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Research Article

Heavy Metals Accumulation by Aromatic Plant *Salvia Officinalis* Irrigated with Treated Wastewater

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Abstract

The use of wastewater for the irrigation of plants will contribute to the beneficial factors of plant growth, but it can damage human health because of the high concentration of toxic elements. The main goal of this research is to assess the concentration of some toxic heavy metals in the Salvia Officinalis plants after their harvest, which are irrigated with treated wastewater. Salvia Officinalis plants leaves are used in culinary and medicine. Sage plants are cultivated in an experiment using pots, in the greenhouse for 180 days. Treatments aim to assess the amount of elements (Cd, Cr, Cu, Pb, Ni and Zn), that are accumulated in plants irrigated with treated and untreated sewage waters. The concentration of these heavy metals in both treated and untreated sewage waters, are below the maximum permissible level in irrigation waters set by the World Health Organization (WHO). The development of plants and the content of heavy metal in their tissues are estimated. The results show that heavy metals content varied from spot to spot, plant to plant, and also in different parts of each plant. This study confirms that domestic sewage can effectively increase water resources for irrigation but the need for continuous monitoring of the concentration of toxic elements in soil, plants and water, still exists. It is required that plants should be checked for contaminant before processing them for pharmaceutical purposes or for human consumption.

Introduction

The use of urban wastewater in urban farming is a century-old practice that is receiving more attention with the increasing scarcity of fresh water resources in many arid and semi-arid areas (Ruma & Sheikh, 2010). Continuous use of wastewater leads to the enrichment of soil with essential macro and micro-nutrients. Micro-nutrients are beneficial for the growth and metabolism of the plants at lower concentrations, but can become toxic when exceeding the requirement level. Several micronutrients are heavy metals and are known to produce undesirable effects on plants at higher concentrations (Singh & Agrawal, 2010). Treated urban wastewater is a common alternative water source for irrigation in arid and semiarid regions. Impacts of using wastewater can be classified in groups, including public health, effecting crop characteristics, effecting soil properties, effecting groundwater resources, property values, ecological impacts and social impacts. Medicinal and aromatic plants and their preparations belong to the oldest known health-care products that have been used by human beings all over the world. With respect to their legal status, these products are either medicines or food, depending on their use and/or therapeutic indication claims, as well as the legal situation in each country, as cited in Karandish (Karandish, 2012). According to the basic European Directives in the pharmaceutical area, medicinal products require pre-marketing approval before gaining access to the market. Documentation of quality, safety, and efficacy as well as respective expert reports is required (Steinhoff, 2005). However, along with beneficial to horticultural crops, wastewater and sewage effluents contain a significant amount of heavy metals and other substances that may be harm and toxic to people (Ali & Shakrani, 2011). The effects of wastewater irrigation on soils have been widely documented, especially on heavy metal concentrations (Dominguez, Carrillo, Ortega, & Orozco, 2004) (Ali & Shakrani, 2011) and also on toxicity. As cited by (Chang, Granato, & Page, 1992), with continuous wastewater applications, heavy metals can accumulate in soil, thus increasing the toxicity of the plant (Chang, Granato, & Page, 1992). Besides, human are subjected to direct impact on health by contamination of soils, due the facts that soils are easily contacted and transferred to them (De Miguel, De Grado, Llamas, Martin-Dorado, & Mazadiego, 1998), (Mielke, Gonzales, Smith, & Mielke, 1999) and (Madrid, Diaz-Barrientos, & Madrid, 2002). Soils act as toxic chemicals filters and may absorb and retain heavy metals from wastewater. Accumulations of metals in crop tissues are different according to species (Datta, Biswas, Saharan, Ghosh, & Rattan, 2000), and metals absorption efficiency by different crops is judged either by plant metal uptake or by metals transfer factor from soil to plant (Rattan, Datta, Chhonkar, Suribabu, & Singh, 2005). Soil pollution by heavy metals is a great concern to public health, as cited in (Goyer, 1997). The source of heavy metal in plant is defined by the environment in which they grow and their growth medium (soil) from which heavy metals are taken up by roots or foliage of plants (Okoronkwo, Igwe, & Onwuchekwa, 2005) and (Naser, Sultana, Mahmud, Gomes, & Noor, 2011). Plant species have a variety of capacities in removing and accumulating heavy metals. So, there are reports indicating that some plant species may accumulate specific heavy metals (Markert, 1993). The uptake of metals from the soil depends on different factors, such as their soluble content in it, soil pH, plant species, fertilizers, and soil type (Lubben & Sauerbeck, 1991). The main goal of this research is to evaluate the uptake of some heavy metals by *Salvia Officinalis* plants, which is irrigated with treated wastewater.

Materials and Methods

Sage plants are cultivated in an experiment using pots, in the greenhouse for 180 days. Treatments aim to assess the amount of elements (Cd, Cr, Cu, Pb, Ni and Zn), accumulated in plants irrigated with treated and untreated sewage waters.

Wastewater Analysis

Sub-surface water samples for physic-chemical analysis were collected between 7:00-9:30 AM, in one-liter plastic bottles, which were previously cleaned with dilute HNO_3 and detergent, followed by distilled water. Before sampling, they were again rinsed with sampling water and 1 ml of concentrated HNO_3 was added in the water sample to keep the same metal concentration, and avoid the microbial activity (Laboratory Training Manual for Wastewater Treatment Plant Operators, 2010). Then, they were brought to laboratory. The critical parameters were tested on the same day while other parameters were tested with in their time limit. The concentration of heavy metals (Cd, Cr, Cu, Zn, Ni, Pb, and Fe) was determined using Atomic Absorption Spectrometer (Model novAA 400P, Analitic Jena). The instrument setting and operational conditions were conducted in accordance with the manufacturers' specifications.

Plant and soil sample collection

The samples were collected carefully using hand trowel to dig the soil around the plant and the plants were pulled out carefully, ensuring that no part of the root was lost. Plant samples were kept in separate polythene bags and were properly labeled. Soil samples were collected at a depth of 0-15 cm from the same point of collecting plant samples.

Preparation and preservation

The vegetable samples were washed in fresh running water to eliminate dust, dirt, possible parasites or their eggs and then again washed with deionized water. The roots and leaves were separated and then thoroughly washed. The clean vegetable samples were air-dried and placed in an electric oven at 65°C until reaching a constant weight. The dried vegetables samples were homogenized by grinding, using a ceramic coated grinder used for metal analysis. The samples were kept in polythene bags and labeled properly. All soil samples are dried at ambient temperature. Air-dried samples were passed through a stainless steel sieve with a 2- mm mesh. Finely ground samples were prepared by grinding a subsample of < 2mm (less than 2mm) soil to a fine powder in a ball mill. The final samples were kept in labeled polypropylene containers at ambient temperature before analysis. The plant and soil samples were analyzed in the laboratory. The concentration of heavy metals (Cd, Cr, Cu, Zn, Ni, Pb, and Fe) were determined by the Atomic Absorption Spectrometer (Model novAA 400P, Analitic Jena).

Digestion of samples

Soil and plant samples (0.3 g) were digested after adding 10 ml of (HNO_3 and H_2O_2) at 180$_o$C until a transparent solution was obtained. After cooling, the digested sample was filtered using Whatman No. 42 filter paper and the filtrate was finally maintained to 50 ml with distilled water.

Results and Discussions

In this paper are investigated the plant leaves, as part of the herb that is mostly used. Concentrations of Ni, Co, Cu, Cr, Cd and Fe in the investigated water samples are presented in Table 1. Heavy metal contents in medicinal plants depend on climatic factors, plant species, vegetation period, air pollution, and other environmental factors, as cited in (Blagojević, Damjanović-Vratnica, Vukašinović-Pešić, & Đurović, 2009). Previously conducted investigations demonstrated that the distribution of the heavy metal among plant organs was selective and depended on the part of the plant, surface characteristics of the plant organ, and the element that was examined.

Nickel (Ni): Most plants of the Albanian serpentine area showed slightly elevated Ni concentrations in comparison with those on other soil types, about 11- 100 mg kg$_{-1}$, rather than 0.5-10 mg kg$_{-1}$ cited by (Bani, et al., 2013). The permissible limit of Nickel in plants, recommended by WHO, is 10 mg/kg. Concentration of nickel in plant samples irrigated with treated wastewater ranged between 3.98 to 6.34 mg/kg, which are within the permissible limit (Table 2). Nickel in water samples ranged between 1.5 to 1.864 μg/L, which is below the permissible limit. The maximum permissible limit for Ni concentration in water is 0.2 mg/L, according to (FAO, Food and Agriculture Organization of the United Nations, 1985)("FAO", 1985). In soil sample the maximum permissible limit set by (86/278/EEC, 2006) for Ni is 30-75 mg/kg dm. In the collected soil samples concentration of nickel ranged between 95 to 96.34 mg/kg, so nickel in the soils is above the allowed limit (Table 3). But, referred to the Albanian soils, there have been found levels of Ni content even higher than these values cited by Bani, et al.2013. We also rely on values proposed in some European countries, compiled from various reports, documents, and internet data for Ni concentration, which is 75-150 mg/kg as recommended by (Kabata-Pentias & Pendias, 1984).

Chromium (Cr): The permissible limit of chromium for plants is 1.30 mg/kg, according to (WHO, 1996). In the leaf of sage plant, concentration of chromium ranged between 1.05 to 1.13 mg/kg, which is below the permissible limit. The maximum permissible limit for Cr in water is 0.1mg/L (FAO 1985). The values of Cr in treated wastewater samples ranged between 0.1 to 0.21 μg/L. In all the collected water samples concentration of chromium was recorded bellow the permissible limit set by FAO. Concentration of chromium in soil samples ranged between 11.508 to 14.998 mg/kg. In all the collected soil samples concentration of chromium was recorded below the permissible limit set by (European, 2002) which is 150 mg/kg.

Cadmium (Cd): The permissible limit of Cadmium in plants, recommended by WHO, is 0.02 mg/kg. In the leaves of sage plant, concentration of cadmium ranged between 0.001 to 0.007 mg/kg which is below the permissible limit. The maximum permissible limit for Cd in water is 0.01 mg/L. Concentration of cadmium in treated wastewater samples was not detected (n.d.) (Table 1). Concentration of cadmium in collected soil samples ranged between 2.4 to 2.5 mg/kg. In all the collected soil samples concentration of cadmium was recorded within the maximum permissible limit set by Directive 86/278/EEC, which is 1-3mg/kg.

Copper (Cu): Contamination of drinking water with high level of copper may lead to chronic anemia, as cited in (Nazir, et al., 2015). Copper accumulates in liver and brain. Copper toxicity is a fundamental cause of Wilson's disease, as cited in (Mebrahtu & Zerabruk, 2011). Copper particulates are released into the atmosphere by windblown dust; volcanic eruptions; and anthropogenic sources, primarily copper smelters and/or processing facilities. The fate of elemental copper in water is complex and influenced by pH, dissolved oxygen and the presence of oxidizing agents and chelating compounds or ions.

The maximum permissible limit for Cu in water is 0.2 mg/L. In the treated wastewater samples, copper concentration was not detected. The concentration of Cu metal in the plant leaves ranged from 1.88 to 4.84 4 mg/kg. These concentration levels are within the average content of Cu in dry plant material, which is reported to be 2.0-20 mg/kg by WHO (1996). Concentration of copper in all the soil samples was below the maximum permissible limit set by Directive 86/278/EEC which is 50-140 mg/kg. Concentration of copper in the soil taken for study ranged between 7.063-8.487 mg/kg.

Lead (Pb): According to WHO standards, permissible limit of lead in water is 5.0 mg/L, and in all the collected water samples concentration of lead was above the permissible limit. The permissible limit in plants recommended by WHO is 2mg/kg. In the sage plant leaves, concentration of lead ranged between 0.555 to 0.716 mg/kg, which levels are below the permissible limit. The low value of Pb in our studied plant samples is mainly due to the location of cultivation area that is away from the road and motor vehicles; the leading factors of plant contamination with Pb, as cited in (Abu-Darwish & Ofir, 2014). Concentration of lead in soil samples was recorded to range between 22.23 to 28.31m/kg. In almost all the collected soil samples, concentration of lead was recorded below the permissible limit set by Directive 86/278/EEC, which is 50-300 mg/kg. Lead as a soil contaminant is a widespread issue. It accumulates with age in bones aorta, and kidney, liver and spleen. It can enter the human body through the uptake of food (65%), water (20%) and air (15%).

Zinc (Zn): Concentration of zinc in water samples is not detected. The permissible limit of zinc in water, according to the FAO (1985) standards, is 2.0 mg/l. Zinc is one of the important trace elements that play a vital role in the physiological and metabolic process of many organisms. Nevertheless, higher concentrations of zinc can be toxic to the organism. It plays an important role in protein synthesis and is a metal which shows fairly low concentration in the surface of water, due to its restricted mobility from the place of rock weathering or from the natural sources. WHO's recommended limit of zinc in plants is 50 mg/kg, as cited by (Shah, et al., 2013). In most of the collected plants, zinc concentration was recorded below the permissible limit, ranging from 3.47 to 3.90 mg/kg. In general, the low contents of Zn in all studied samples indicated a non polluted soil environment and/or of locations far away from industrial and heavy traffic activities, which were reported as the main factors of plant growth soil pollution with heavy metals, as cited in (Abu Darwish, 2014). Zinc concentration in soil samples ranged between 55.399 to 63.965 mg/kg. In all the soil samples, concentration of zinc was recorded below the permissible limit set by Directive 86/278/EEC, which is 150-300 mg/kg.

Iron (Fe): The maximum allowed concentration of iron in drinking water is 1.0 mg/L, according to the WHO report, as cited in (Nazir, et al., 2015). In all the collected water samples, concentration of iron was recorded below the permissible limit, according to WHO. The recommended level of iron in plants by (WHO / FAO, 2007) is 450 mg/kg. According to (EPA, 2003), iron is considered a plant micronutrient. Iron is absorbed by plants as the ferrous ion (Fe+2), which is necessary for the formation of chlorophyll and functions in some of the enzymes of the plant's respiratory system. Much of the iron in well-drained soils is in the ferric (Fe+3) form, which is unavailable to plants. Iron deficiencies may result if the soil minerals do not gradually release ferrous (Fe+2) iron to replace that, which is being oxidized to ferric iron over time. The results showed a wide variation of Fe concentration in S.Officinalis. In all collected plant leaves, concentration of iron was recorded ranging from 416.68 to 551.73mg/kg. High concentration of Fe in plants may be due to the foliar absorption from the surroundings air, as cited in (Shad, Lajbar, Iqbal, Khan, & Naveed, 2008). This is supported by the results of Fe concentrations in S.Officinalis cultivated in worldwide regions, which revealed a wide variation in the values of Fe concentrations. In soils, Fe is believed to occur mainly in the forms of oxides and hydroxides as small particles or associated with the surfaces of other minerals. Iron concentrations in most soils ranged between 0.5-5 percent, referred to (Lafta., Sabbar, Mazoor, & Qadir, 2011). Concentration of total iron in all the collected soil samples ranged between 3944.13 to 4667.86 mg/kg. The amount of iron is different in soils of various origins and used differently. Its natural, average content in soil is 0, 6%, reported by (Kabata-Pendias & Pendias, 1992), and may undergo significant changes due to the high vertical mobility of iron in soil profiles.

Table 1. Mean concentration of heavy metals in treated and untreated urban sewage waters, used for the irrigation of sage plants

Irrigation waters	Mean concentration of metals (µg/L)							
	Zn	Pb	Cd	Cr	Cu	Ni	Co	Fe
Untreated urban sewage water	0.0679	0.2750	<0.01	0.1557	<0.2	18.7	n.d	<5.0
Treated sewage water	0.1664	0.6962	<0.01	0.2816	<0.2	23.1	n.d	<5.0
Recommended Maximum Concentration[1] (mg/l)	2.0	5	0.01	0.1	0.2	0.2	0.05	5.0

[1] The maximum concentration is based on a water application rate, which is consistent with good irrigation practices (10 000 m^3 per hectare per year). If the water application rate greatly exceeds this, the maximum concentrations should be adjusted downward accordingly. No adjustment should be made for application rates less than 10 000 m^3 per hectare per year.

Table 2. Heavy metal accumulation in Sage plant leaves (mg kg^{-1})

Element	Zn	Cu	Cd	Cr	Pb	Ni	Fe
Range	3.47-3.90	1.88-4.84	0.001-0.007	1.05-1.13	0.555-0.716	3.98- 6.34	416.68-551.73
Average	3.7415	3.2669	0.0051	1.1970	0.6097	2.4417	485.8281
STDV	0.2353	2.9729	0.0035	0.1802	0.0922	2.5110	67.5875
Permissible limits of heavy metals in plants by WHO[1](mg/kg)	50	10	0.02	1.30	2	10	-
Directive 86/278/EEC [2](mg/kg dm)	150-300	50-140	1-3	-	50-300	30-75	-

[1] **SOURCE:** WHO (1996).

Table 3. Metal contents in soil samples & Maximum Allowable Limits of Heavy Metal in Soils (mg/kg)

Code	Ni	Pb	Zn	Cr	Cu	Cd	Fe
T10	95.8462	25.2780	59.6823	73.1920	7.7756	2.5457	4305.99
Directive 86/278/EEC [2](mg/kg dm)	30 – 75	50 – 300	150 - 300	-	50 – 140	1 – 3	-

[1] Directive 86/278/EEC (2006)

Conclusion

The main goal of this research is to assess the concentration of some of the toxic heavy metals in plants, after the harvest period, irrigated with treated wastewater. The concentration of these heavy metals in both treated and untreated sewage water is below the maximum permissible level set by the WHO. The assays of heavy metals varied from spot to spot, plant to plant, and also in different parts of each plant, depending upon the chemical composition of soil and absorption rate by plants. The results of this research show that the treated wastewater can be used to irrigate plants, considering the concentration of heavy metals in them. Though the study confirms that the domestic sewage can effectively increase water resource for irrigation, there is a need for continuous monitoring of the concentrations of potentially toxic elements in soil, plants and ground water. Thus, considering the results, it is essential to check every medicinal plant for contaminant load, before processing it for further pharmaceutical purposes or for local human consumption.

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