxidant enzyme activity was essential to maintaining cellular homeostasis and assisted in the arid plant's tolerance to Pb stress.

Cadmium (Cd)

Cadmium is a non-redox heavy metal which can indirectly increase the production of reactive oxygen species (ROS), inducing cell death. The Cd stress significantly reduced the growth and increased the electrolyte leakage (EL), hydrogen peroxide (H_2O_2) concentration and malondialdehyde (MDA). Moreover, Cd stress, particularly 50 μM and 100 μM, decreased the activity of different antioxidant enzymes, including superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) [19]. The analysis of the effects of cadmium (Cd) on plant cells is crucial to understand defense mechanisms and adaptation strategies of plants against Cd toxicity. The results of research carried out by [20] show that Cd application in concentrations of (10, 50, 100, and 200 μMCdSO4) on Pisum sativum L. caused a decrease of all investigated antioxidative enzyme activities such as catalase (CAT), ascorbate peroxidase (APX), and guaiacol peroxidase (GPX).

Sharma., *et al*. 2020 [21] highlight that micronutrients are trace elements required in very small amounts in the diet and metals such as copper (Cu), iron (Fe), and zinc (Zn) are essential nutrients that are required for various biochemical and physiological functions.

In another study carried out by the same authors, the concentrations of Cu, Fe, Zn and Cd have been determined in the white quinoa and amaranth by ICP-MS analysis [22].

Concentrations of all examined metals were higher in the amaranth. This research has shown that amaranth and white quinoa could be good sources of essential micronutrients. The concentration of cadmium in amaranth was very close to maximum permitted concentration in food.

Recently, [23] discuss the most common and significant antioxidant enzymes which include glutathione peroxidase, catalase, superoxide dismutase, glutathione reductase, thioredoxin reductase, heme oxygenase, and biliverdin reductase. High levels of antioxidant enzymes are available from some food such as raw vegetables, barley grass, and wheatgrass, but also from algae, yeast, and sprouts. Recently lead Pb, cadmium Cd and arsenic As were tested in 177 samples of fresh vegetables produced in the area of Zenica [24]. In this research were found that different types of vegetables have different ability to adopt and accumulate heavy metals. The intensity of adoption is primarily influenced by the concentration of heavy metals in the external environment, especially the concentration of dissolved (active) forms of metal, the pH value of the soil, the carbonate content and organic matter in the soil and the degree of soil moisture.

Arsenic (As)

Arsenic occurs naturally in air, water and soil, but it can enter the environment through certain agricultural and industrial processes, such as mining and metal smelting. Arsenic is present as a contaminant in food, water and the environment. Arsenic as a harmful heavy metal appears in two forms (organic and inorganic). The inorganic form is more toxic than the organic form. The order of increasing toxicity of As compounds is defined as organic arsenic < metalloid (As^0) < inorganic species (As^{5+} < As^{3+}) < arsine. [25].

The objective of this work was to determine the accumulation of heavy metals in lettuce leaves, grown in greenhouses.

Materials and Methods

Lettuce was collected from greenhouse placed in Tuzla, (44°32'49.1"N 18°39'57.8"E) in Bosnia and Herzegovina. The lettuce is harvested in its full ripening phase. After the lettuce was harvested, the root was removed and the lettuce leaves were washed several times with clean tap water. Therefore, the sample was prepared from the leafy part of the plant. After washing, lettuce leaves were air-dried for 14 days and then ground using a grinder AD 443, Adler Europe, 150 W.

Heavy metal	Critical concentration (mg/kg)	Toxic concentration (mg/kg)
Pb	10	20
Cd	5	10

Table 1: Average critical and toxic concentrations of heavy metals for cultivated plants [26].

In figure 1 is shown the the appearance of fresh lettuce and after drying.

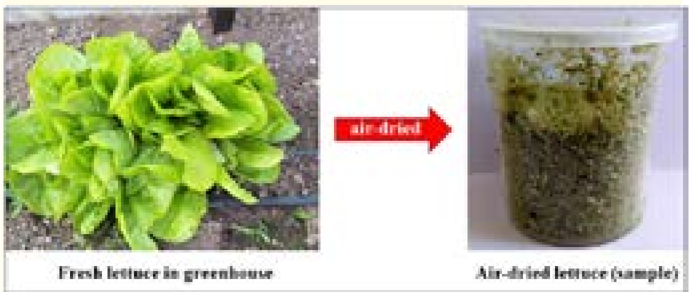

Figure 1

The air-dried sample were oven-dried at 105 °C to remove all moisture content.

Sample preparation for AAS

Digestion of lettuce samples was done in a Perkin Elmer Titan MPS microwave oven. 0.4 g of the sample was weighed, and 10 ml of concentrated TRACE METAL HNO_3 produced by FISHER was added. After the digestion under pressure, the solutions were transferred to 50 ml flasks and diluted with ultrapure water to the mark.

Sample preparation for ICP-MS

A sample of 0.5 g is weighed according to the recommendation of the microwave oven manufacturer into Teflon containers for the microwave digestion workstation, Sineo, Jupiter-B. Weighing is done on an analytical balance with a sensitivity of 0.1 mg. Add 8 ml of nitric acid min. 65% (high degree of purity) for a pre-treatment of 20 minutes, then 3 ml of the same nitric acid and 1 ml of hydrogen peroxide 30%. Hydrogen peroxide in the amount of 1.0 ml is added to prevent the samples from sticking to the walls of the digestion vessel and to achieve complete mixing of the samples with the acid. To check for contamination, prepare a blank in the same way as the sample (it contains the same amount of solvent, except for the analyzed sample). The sample is quantitatively transferred into a 50 ml measuring vessel and topped up to the mark with the standard blank solution.

Analysis by AAS

The samples thus prepared were analyzed on AAS PINAACLE 900 T, manufactured by Perkin Elmer. The test was performed using the graphite technique using lamps with a hollow cathode for Pb and Cd, and EDL lamps for As. The solutions for the calibration curve are made from a multi-element standard solution containing the As, Cd and Pb elements, and 1% TM nitric acid, which is made from ultrapure water and concentrated TM nitric acid.

Argon was used as gas medium. A mix matrix of palladium nitrate and magnesium nitrate modifiers were used for stabilization.

Hg analysis was performed using the hydride technique on a FIMS 100 device manufactured by Perkin Elmer. The solutions used for calibration were made from the same multielement standard. Sodium boron hydride ($NaBH_4$) solutions, produced by ACROS ORGANICS, were used for the analysis as a reducing agent, and 3% HCl, which was made from ultrapure water and concentrated Trace metal hydrochloric acid manufactured by FISHER.

Analysis by ICP-MS

Three different concentrations of calibration solutions were used to calibrate the ICP-MS 7700 Agilent Technologies instrument. The range of concentrations was chosen in relation to the expected concentration of the analyte in the tested sample. The acid concentration in the sample solution and the calibration solution were approximately the same. The standard blank contains the same amount of acid used in the calibration solutions. It is prepared in such a way that the final solution should contain 2% (w/w) nitric acid and 0.5% (w/w) hydrochloric acid. Digested samples were then analyzed for the metal content.

Results and Discussion

Moisture content

Moisture content was determined by weighing the sample before and after drying at 105 °C. Two measurements were taken and the mean value of the moisture content was 10.7%.

Content of heavy metals

Results of mean value of two measurements for Pb, Cd, and As were measured by using AAS (Table 2) and ICP-MS (Table 3).

Method AAS	Pb	Cd	As
[mg/kg]	0.01	1.019	0.01

Table 2: Results of mean value of two measurements obtained by AAS.

Method ICP-MS	Pb	Cd	As
[mg/kg]	0.044	1.2	0.186

Table 3: Results of mean value of two measurements obtained by ICP-MS.

From all examined heavy metals the cadmium (Cd) concentration was the highest (1.019 mg/kg dry matter), followed by, lead (Pb) and arsenic (As) were equal (0.01 mg/kg dry weight) determined by AAS method.

The cadmium concentration was also the highest determined by ICP-MS method, and then followed by arsenic (0.186 mg/kg dry matter) and lead (0.044 mg/kg dry matter). It is noticeable that those concentrations determined by ICP-MS are slight higher than those determined by AAS. Such results can be explained by the greater sensitivity of the GS-MS method.

Sharma., *et al*. (2016) [26] found that not only the crops irrigated with wastewater are hazardous and not safe for human consumption, but also those growing in vicinity of wastewater drain. They also determined hazard quotient and results revealed that it was higher for leafy and tuberous vegetables than the safe limits in all the sites irrespective of mode of irrigation. The concentration of Cadmium (1.20 mg/kg) was higher than permissible limits in many vegetables.

Cadmium is a possible carcinogen and dietary intake of cadmium affects kidneys and liver. Permissible limit for cadmium in leafy and tuberous vegetables is 0.1 mg/kg (FAO/WHO 2014) [27].

Zubillaga and Lavado, (2002) [28] studied the application of different proportions (0-100%) of composted biosolids on the accumulation of heavy metals (Cd, Cu, Ni, Pb and Zn) in lettuce leaves.

They determined mentioned metals uptake after harvest in dry and fresh weight, leaf area. Therefore, they found 0.05 mg/kg of Cd and Pb concentrations in both fresh and in air – dried leaves of lettuce under greenhouse conditions. Zn concentration increased in leaves by decreasing of compost addition, ranging from 57.2 to 80.4 mg/kg. In all treatments the proportions of heavy metals in plants were below the international standards of toxicity.

The heavy metals in the vegetables appeared to originate primarily from fertilizers (mainly organic manure from poultry, and chemical fertilizers) [29]. Contributions from pedogenic weathering of minerals was significantly observed for Fe in this study. Lente., *et al*. 2012 and Alloway, 2013 [29,30] showed that fertilizers such as phosphate, NPK, copper sulphate, iron sulphate, and animal manure commonly introduce Hg, Cd, Ni, Zn, and Cd into the soil.

In figure 2 The concentrations of cadmium in lettuce, chia, amaranth and white quinoa are presented.

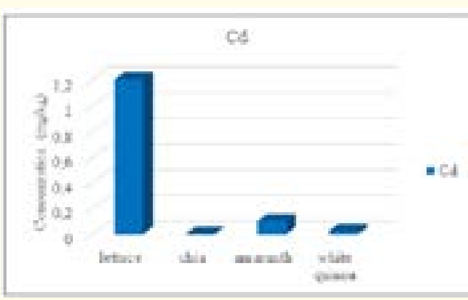

Figure 2

This high concentration of cadmium 1.2 mg/kg in lettuce is significantly higher, while in amaranth was 0.1 mg/kg, in white quinoa seeds was 0.026 mg/kg and in chia was 0.002 mg/kg.

The other heavy metals studied were arsenic (0.186 mg/kg dry matter) and lead (0.044 mg/kg dry matter) in lettuce.

In figure 3 the concentrations of cadmium, arsenic and lead in lettuce determined by ICP-MS and by AAS.

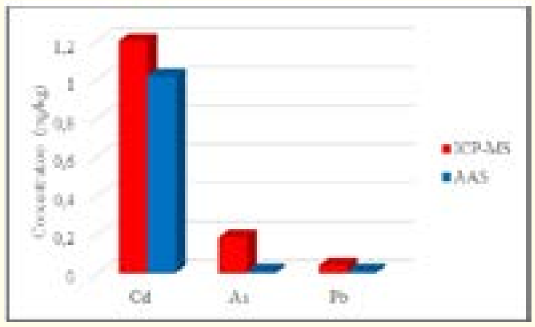

Figure 3

Conclusion

In this paper, the concentrations of lead, cadmium and arsenic were determined using AAS and ICP-MS techniques. All three tested heavy metals are very toxic and are rarely determined in food products. We were interested in determining their concentration in a sample of lettuce, which is known to absorb heavy metals very easily. The lettuce sample was grown in a greenhouse. Certain concentrations of heavy metals were found, but within the permitted limits according to the rulebook we referred to. The research results showed a higher sensitivity of ICP-MS compared to the AAS technique.

Conflict of Interest

The Authors declare no conflict of interest exists.

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