AMERICAN UNIVERSITY OF BEIRUT

UPTAKE OF CADMIUM, LEAD, AND NICKEL BY *ORIGANUM SYRIACUM* PRODUCED IN LEBANON

by

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A thesis

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AN ABSTRACT OF THE THESIS OF

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Heavy metals are elements found naturally in the soil. However, the increase in polluting activities such as industrial, agricultural, medical, technological, and domestic has increased heavy metal contamination to an extent where human health, animal health, and the ecosystem as a whole has been put at risk. This issue has raised high concerns and caused high awareness assessments in the public health sector. This study was divided into three parts. The first part was conducted in a pot experiment at the greenhouse at AUB, Beirut, to study the effect of applying different concentrations of cadmium, lead, and nickel in soil on their absorption by *Origanum syriacum* plants. The second part was done to measure the concentrations of cadmium, lead, and nickel in selected soil and *Origanum* samples collected from several fields of different soil types in Lebanon. The third part was done to measure the concentrations of cadmium, lead, and nickel in *Origanum syriacum* selected from different sale outlets in Lebanon. Results for the first part showed that there was a positive relationship between the concentrations of cadmium, lead, and nickel in soil and that in *Origanum syriacum* tissue. In other words, the higher the concentrations of cadmium, lead, and nickel in soil, the higher the concentration was in *Origanum* plants. A significant difference was clearly seen between the control and the rest of the applied treatments. Results for the second part showed that none of the selected soil samples from fields in Lebanon were contaminated with cadmium, lead, and nickel because the levels did not exceed the maximum limits for any of the three elements. Results of the third part showed that the concentrations of cadmium, lead, and nickel in most of *Origanum* samples which were collected from different stores in Lebanon did not exceed the maximum limits set by the international organizations and governmental authorities; except one sample purchased from a major store in Beirut that was more than double the maximum limit for lead.

Keywords: *Origanum syriacum*, Heavy metals, Cadmium, Lead, Nickel, Environmental Pollutants, Pot Experiment, Lebanon

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ABBREVIATIONS

°C	Degrees Celsius
µg/Kg	Microgram per Kilogram
ATSDR	Agency for Toxic Substances and Disease Registry
Cd	Cadmium
Du	Dunum
EFSA	European Food Safety Authority
EPA	Environmental Protection Agency
ESCWA	Economic and Social Commission for Western Asia
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
g/cm^3	Gram per centimeter cubed
KAS CRSL	Kamal A. Shair Central Research Science Laboratory
Km	Kilometer
mg/Kg	Milligram per Kilogram
NGO	Non-governmental Organization
Ni	Nickel
NPL	National Priorities List
O. *syriacum*	*Origanum syriacum*
Pb	Lead

ppm	Parts per million
spp.	Species
Trt.	Treatment
UNDP	United Nations Development Programme
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

Heavy metals or trace metals are natural elements present in Earth's soils. However, the increase in anthropogenic activities such as industrial, agricultural, medical, technological, and domestic have caused severe contamination of these metals in some soils. Heavy metals are those elements that have densities and atomic weights five times more than that of water (Tchounwou et al., 2012). These metals have reached high levels that have caused high awareness assessments in the public health sector. Even the agricultural sector has highlighted this issue as a major one because high heavy metal levels may cause human defects and therefore, cause major implications in the food chain as a whole. Some plants have the ability to accumulate heavy metals in their system. Some plants accumulate heavy metals in the root zone while others accumulate them in the shoots. What happens to humans when they consume contaminated goods has raised high concerns by the public. Especially that those heavy metals have caused major human problems such as renal, neurological, carcinogenic, and mental disorders. They have been found to cause negative effects on cellular organelles, enzymes, DNA, and nuclear proteins (Tchounwou et al., 2012).

Origanum spp. is one of the basic spices consumed in the Middle East. It is considered a cheap staple food that is consumed by everyone, especially the poor. Furthermore, *Origanum spp*. is not only used for consumption purposes but it is also used widely in the world of medicine. It has been reported by Lukas et al. (2009) that *Origanum* *spp*. treats gastrointestinal problems, respiratory diseases, hemorrhoids, sexual diseases, and other pains such as animals' bites and poison. Studies have also proved the effect of *Origanum spp*. against *Candida albicans*, a yeast infection (Zeina Kassaify et al., 2008).

Can a medicinal herb such as *Origanum spp*. treat many diseases and at the same time be a pathway to heavy metal accumulation in the human body is a question being raised. If the answer to the question is true, then *Origanum spp*. consumption should be reduced to a certain level in which chronic disorders that are caused by heavy metal accumulation become limited.

International organizations and governmental authorities have set regulations or standards of heavy metal exposure through inhalation and consumption (air, water and food). These standards differ from one country to another. Some of these international organizations and governmental authorities are FAO, WHO, EPA, FDA, Canadian government, Australian government, and CODEX. A list of tables is presented in chapter 2 (Literature Review) that shows the recommended limits of cadmium, lead, and nickel in food and their daily tolerable intake for humans.

The objectives of this study are to (1) measure the concentrations of cadmium, lead, and nickel in selected tissue and soil samples collected from fields that are planted with *Origanum syriacum* in Lebanon, (2) measure the concentrations of cadmium, lead, and nickel in selected tissue samples collected from sale outlets in Lebanon, and (3) study the effect of applying different levels of cadmium, lead, and nickel in soil on *Origanum syriacum* plants.

CHAPTER 2

LITERATURE REVIEW

In this chapter, an overview of *Origanum spp*., its botanical classification, description, use, and productivity in South Lebanon will be covered. Also, an overview of 3 heavy metals (Cd, Pb, and Ni), their presence in soils and plants, and their effect on the human body will be reviewed. Finally, the last section in this chapter will show the maximum limits of these heavy metals according to several international organizations and governmental authorities.

A. *Origanum*

1. Origanum General View

According to the Herb Society of America (2005), the genus *Origanum* belongs to the *Lamiaceae* family or what is commonly known as the mint family. The *Lamiaceae* family consists of about 200 genera. The *Origanum* genus was first classified by Linnaeus in the fifth edition of Genera Plantarum in the year 1754 (Ietswaart, 1980). Later on, the plants in the genus have been placed under different botanical names like *Amaracus*, *Origanum*, and *Majorana*. The *Origanum* genus contains about 43 species and 18 hybrids that are arranged in three groups and ten sections as stated by Lukas et al. (2009) in the article "Composition of Essential Oil Compounds from Different Syrian Populations of *Origanum syriacum* L. (Lamiaceae)". The article has also stated that *O. syriacum* is placed in the Majorana section along with other species like *O. majorana L*. and *O. onites L*. *Origanum syriacum* has three varieties, *var. syriacum, var. beVanii* (Holmes) *Ietswaart*, and *var. sinaicum* (Boissier) *Ietswaart*, that are present in many countries of the Eastern Mediterranean like South Turkey, Cyprus, Syria, Lebanon, Palestine, and Jordan. These varieties can be grown at areas close to sea level and can tolerate up to at least 2000 meters above sea level. Several common names go with *O.syriacum* such as "**زوبع**" which means whirlwind and "**زعتر خليل**" (Lukas et al., 2009).

O. syriacum has white flowers that occur in spikes. Its flowering part and leaves are what give it its unique smell due to the presence of the essential oil glands that release volatile oils. Its non-woody stems and oval shaped leaves contain small hairs. It can be sown using seeds or cuttings. *Origanum* species can grow on different types of soils and climates but their best growing conditions are dry, sandy, gravelly loam, well drained soils, and neutral to alkaline pH. Most importantly, *Origanum* species require a good aeration system. *Origanum* species require full sun to half shade. Lighting conditions play a key role in the scent and flower color. When subjecting the plants to half day of full sun, the fragrance and pigmentation of the flowers enhances (The Herb Society of America, 2005). *Origanum* species are considered tolerant plants. Even though they prefer drained soils, they can tolerate moist or dry conditions. They can also tolerate low nutritional quantities. Moreover, *Origanum* species can also tolerate a wide range of pests and diseases but some are susceptible to diseases such as rust fungi (*Puccini spp*.), *Fusarium oxysporum, F. solani*, alpha mosaic virus, *Botrytis spp*., *Phytopthera* and *Pythium*. Susceptibility to diseases is dependent on the propagation method. For example, as reported in the article Oregano and Marjoram (The Herb Society of America, 2005), *O. majorana* becomes more tolerant to root and stem diseases when propagated as cuttings compared to seeds. In addition, growing *Origanum* plants in containers make them more susceptible to fungal diseases.

Figure 1. *Origanum syriacum* plant

Source: retrieved from http://www.impecta.se/sv/artiklar/syrisk-oregano-zaatar.html

Origanum is mainly used for consumption purposes. It is considered one of the most important staple herbs in the world because it is cheap, tasty, and found in most of the kitchen houses. It is consumed in the Mediterranean countries almost on a daily basis and commonly goes by the name "زعتر". The basic mixture of *"*Za'atar*"* is *Origanum* leaves, sesame seeds, sumac (Rhus), and salt. *O. syriacum* is mostly consumed in man'oushe

(منقوشه), the famous Mediterranean pastry. Laborers, students, and employees would grab a man'oushe on their way to work/school firstly because it's very cheap and secondly because it's widely available in the market. *O. syriacum* is also consumed as leaves. Many people would have it for breakfast with chopped onions and olive oil mixture and with labne (strained yogurt) on the side. Others would have it for lunch as a salad or add it to a salad recipe.

Traditionally, *Origanum* species were used to treat internal diseases such as gastrointestinal problems, respiratory diseases, hemorrhoids, sexual diseases, and other pains such as animals' bites and poison (Lukas et al., 2009). Zeina Kassaify et al. (2008) reported that *O. syriacum* has been proved to be effective against *Candida albicans*, a yeast infection, that appears on the skin and mucous membranes of females mainly. Essential oil of *O. syriacum* has been tested on female hamsters and results have shown that the colonization and adaptability of Candida albicans is reduced; however, when the essential oil of O.*syriacum* has been diluted with dimethyl sulfoxide, total elimination of C. albicans occurs in comparison to control treatments. Another study by Ayesh (2014) proved that O.*syriacum* extracts reduces the viability and cytotoxicity of THP-1 cells and PBMC's; however, they have not been selective against leukemic cells. Research is still ongoing on O.*syriacum* and it is considered a very beneficial herb that may be used in treating several diseases.

2. Origanum Plantation and Productivity in South Lebanon

The Planting area of *Origanum* in Lebanon is not exactly known because they are planted on a small scale all over Lebanon. A study has been initiated by ESCWA in the year of 2009 in South Lebanon explaining in details about the production of *Origanum* there, yet no planting area has been stated in the study (ESCWA, 2010). Moreover, a farmer in South of Lebanon, goes by the name Ali Nehme, agrees that it is difficult to know the exact planting area but he stated that many tobacco farmers are shifting to *Origanum* plantation because of its high yield and low input cost. Ali Nehme is one of the first farmers (if not the first to start *Origanum* production on a commercial scale) in South of Lebanon to start the *Origanum* plantation. In 2000, he started to produce with some technical support from UNDP and NGOs (non-governmental organization). Later, in year 2006, most of his *Origanum* fields were destroyed during the war between Israel and Hezbollah in South Lebanon. Afterwards, Ali Nehme became a producer of *Origanum* seeds in the South and most farmers buy seeds from him. He became a reliable source of information about growing *Origanum* in Lebanon and he established a small company to sell his products. He explains that irrigated za'atar in two cuts per year produces up to 350 Kg/ du, while rain fed *Origanum* may produce up to 125 Kg/ du in only one cut per year. The first year of growing is the year of establishing the crop and there will be no production. The production will start in the second year and will continue for 5 years without the need to add any fertilizers. However, after 5 years, the addition of fertilizers will help in stabilizing the production; otherwise the yield will drop gradually. No addition of pesticides or fungicides is required for *Origanum* planation other than copper and sulfur for protection against fungal infections (Ali Nehme, personal communication). The ESCWA study (2010), on the other hand, explains that the productivity of *Origanum* (za'atar) depends on the variety and on the water availability. Briefly, the production of *Origanum* grown under rainfed conditions differs from that of irrigated conditions in yield and number of cuts per season. The yield of *Origanum* can reach 1.8 and 4.5 tons for rainfed and irrigated fields respectively. Moreover, this study indicated that the number of cuts in irrigated fields may reach three while it can only reach two in rainfed fields.

B. Heavy Metals

1. General View

Heavy metals are those elements that are present naturally in the soil and have high atomic weight and density. According to Tchounwou et al. (2012), heavy metals have densities and atomic weights five times more than that of water. Moreover, Hogan (2011) stated that heavy metals are referred to those elements that have an atomic number of 21 and higher. Therefore, the elements that are considered heavy metals, according to Hogan (Encyclopedia of Earth), start from the element scandium (atomic weight of 44.566) and above. Years of research have proven that some heavy metals are required by humans and plants in low amounts and without them, deficiencies occur. These heavy metals are as follows: chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu) zinc (Zn), selenium (Se), and molybdenum (Mo). These elements have been found to be essential for several biochemical and physiological processes. Some play an important role in enzymatic processes and others are required in oxidation-reduction reactions. In contrast, any excess in the quantities of these elements causes toxicity (Tchounwou et. al., 2012).

Even though heavy metals are naturally occurring elements in the environment, the increase of human activities - industrial, agricultural, medical, technological, or domestic - have increased their presence and availability in the environment, sometimes to dangerous levels; especially that many of these heavy metals are considered non-essential and have no role in biological or physiological processes in human or plant systems. Some of these non-essential heavy metals are aluminum (Al), arsenic (As), cadmium (Cd), and lead (Pb). High concentrations of heavy metals have been found to have negative effects on cellular organelles (cell membrane, mitochondria, lysosome, endoplasmic reticulum, and nuclei) and on some enzymes that are in charge of metabolism, detoxification, and damage repair. Heavy metals have also been found to interfere with DNA and nuclear proteins and to cause DNA destruction leading to carcinogenesis and other serious diseases (Tchounwou et al., 2012).

Five heavy metals have been found to be very dangerous ones because they are reactive oxygen species producers and cause high toxicity levels. They are a priority in comparison to the rest of the heavy metals and they have brought significant public health concerns. These heavy metals are arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb), and mercury (Hg) (Tchounwou et al., 2012).

Su et al. (2014) gives an overview of the levels of heavy metals in several countries in an article entitled “A Review on Heavy Metal Contamination in the Soil Worldwide: Situation, Impact and Remediation Techniques”. Data in tables 1 and 2 collected from different references give an overview of the heavy metals in urban and agricultural soils present in several Chinese cities, Spain, America, Korea, Slovakia, India, and Iran. The two tables also show the environmental capacity which explains how much the soils are contaminated with the mentioned heavy metals.

Table 1. The Content of Heavy Metals in Urban Soils in Different Locations in the World. Concentrations are in mg/Kg.

City/Country	Pb	Ni	Cd	Reference
Beijing, China	28.60	27.80	0.15	Zheng et al., 2008
Guangzhou, China	108.55	25.67	0.50	Lu et al., 2007
Shanghai, China	70.69	31.14	0.52	Shi et al., 2008
Changsha, China	89.40	-	6.90	Xi et al., 2008
Hong Kong, China	94.60	12.40	0.62	Li et al., 2004
Qingdao, China	62.00	17.30	0.30	Yao et al., 2008
Baoji, China	25380.55	72.10	-	Li and Huang, 2007
Luoyang, China	35.92	-	1.71	Lu et al., 2007
Wenzhou, China	65.22	-	-	Chen et al., 2007
Nanjing, China	107.30	-	-	Lu et al., 2003
Cincinnati, Ohio	41.00	19.00	-	Turer et al., 2001
Syria	17.00	39.00	-	Moller et al., 2005
France	43.14	14.47	0.53	Hernandez et al., 2003
Spain	1505.45	-	3.76	Rodrguez et al., 2009
Iran	46.59	37.53	1.53	Sayadi and Rezaei, 2014
Turku, Finland	17.00	24.10	0.17	Salonen and Korkka-Niemi, 2007
Range	17-25380.55	12.40-72.10	0.15-6.90	
Average	1733.94	29.14	1.52	
Background	26.00	26.90	0.10	CEPA, 1990
Environmental capacity	300.00	50.00	0.30	CEPA, 1995

Source: (Su et al., 2014)

Table 2. The content of heavy metals in agricultural soils in different locations in the world. Concentrations are in mg/Kg.

City/Country	Pb	Ni	Cd	Reference
Beijing, China	18.48	-	0.18	Liu et al., 2005
Guangzhou, China	58.00	-	0.28	Liu et al., 2009
Yangzhou, China	35.70	38.50	0.30	Huang et al., 2007
Wuxi, China	46.70	-	0.14	Zhao et al., 2007
Chengdu, China	77.27	-	0.36	Liu et al., 2006
Kunshan, China	30.48	31.08	0.20	Chen and Pu, 2007
Xuzhou, China	56.20	-	2.57	Liu et al., 2006
Changde, China	-	-	-	PSTV, 2014
Spain	213.93	34.75	1.42	Zimakowska-Gnoinska et al., 2000
America	23.00	57.00	0.78	Han et al., 2002
Korea	5.25	-	0.12	Kim and Kim, 1999
Slovakia	139.00	29.00	-	Wilcke, 2005
USA	55.00	29.00	13.5	Jean-Philippe et al., 2012
India	0.95	4.34	0.82	Raju et al., 2013
India	2.82	0.14	0.05	Prajapati and Meravi, 2014
Iran	5.17	11.28	0.34	Sayyed and Sayadi, 2011
Iran	-	-	-	Zojaji et al., 2014
Range	0.95-213.93	0.14-57.00	0.05-13.50	
Average	51.19	26.12	1.50	
Background	26.00	26.90	0.097	CEPA, 1995
Environmental capacity	300.00	50.00	0.30	Zheng et al., 2008

Source: (Su et al., 2014)

The level at which heavy metals affect humans is known as the level of exposure. At this level, heavy metals come in contact with the human body through eating, drinking, breathing, or even through contact. What really determines whether this exposure will be harmful or not is the dose, the time, and the way the contact has occurred. In the United States, before the contamination level reaches, the EPA (Environmental Protection Agency) would mark these places and put them on the NPL (National Priorities List) to be put for long-term federal clean-ups (ATSDR, 2102).

Heavy metal contamination is a life-threatening issue that affects the ecosystem as a whole through eating and drinking, breathing, or even getting in contact with contaminated soils. This poses a risk to the food chain starting from soil and ending with animals and human beings. This issue has led to the decrease in food quality, quantity, and in the soils' health and production level causing food insecurity concerns (Wuana and Okieimen, 2011).

2. Cadmium General View

Cadmium (Cd) is one of the most significantly hazardous heavy metals known. It is a divalent cation that has an atomic number of 48 and atomic mass of 112.41(ATSDR, 2012). It has a density of density 8.65g/cm^3, a melting point of 320.9°C, and a boiling point 765°C (Wuana and Okieimen, 2011). Cadmium is naturally present in Earth's crust, at an average concentration of about 0.1 mg/Kg, as a soft silver white metal that has no distinct taste or smell. It is usually found as cadmium oxide, cadmium chloride, and cadmium sulfide in the environment. It is known to be the highest in sedimentary rocks (marine phosphates contain 15 mg Cd/Kg (ATSDR, 2012). Even though cadmium is naturally found in Earth's crust, it's found in toxic quantities near industrial sites where its use is required for production of batteries, pigments, metal coatings and plastics (Martin and Griswold, 2009). Cadmium can also be found in toxic quantities in soils near roads due to car pollution, mainly tires and oil lubricants (Wuana and Okieimen, 2011).

3. Cadmium in Soil and Plants

Cadmium and many other heavy metals have been found to accumulate in soils due to the increase in industrial areas that emit them. The increase application of agricultural chemicals such as organic and inorganic fertilizers (mainly phosphorous), pesticides, sewage sludge, and wastewater irrigation have also been found to increase cadmium concentrations in soils. Other contaminations can come from combustion of coal, spillage of petrochemicals and atmospheric deposition. The reason why cadmium and other heavy metals accumulate in the soil is due to the fact that they do not get degraded by microbes or chemical reactions (Wuana and Okieimen, 2011). Airborne cadmium has not been a major cause of accumulation by plants in soils contaminated by high metal sludge since only about two grams per hectare per year has come from it. However, in areas where cadmium is highly emitted (ten folds higher) and soil cadmium is low, airborne cadmium becomes considerable (Smolders, 2001).

According to the article "Cadmium in plants on polluted soils Effects of Soil Factors, Hyperaccumulation, and Amendments" written by Kirkham (2006), several factors control the uptake of cadmium from soils. These factors are pH, phosphate, zinc, and organic matter; however, the major factor is pH. The lower the pH, the more the cadmium uptake becomes. Moreover, high levels of phosphate and zinc decrease the uptake of cadmium due to oxidation reaction and compatibility respectively. The study also has shown that chloride increases the presence of cadmium while silicon reduces it. According to another article "Cadmium Uptake by Plants" (Smolders, 2001), the bioavailability of cadmium is what determines its uptake by plants and not the total cadmium level in the soil.

Cadmium and zinc belong to the same group in the periodic table. Elements of the same group on the periodic table have similar physical and chemical properties. For this reason, cadmium is compatible with zinc, an essential micronutrient, and can replace it in the soil solution. Zinc is known to be an essential element that is required in metabolic processes and the compatibility of cadmium with an essential element hinders metabolic processes and thus causes toxicity (Wuana and Okieimen, 2011). Deficiency of zinc in soils leads to increased uptake of cadmium by plants. Treatments of zinc deficiency have been found to reduce cadmium in wheat grains by about two times in Australia (Smolders, 2001).

High concentrations of cadmium salt (NH_4NO_3, $NaNO_3$ or $CaCl_2$ extracts) in the soil leads to high concentrations of cadmium in plants. A study done on potato in Australia has revealed that cadmium concentrations in potato tubers are about five folds higher in treatments with saline soils than with non-saline soils. This study shows a positive linear relationship between soil cadmium salts and crop cadmium and also it shows that cadmium availability and mobility are interrelated. The ratio between cadmium concentration in soil and that in plants is known as the transfer factor (TF) (Smolders, 2001). The plant accumulates cadmium the most in roots and the least in fruits, grains, and tubers. Cadmium accumulation in plants is in the following order: new roots >old roots> rhizomes> stems and leaves > grain and tubers (Krishnamurti et al., 2005).

Organic matter is also a factor that affects cadmium availability for plants. Cadmium has a strong ability to bind to organic matter which will cause itself to become unavailable to plants (immobile) and remains in the upper layer of the soil(ATSDR, 2012; Krishnamurti et al., 2005).

4. Cadmium in Human Body

Cadmium is considered a toxic element to the human body. Once it's present, cadmium persists for years in the body. Cadmium is introduced to the human body via several ways such as inhalation from air and ingestion of contaminated food and water. About 5-50% of the cadmium that people breathe can be found in the lungs. Inhalation of cadmium is a major problem to those who live near industrial places or work in metal industries; however it's not a concern to those living far away from these areas. Cigarettes have cadmium sources and are major contributors as well. Those who smoke have higher cadmium levels in their bodies compared to nonsmokers (EFSA, 2009; ATSDR, 2012). Ingestion of cadmium by food, on the other hand, has become a serious issue due to the fact that cadmium accumulation in plants increases with increasing cadmium levels in the soil. Cadmium has been found in trace amounts in leafy vegetables such as lettuce and spinach and other crops such as tobacco, potatoes, soybeans, peanuts, kidneys, liver, and seafood such as mollusks and crustaceans (ATSDR, 2012; Tchounwou et al., 2012). Cadmium in water is a concern when water supplies are close to industrial areas. In this case, cadmium in water will not only affect humans but will also affect aquatic organisms (ATSDR, 2012).

Cadmium that enters the body is then moved to the liver and kidneys. It stays there for a long time (Dghaim et al., 2015; Smolders, 2001) but some will be excreted in the urine and feces. Moreover, the body has the ability to convert cadmium into a nontoxic form only if the quantity is little. The tolerable daily intake of cadmium for the human body is 1 µg Cd/Kg per body weight. Therefore, 70 µg Cd is acceptable for a 70 Kg adult (Smolders, 2001).

Testing cadmium presence in the body is done either by blood or urine tests. Blood tests determine recent exposures to cadmium while urine tests determine chronic exposures. Cadmium toxicity is found to cause serious illnesses to the human body. Cadmium inhalation causes severe lung damage and has also lead to death at high inhalation levels. Cadmium has also caused stomach irritation, vomiting, and maybe death if highly ingested from food or water (ATSDR, 2012). Cadmium has also been found to affect several enzymes of the body. Kidney failure occurs when little amounts of cadmium are ingested over a long period of time. Cadmium levels affect the enzymes responsible for the reabsorption of proteins in the kidney tubules causing proteinuria. For that reason, urine tests are the means of diagnosis to chronic exposures. A famous itai itai disease that has affected the Japanese people as a result of cadmium toxicity as well as lead and zinc from contaminated water has shown severe symptoms of osteomalacia and kidney failure (Wuana and Okieimen, 2011).

Cadmium toxicity symptoms are the same for children, however, children are more vulnerable to diseases than adults. Cadmium can also be passed through breast milk which can affect infants depending on the dosage the mother has had (ATSDR, 2012).

5. Lead General View

Lead (Pb) is considered a toxic heavy metal that is naturally present in small amounts in Earth's crust and has a bluish grey color. It has an atomic number of 82, atomic mass of 207.2, density 11.4g/cm^3, melting point 327.4°C, and boiling point 1725°C. Lead complexes with sulfur, sulfates, phosphates and carbonates. It is considered the second most hazardous element after arsenic and the fifth most used metal in industry (ATSDR, 2007; Wuana and Okieimen, 2011). It is highly used in the production of batteries, armaments, metal production, and other devices such as x-ray covers. In the year 2004, it has been estimated that about 1.5 million metric tons of lead are used in the US for industrial purposes, mainly for battery manufacturing (Tchounwou et al., 2012). Other sources of lead are mining and smelting of lead ores, lead containing paints, gasoline, sewage sludge, fertilizers, and pesticides. Several measures have been taken in order to decrease lead levels in the environment, yet it is still considered an environmental problem worldwide (Sharma and Dubey, 2005).

6. Lead in Soil and Plants

Lead is mostly present on the surface of the soil and decreases with increasing soil depth. Not more than 50 mg Pb/Kg is present naturally in the environment (Pourrut et al., 2011). However, high levels of lead can be present in the environment from anthropogenic activities near industrial areas. Moreover, soils near highways are also highly contaminated with lead from automobile exhausts which may later contaminate water streams and other lands around that can be used for agricultural purposes. In that case, crop production can be hindered depending on the level of contamination. Another cause of lead contamination is the pesticide use of lead containing chemicals such as lead arsenate (Sharma and Dubey, 2005).

Lead is present freely as an ion in the soil or it can be combined with organic or inorganic components such as bicarbonates, carbonates, sulfates, amino acids, fulvic acids, iron oxides, organic matter and clay particles of soil (Pourrut et al., 2011). Lead is not usually available in its soluble form in the soil. Its solubility in the soil is limited and only a small fraction is soluble for plant uptake (Punamiya et al., 2008). When lead is in its soluble form in the soil solution, the roots are able to accumulate high amounts of lead in their system preventing lead movement to the upper part of the plants. When high levels of lead are present, the ability of roots to stop lead movement to the upper part of plants becomes weak. Therefore, the higher the available lead is present in the soil, the more lead can be found in the shoots. In addition, some plant species are able to absorb lead from the air sources (Sharma and Dubey, 2005).

What determines the availability of lead in the soil is soil factors such as organic matter, root surface area and exudates, cation exchange capacity, mycorrhizae, rate of transpiration, pH, biological and microbial conditions, redox reactions, and level of lead present (Sharma and Dubey, 2005; Pourrut et al., 2011) .

When lead is present outside the roots, it is bound to the carboxyl group of the mucilage uronic acids thus preventing its entrance to the roots system. This can be considered a protection mechanism that plants possess to prevent heavy metal movement into their system. However, when lead is able to enter the root system, it gets deposited as lead carbonate in the cell walls. This is another mechanism plants have as protection. Lead can always find a way to enter the plant system. When factors like EDTA chelates and low pH are available, lead will have the ability to reach the shoots of plants (Sharma and Dubey, 2005).

Lead is mostly found (96%) in the cell wall and vacuoles of plants. Plants accumulate lead in the following order: Roots > leaves > stem > inflorescence > seeds. However, the order is dependent on plant species and age. Dicots, for example, have been found to accumulate more lead in roots than monocots do. Seeds rarely have heavy metals present in them because the seed coat (testa) protects them greatly. Yet, when the testa is shattered due to the formation of the radicle, lead will easily enter the seed and accumulate inside excluding the radicle and hypocotyl (Sharma and Dubey, 2005).

Lead toxicity in plants causes significant concerns because of its lethal effect on plant species. Lead levels in plants may cause several disturbances such as enzyme activity prohibition, water and nutritional imbalances, changes in the membrane permeability, stunted growth, cell division, inhibition of root elongation, seed germination, chlorophyll production (this explains the loss of green color), and in severe cases death of the plant (Sharma and Dubey, 2005; Pourrut et al., 2011). However, some plants have the ability to form mechanisms to protect themselves from high levels of heavy metals. Plants either use the avoidance mechanism, vacuolar compartmentalization, or cellular sequestration (Maestri, 2010).

7. Lead in Human Body

Lead is considered one of the most significant heavy metals that have caused severe poisoning symptoms. Even though several measures have been taken to decrease lead exposure from industries such as reducing lead containing material, traces of lead have still been found in the environment. Lead has shown several serious symptoms on children and adults. Lead's main target is the central nervous system. It has been found to affect the brain and kidneys, cause high blood pressure, anemia, and maybe death. It has also been found to cause miscarriage in pregnant women and low sperm production in men (ATSDR, 2007).

What determines the level of lead toxicity is the means of exposure. Inhalation and ingestion are the highest cause of lead toxicity (ATSDR, 2007; Patrick, 2006). Small particles of lead that are inhaled from contaminated dust or soil enters the lungs and then moves to the bloodstream. About 30-40% of the lead inhaled is moved to the bloodstream (Patrick, 2006). Lead is ingested from the mouth when consuming lead contaminated goods. How much lead enters the bloodstream from the gastrointestinal tract depends on the time a person has had his last meal. Studies show that people can absorb more lead when they are fasting. About 60-80% of lead absorbed from the gastrointestinal tract travels into the bloodstream of fasting people; however, 6% of the lead moves from the gastrointestinal tract into the bloodstream when people have just had a meal. Also, iron levels are believed to hinder lead absorption in the gastrointestinal tract. In children, iron deficiency causes lead levels in the blood to rise. Studies show that children are able to absorb 50% more lead than adults. Moreover, high calcium levels in the diet of children can limit lead absorption. Other factors that can limit lead absorption in the gastrointestinal tract are improved magnesium and phosphate, alcohol, and fat containing food intake. 99% of lead that enters the body of an adult leaves through waste in about two weeks, while only 32% of the lead that enters the body of a child leaves through waste and the rest accumulate in the bones mainly (ATSDR, 2007; Patrick, 2006).

Lead can enter the skin but studies show that insignificant amounts reach the bloodstream. The European Food Safety Authority (2010) has estimated absorption of lead to be 0.06%.

Lead moves into the bloodstream after it has been ingested or inhaled and binds to the red blood cells. 99% of the lead in the bloodstream is bound to red blood cells and only

1% is present in the plasma and serum (Patrick, 2006; IARC, 2006). Lead stays in the blood for about 30-35 days and then moves to the soft tissues like liver, renal cortex of the kidney, lungs, brain, teeth, and bones. However, it has been found that infants can circulate lead in the bloodstream for up to 90 days (IARC, 2006). Moreover, lead stays in the soft tissues for about 4-6 weeks. Adults hold about 80-95% of the accumulated lead in the bones whiles children hold lower levels (70%). Lead stays in the bones for as long as 20-30 years (Patrick, 2006; IARC, 2006; EFSA, 2012).

8. Nickel General View:

Nickel has been considered an essential element for plants a few years ago and is ranked the 24th in its richness in Earth's crust. Earth's crust encompasses about 0.008% Nickel (He et al., 2012; Ahmad et al., 2011). Nickel is a transition metal that has an atomic number of 28, atomic mass of 58.69, melting point of 1,453.0 °C, and boiling point of 2,732.0 °C. Nickel is used mainly in manufacturing stainless steel products and nickel alloys. It is also used in other industries such as the chemical and food processing industries as catalysts and pigmentation (Cempel and Nikel, 2005; He et al., 2012). Nickel salts such as nickel chloride, nickel nitrate, and nickel sulfate are widely used and are considered commercially important (Cempel and Nikel, 2005). Agricultural operations such as fertilizer and pesticide use, combustion of waste, and sewage sludge use have increased nickel accumulation in soils. Not to mention, the industrial operations that have significantly polluted the environment such as mining and smelting operations (He et al., 2012).

9. Nickel in Soil and Plants

The natural concentration of nickel in soils is between 1-450 mg/Kg but when soils are polluted, nickel concentrations can reach 26,000 mg/Kg. Moreover, nickel can be highly present naturally in the soil. For example, soils formed from serpentine minerals have been found to contain high nickel levels (1-3%) and soils formed from limestone contain lower levels of nickel (Kopittke, 2007; He et al., 2012).

Nickel is present in three forms in the soil, available, potentially available, and unavailable. When nickel is in the available form, it is readily available for plant uptake. Nickel in the potentially available form is first adsorbed to soluble minerals or organic complexes but as soon as the available form is spared, the potentially available form becomes available for plants. On the other hand, the unavailable nickel form is not available to plants because it is complexed with oxides or with water insoluble compounds. Several factors, however, control the availability of nickel. These factors are pH, CEC, moisture, organic matter, presence of organic substances, redox reactions, temperature, and the concentration of macro and micronutrients (He et al., 2012; Seregin and Kozhevnikova, 2006). For example, a study by Seregin and Kozhevnikova (2006) showed that nickel absorption is the highest between 23 and 30°C while its absorption is low at temperatures like 2°C. Also, the presence of 2, 4-dinitrophenol in the soil solution blocked nickel uptake by 91%. The presence of nutrients such as zinc, copper, magnesium, and calcium has been found to stop nickel uptake, and zinc and copper having competitive effects on nickel absorption (He et al., 2012). Nickel is bound to magnesium and calcium oxides in most pH ranges, but nickel is bound to iron oxides only at pH above 5.5. Therefore, plant roots are covered with a coat of insoluble iron compounds that bind to nickel and prevent its entrance to the plant tissues but high pH levels in soil solution will allow nickel to enter plant tissues and eventually reach the shoots (Seregin and Kozhevnikova, 2006).

Plants can absorb nickel in the ionic form and in the complex form like nickel sulfate and nickel hydroxide (He et al., 2012). The means of absorption can be through the process of passive diffusion or active transport. However, active transport surpasses passive transport since factors like temperature and organic solvents are capable of stopping nickel absorption into the plant roots. Moreover, active transport takes up nickel ions when the concentration of nickel is low while passive transport increases at high nickel levels (Seregin and Kozhevnikova, 2006).

Nickel's approach to enter plants is not only through roots but through plant leaves as well. Studies have shown that 37% of nickel salts applied on sunflower leaves is found in other plant organs. The study has also been conducted on other crops like eggplants and tomatoes which has given similar results (Seregin and Kozhevnikova, 2006).

Even though it is considered an essential micronutrient to plants, nickel can show toxic symptoms on plants. The vegetative parts of plants contain about 1-10 mg Ni/Kg dry weight. Nickel plays an important role in plants. It is present in many enzymes of plants and it also serves as a protector against pathogens (rust) and herbivores. Studies have shown the positive effects of nickel availability on plant health. For example, alyssum species (Brassicaceae family) such as Alyssum *murale* and Alyssum *serpyllifolium* have been protected from fungal attack when nickel concentrations are higher than usual, however the exact concentration needed to show such effects is still unknown and studies are being conducted for better understanding on the subject (He et al., 2012).

It is very rare for plants to be deficient in nickel because it is needed in very low levels. However, when plants are deficient in nickel, they show symptoms in their growth and development. In addition, they show reduction in yields. Toxicity symptoms that also appear are chlorosis, necrosis, wilting and inhibition in growth. Moreover, a study has shown that high concentrations of nickel in plants cause significant reductions in competing nutrients like iron, manganese, and calcium. Also, as nickel levels increase, nutrients like potassium, zinc, copper, and nitrogen decrease (Ahmad et al., 2011). Another study that has been conducted on sunflower (*Helianthus annuus L*.) has resulted in the decrease of all plant nutrients in vegetative part and achenes of sunflower due to high nickel levels. This has led to decreased fresh weight of both shoots and roots (He et al., 2012).

10. Nickel in Human Body

Nickel's effect on the human body is not as harmful as the effect that cadmium and lead cause. Nickel is usually present in the human body. A healthy human body possesses about 0.2 µg/L in the blood and 1-3 µg/L in urine. However, outside exposure to nickel increases its levels, maybe to unwanted quantities. These outside exposures occur either through inhalation or through ingestion of food and water. Furthermore, the main means of exposure is ingestion through food, then through water, and lastly through inhalation from air (Cempel and Nikel, 2005; ATSDR, 2005). The tolerable daily intake of nickel is 2.8 µg Ni/Kg body weight according to EFSA (2015). Many food types contain different levels of nickel such as chocolate, soybeans, nuts, and oatmeal (ATSDR, 2005). Coming in contact with coins or jewelry containing nickel or even with soils containing nickel sources result in people's exposure to such a metal. The main health problems that come from nickel compounds and metals are mainly skin diseases such as skin rash, eczema, and contact dermatitis. Such irritations that nickel cause usually affect people sensitive to nickel or allergic to it. People are either born with nickel allergy or they may become allergic to it due to continuous contact with the skin, such as jewelry. In fact, some jewelry that contains nickel doesn't release much nickel to cause an allergy. Nickel can also be exposed to the fetus from the mother's blood. Nursing infants can also get exposed to nickel from the mother breast milk but in a lesser concentration (ATSDR, 2007; Seregin and Kozhevnikova, 2006).

Absorption of nickel in the stomach depends on several factors such as the amount of food present, acidity, dietary and other components that can bind to nickel (Cempel and Nikel, 2005). A fasting person is able to absorb more nickel than a person who has just eaten. Even though nickel's main exposure is the gastrointestinal tract, it has an insignificant absorption rate. However, a study has shown that nickel is able to play an important role as a co-factor in the intestines to absorb iron more efficiently. This study has been done on rats deficient in iron. Results have proved better iron absorption in the presence of nickel but this was seen only when the dietary iron was in the unavailable form. When the dietary iron consumed was a mixture of both available and unavailable forms, no effect has been observed. Also, most of the absorbed nickel leaves the body through urine regardless of the way the body has been exposed to it while unabsorbed nickel is excreted through feces (Punamiya et al., 2008).

Nickel has been found to target the respiratory tract when inhaled. When it is ingested, nickel targets the kidneys (Cempel and Nikel, 2005). Whether under long term exposure or short term, nickel has been seen to target the kidneys predominantly, when ingested, and then it goes to other parts of the body such as the cardiovascular system and immune system. Nickel is found to affect the body in the following order: kidneys > lungs> liver >heart> testicles. When nickel is inhaled and passed to the lungs, 20-30% of it is absorbed into the blood.

Nickel can enter the body from the skin fast only when nickel is in the form of nickel chloride or nickel sulphate. In this way, the skin can absorb 77% of the nickel in 24 hours. The five most dangerous nickel compounds assigned by the World Health Organization (WHO) are nickel powder, nickel sulphate, nickel chloride, nickel carbonate and nickel nitrate. Nickel powder has been classified under the chronic toxicity group while the rest of the nickel compounds are considered carcinogenic when inhaled. They are also considered reproductive toxicants and chronic toxicants (Punamiya et al., 2008). Nickel compounds are also considered carcinogenic to humans according to the International Agency for Research on Cancer (IARC, 2012). Because nickel has been found to interfere with the physiological process of several elements needed by the body such as iron, copper, manganese, zinc, calcium, and magnesium, its carcinogenic effect becomes elevated (Punamiya et al., 2008; Cempel and Nikel, 2005) .

C. Uptake of Cadmium, Lead, And Nickel in *Origanum Syriacum*

Origanum syriacum (za’atar) is one of the most consumable herbs in the Arab region. It is used mainly in the man’oushe, a type of pastry vastly consumed by all classes of people and especially the lower class. “What if *Origanum* has the ability to accumulate heavy metals in its system?” is a question that many experts have asked. For that reason, scientists have done many experimental researches to answer the question. Results, however, have not been as optimistic as needed and at the same time they are not very hazardous as well. Nonetheless, how dangerous *Origanum* is to the human body depends on the type of heavy metal accumulated in it. Some heavy metals are more toxic and have more detrimental effects than others. Moreover, some heavy metals have been observed to accumulate more than others in the edible parts of *Origanum* and at different levels of concentrations. For example, some heavy metals have been found to accumulate only in the roots while others have been able to reach the shoots.

Many studies have been conducted to test several herbs and medicinal plants present in the market. Also, herbs and medicinal plants were planted in heavy metal contaminated soils to determine the effect of such metals on the productivity and quality of such plants. In addition, the objective of these studies is to determine whether such plants can accumulate heavy metals and in what quantity. At the same time, these studies are especially important to check whether the levels of accumulation would have negative and detrimental effects on humans, primarily, and on the food chain and the ecosystem as a whole.

1. Research Experiments

In their research, Al-Eed et al. (1997) investigated the availability of 4 heavy metals (Cd, Co, Pb, and Se) in 18 different spices in the Saudi Arabian market. Of these 18 spices is thyme (*Thymus vulgaris*) where its leaves have been tested for heavy metal presence. Results have shown that lead levels are between 0.4 and 0.9 mg/Kg for all the tested samples. These levels exceed 0.3mg/Kg, the amount set by FAO and WHO. As for cadmium, results for the tested samples did not exceed the maximum permissible concentration (0.20 mg/Kg), also set by both FAO and WHO. Cadmium levels have ranged between 0 and 0.14 mg/Kg. However, cadmium levels in thyme have not been detected. Another study that has been done by Dghaim et al. (2015) has tested a total of 81 samples for 7 different herbs that are commonly consumed in the UAE (United Arab of Emirates) market. Of these 81 samples are 11 *Origanum vulgare* and 13 *Thymus vulgaris*. These traditional herbs have been tested for several heavy metals including cadmium and lead. Results have shown that the maximum cadmium level for *Origanum* vulgare is 0.35 mg/Kg and for *Thymus vulgaris* is 0.63 mg/Kg. In other words, 9% and 27% of the tested *Origanum* vulgare and *Thymus vulgaris* samples, respectively, exceeded the maximum permissible concentrations of permissible limits (PL) set by the FAO and WHO for cadmium in medicinal herbs and plants. As for lead, its concentration in *Origanum* vulgare and *Thymus vulgaris* is 18.06 and 23.52 mg/Kg respectively. Both results have exceeded the permissible limits set by the FAO and WHO which is 10 mg/Kg. Moreover, 90% of the *Origanum* vulgare samples and 91% of *Thymus vulgaris* samples that have been tested have given lead levels above 10 mg/Kg. These levels have been detected and reported in many Middle Eastern countries. For example, in Jordan, Egypt, and Iran, the lead levels have been reported to be 13.1, 14.4, and 21.7 mg/Kg respectively.

In Ash-Shoubak area in South of Jordan, a study has been conducted by Mohammad Sanad Abu-Darwish in 2009 in which he investigated the content of 9 heavy metals, including Cd, Pb, and Ni, in three aromatic medicinal plants, two of which are from the *Thymus species* (*Thymus vulgaris L. and Thymus serpyllum L.*). Results have shown that cadmium content was undetectable in both thymus species, lead content was much higher in *Thymus vulgaris* (32.03 mg/Kg) than in *Thymus serpyllum* (1.26 mg/Kg) where it exceeded the acceptable level set by WHO, and nickel content was high in *Thymus vulgaris* (23.85 mg/Kg) and not detectable in *Thymus serpyllum*. The permissible limit for nickel is 10 mg/Kg as recommended by WHO.

Bulgarian herbs used in tea bags have been tested by Arpadjan et al. (2008) for heavy metals contents. In this study, several herbs were analyzed including 14 *Origanum vulgare* and 12 *Thymus serpyllum*. Results have shown that maximum heavy metal levels have been found to be 0.25 mg/Kg for cadmium and 8.6 mg/Kg for lead. These levels are still below the maximum permissible level set by WHO. These results have been similar to other research conducted in different countries like the works done by Lozak et al. in 2002 in Poland, Abou-Arab et al in 1999 in Egypt, and Khan et al. in 2001 in the United States.

In Plovdiv, Bulgaria, an experiment was conducted near a non-ferrous metal combine (Pb-Zn) smelter by Zheljazkov et al. (2008) to study the relationship between heavy metals availability in the soil plots at different distances from the smelter and the heavy metal content in 5 medicinal plants planted in these plots. The distances that have been chosen are 0.5 Km (soil 1), 3 Km (soil 2), 6 Km (soil 3), and 9Km (control) away from the smelter. Results have shown that plants in soil 1 gave about 32% lower yields compared to the control plots while those in soils 2 and 3 gave similar results as the control. As for the heavy metals contents, plants had high cadmium and lead content in soil 1 relative to soils 2, 3 and the control. However, *Origanum heracleoticum* present in soil 1 contained high levels of cadmium and lead relative to the other medicinal plants that have been tested.

A pot experiment was conducted by Lydakis-Simantiris et al. (2012) to determine the effect of cadmium, lead, and nickel on growth and quality of three different crops including thyme (*Thymus vulgaris*). Results have shown that cadmium accumulates in roots and increases with increasing cadmium levels in soil. Furthermore, cadmium levels in leaves are the highest (8.65 mg/Kg) when cadmium levels in the soil are high (30 mg/Kg) while cadmium levels in leaves do not exceed 1 mg/kg in the 0, 1, 3, and 10 mg/Kg concentrations in the soil. However, all cadmium levels in leaves of thyme exceed the limit set by WHO. As for lead, its concentration in the roots is high especially when the concentrations in the soil are 600 and 1800 mg/Kg. Also, all lead levels in thyme leaves have given relatively similar results and all are higher than the permissible limits set by the WHO excluding the control. Nickel levels are similar to lead levels in roots. Nickel content in the roots is highest in high nickel content in soil. As for nickel levels in leaves, all nickel levels in soil gave relatively similar concentrations in leaves (27-46 mg/Kg) except for control (0.55 mg/Kg).

D. Limits of Cadmium, Lead, and Nickel in Food

The maximum permissible limit is the level of toxic material at which a person is not health threatened by it. Basically, those who set these limits are recommended by the government or international organizations such as the FAO and WHO. Those limits differ from one organization or government to the other. The following tables (tables 3-11) show the limits of cadmium, lead, and nickel for herbal medicines and leafy vegetables the daily allowable intake is also reported. These data are acquired from different sources and they are JECFA, Canadian government, WHO, ATSDR, Australian government, CODEX, and EFSA. One table has also been extracted from an article written by Ezeabara et al. (2014).

According to the European Food Safety Authority (EFSA), there is no maximum limit for nickel in food. However, an oral exposure of 1.1 µg Ni/Kg body weight was found to cause systemic contact dermatitis on human volunteers (EFSA, 2015). However, for those who are not sensitive to nickel, a tolerable daily intake of 2.8 µg/Kg body weight is suitable.

Because heavy metals limits differ from one source to another, it is always best to use the lowest limits possible. People should have zero tolerance to products containing heavy metals; however, air, food, and water are all contaminated with heavy metals and that is why people should accept this fact and try to cope with the situation. Therefore, the lower the daily intake of heavy metals, the better and the healthier for people. These words have been stated by Rick Liva, a naturopathic doctor, in his article “Facing the Problem of Dietary-Supplement Heavy-Metal Contamination: How to Take Responsible Action” (2007).

Table 3. Oral Limit for Cadmium (μg/Day) Set by Different International Organizations and Governmental Authorities.

	Established oral limits for cadmium (μg/day)			
		μg/day	**Notes**	**Reference**
Limit for Finished Product: daily dose	**AHPA**	4.1	Maximum quantitative limit for cadmium in a dietary supplement	AHPA executive committee action, November 16, 2009
	NSF/ANSI 173	6	Maximum level of undeclared cadmium	NSF International Standards/American National Standard for Dietary Supplements; Approved by ANSI and designated as an ANSI Standard on April 14, 2008
	Canada natural health products directo rate	6	Established tolerance of < 0.09 μg/kg bw for cadmium multiplied by 70 kg for an adult to reflect adoption from NSF/ANSI 173	Natural Health Products Compliance Guide, Version 2.1, January 2007.
	California Prop 65 reproductive toxin	4.1	Maximum Allowable Dose Level (MADL) of cadmium above which warning is required	Proposition 65 Safe Harbor Levels, March 2008. Listing is for "cadmium" and specifies "developmental, male."
	California Prop 65 carcinogen	-	No Significance Risk Level (NSRL) for cadmium exists only for inhalation (0.05 μg/day); no level is given for oral consumption	Proposition 65 Safe Harbor Levels, March 2008. Note that though cadmium is listed for cancer, this chemical is not generally considered carcinogenic by the oral route. the listing of cadmium in the Proposition 65 list does not, however, state this clearly.
Limit for total daily consumption	**US agency for toxic substances and diseases (ATSDR)**	14	Minimal Risk Level (MRL) of 0.2 μg/kg bw for chronic cadmium oral consumption multiplied by 70 kg for an adult	ATSDR Minimal Risk Levels, November 2007; http://www.atsdr.cdc.gov/mrls/pdfs/mrllist_11_07.pdf.
	US Environmental Protection Agency (EPA)	70	Reference Dose (RD) of 1.0 μg/kg bw for dietary exposure to cadmium multiplied by 70 kg for an adult re: chronic effects (noncancer)	EPA's Integrated Risk Information System: Cadmium, February 1994; http://www.epa.gov/iris/subst/0141.htm; also see: http://www.epa.gov/ttn/atw/hlthef/cadmium.html).
	US FDA Tolerable daily intake	55	The cited references gives this level for cadmium, and states, "since cadmium toxicity is expressed only after chronic exposure, separate figures for the age category 2-5 years are not warranted	Guidance Document for Cadmium in Shellfish, 1993; http://www.cfsan.fda.gov/~frf/guid-cd.html. Note that this document "no longer represents the current state of science and is presented here for the historical record only."
	Joint FAO/WHO Expert Committee on food Additives (JECFA)	70	Provisional Tolerable Weekly Intake (PTWI) of 7 μg/kg bw for cadmium multiplied by 70 kg for an adult and divided by 7 to obtain a daily limit	WHO Food Additives Series 52; Safety evaluation of certain food additives and contaminants; 2004; http://whqlibdoc.who.int/publications/2004/924166052X.pdf page 556.
	European Union (EU)	70	The European Commission endorsed JECFA's PTWI of 7 μg/kg bw for cadmium on June 2, 1995; multiplied by 70 kg for an adult and divided by 7 to obtain daily limit	2006: Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs; in the Official Journal of the European Union, 20.12.2006 (EN), L 364/5.

Source: (AHPA, 2009)

Table 4. Oral Limit for Lead (μg/Day) Set by Different International Organizations and Governmental Authorities

	Established oral limits for Lead (μg/day)			
		μg/day	**Notes**	**Reference**
Limit for Finished Product: daily dose	**AHPA**	10	Maximum quantitative limit for lead in a dietary supplement	AHPA executive committee action, November 16, 2009
	NSF/ANSI 173	20	Maximum level of undeclared cadmium	NSF International Standards/American National Standard for Dietary Supplements; Approved by ANSI and designated as an ANSI Standard on April 14, 2008
	Canada natural health products directo rate	20	Established tolerance of < 0.29 μg/kg bw for lead multiplied by 70 kg for an adult to reflect adoption from NSF/ANSI 173	Natural Health Products Compliance Guide, Version 2.1, January 2007.
	California Prop 65 reproductive toxin	0.5	Maximum Allowable Dose Level (MADL) of lead above which warning is required	Proposition 65 Safe Harbor Levels, March 2008. Listing is for "lead" and specifies "developmental, female, male."
	California Prop 65 carcinogen	15	No Significance Risk Level (NSRL) of lead above which warning is required	Proposition 65 Safe Harbor Levels, March 2008. Note that each of the following is listed as a carcinogen: "lead and lead compounds," "lead acetate," "lead phosphate," and "lead subacetate."
Limit for total daily consumption	**US agency for toxic substances and diseases (ATSDR)**	-	No Minimal Risk Level (MRL) established for lead "because a clear threshold for some of the more sensitive effects in humans has not been identified."	August 2007: ATSDR's Toxicological Profile for Lead, Draft from Public Comment; Section 2: Relevance to Public Health; page 31.
	US Environmental Protection Agency (EPA)	-	EPA has not established a Reference Dose (RfD) for lead; RfD Work Group (1985) stated "inappropriate to develop an RfD for inorganic lead." Cited reference notes it appears that some of lead's adverse effects "may occur at blood lead levels so low as to be essentially without a threshold."	EPA's Integrated Risk Information System: Lead and compounds (inorganic); July 2004 (last revised); http://www.epa.gov/iris/subst/0277.htm; also see: http://www.epa.gov/ttn/atw/hlthef/lead.html).
	US FDA Tolerable daily intake	75	Level here is for adults. Level is 25 μg/day of lead for pregnant women, 15 μg/day for children 7 and older, and 6 μg/day for children under 6.	1993: Guidance Document for Lead in Shellfish; http://www.cfsan.fda.gov/~frf/guid-pb.html. Note that this document "no longer represents the current state of science and is presented here for the historical record only."
	Joint FAO/WHO Expert Committee on food Additives (JECFA)	250	Provisional Tolerable Weekly Intake (PTWI) of 25 μg/kg bw for lead multiplied by 70 kg for an adult and divided by 7 to obtain daily limit	Safety Evaluation of Certain Food Additives and Contaminants, WHO Food Additives Series: 44; 2000; http://www.inchem.org/documents/jecfa/jecmono/v44jec12.htm
	European Union (EU)	250	The European Commission endorsed JECFA's PTWI of 25 mcg/kg bw of lead on June 19, 1992; multiplied by 70 kg for an adult and divided by 7 to obtain daily limit.	Commission Regulation (EC) No 1881/2006 of 19 December 2006; maximum levels for certain contaminants in foodstuffs; Official Journal of the EU, 20.12.2006 (EN), L 364/5.

Source: (AHPA, 2009)

Table 5. JECFA (and EU as indicated) Heavy Metal Limits

Heavy Metal	Stated Limit (PTWI-weekly)	Calculated Daily Limit (adult, 70 Kg)	EU Status
Cadmium (Cd)	7 µg cadmium / Kg bw	70µg	Endorsed 6/2/1995
Lead (Pb)	25 µg lead / Kg bw	250 µg	Endorsed 6/19/1992

Source: (AHPA, 2009)

Table 6. Heavy Metal Limits for Canada's Natural Health Products

	Stated limit	Calculated daily limit (adult, 70 Kg)
Cadmium (Cd)	0.09 µg cadmium/ Kg bw	6 µg
Lead (Pb)	0.29 µg lead/ Kg bw	20 µg

Source: (AHPA, 2009)

Table 7. Australian Government Maximum Levels

Heavy metal	Food	Maximum level (mg/Kg)
Cadmium (Cd)	Leafy vegetables	0.1
Lead (Pb)	Vegetables	0.1

Source: (Federal Register of Legislation, 2011)

Table 8. Codex standards with reference to JECFA

Heavy metal	Product	Maximum level (mg/kg)
Cadmium (Cd)	Leafy vegetables	0.2
Lead (Pb)		0.3

Source: (CODEX, 2009)

Table 9. National Limits in Herbal Medicines and Products

		Lead (Pb)	**Cadmium (Cd)**
For herbal medicines			
Canada	Raw herbal materials	10 ppm	0.3 ppm
	Finished herbal products	0.02 mg/day	0.006 mg/day
China	Herbal materials	10 ppm	1 ppm
Malaysia	Finished herbal products	10 mg/day	-
Singapore	Finished herbal products	20 ppm	-
Thailand	Herbal material, Finished herbal products	10 ppm	0.3 ppm
WHO Recommendations		10 mg/kg	0.3 mg/kg
For other herbal products			
National sanitation foundation draft proposal (Raw dietary supplement)		10 ppm	0.3 ppm
National sanitation foundation draft proposal (Finished dietary supplement)		0.02 mg/ day	0.006 mg/day

Source: (WHO, 2007)

Table 10. Toxic Limit and Recommended/Safe Intake of Heavy Metal

Heavy metal	**Toxic limit**	**Recommended intake (safe intake)**
Cadmium (Cd)	200 µg/Kg of fresh weight	15-50 µg/day adults 2-25 µg/ day children
Lead (Pb)	≥ 500 µg/L (blood)	20-280 µg/day adults 10-275 µg/day children

Source: (Ezeabara et al., 2014)

Table 11. Tolerable daily intake set by EFSA and ATSDR

Heavy metal	Reference	Tolerable daily intake (TDI)
Nickel (Ni)	ATSDR	101-162 µg/day for adults 136–140 µg/day for males 107–109 µg/day for females 121 µg/day for pregnant women 162 µg/day lactating women
	EFSA	2.8 µg/ Kg bw[1]

Source: (EFSA, 2015; ATSDR, 2005)

[1] TDI applies on humans not sensitive to Nickel

CHAPTER 3

MATERIALS AND METHODS

In this chapter, a clear description of the materials and methods used in this study will be presented. The study was composed of three parts:

A. Conducting a pot experiment at FAFS greenhouse in Beirut

B. Collecting soil and *Origanum* samples from different regions in Lebanon

C. Collecting *Origanum* samples from different sale outlets in Lebanon

Moreover, the procedure for soil analysis used in the pot experiment and those selected from several fields of different soil properties in Lebanon is indicated. Following soil analysis, the statistical analysis of the pot experiment results is illustrated.

A. Pot experiment at FAFS Greenhouse in Beirut

A pot experiment was conducted at the greenhouse area at AUB, Beirut. This study was done to examine the effect of cadmium, lead, and nickel in the soil on their absorption by *Origanum syriacum* shoots (stems and leaves).

1. Experiment Preparation

A total of 180 Kg of soil brought from AREC (Agricultural Research and Educational Center) were used in the experiment. The soil was sieved with a 4 mm size opening to remove large gravels and other unwanted material. The soil was then distributed into 45 pots, 4Kg of soil each. The pots were divided into 3 sets. First set was for cadmium

(Cd), 2nd for lead (Pb), and 3rd for nickel (Ni). Each set encompassed a total of 15 pots that were divided into 5 rows of 5 different concentrations replicated 3 times. After the pots were filled with soil, they were mixed with a solution containing the required amount of heavy metal concentrations and fertilizers. The control pots however, were only mixed with fertilizers and distilled water.

a. Distribution of the Pot Experiment

Table 12 shows the treatments used for each heavy metal and the tested concentrations. Table 13 shows the randomized distribution of the pots in the greenhouse at AUB, Beirut.

Table 12. Concentrations of Cd, Pb, and Ni Used in the Pot Experiment

Treatments		
Cadmium (mg/Kg)	**Lead (mg/Kg)**	**Nickel (mg/Kg)**
0	0	0
10	100	50
20	200	100
50	500	200
100	1,000	500

Table 13. Distribution of Pots in the Greenhouse.

Cd (mg/Kg)			**Pb (mg/Kg)**			**Ni (mg/Kg)**		
C	C	100	1,000	500	500	200	50	50
10	100	20	500	1,000	1,000	C	C	100
100	10	C	100	100	100	50	100	C
50	50	10	200	C	C	500	500	200
20	20	50	C	200	200	100	200	500

Figure 2. Pot Experiment at the Greenhouse, Beirut (April 1, 2015).

b. Heavy Metal Solutions

The sources of the heavy metals used to prepare the stock solutions added to the pots were:

- Cadmium: 16.46g/L of cadmium nitrate $Cd(NO_3)_2 \bullet 4H_2O$
- Lead: 96g/L of lead nitrate $Pb(NO_3)_2$
- Nickel: 134g/L of nickel sulfate $Ni(SO_4)_2 \bullet 6H_2O$

c. Fertilizers Preparation

40 g of soluble fertilizer (20-20-20 + TE) were dissolved in 2L of water and 60 ml of this solution were applied to each pot (1.2g fertilizer per 4 Kg pot), as a source of supplemental nutrients for the plants during the study

2. Planting and Harvesting

Origanum syriacum seedlings were brought from Al-Mona Nursery in Jiyyeh. Two seedlings were planted in each pot on December 25, 2014. The seedlings were left for four months before harvest on April 1, 2015. The shoots (stem and leaves) of the plants were harvested and were placed in envelopes, washed, and dried in an oven for 24-48 hours at a temperature of 60°C, then ground and stored in 50 ml clean falcon tubes for analysis.

3. Experimental Digestion

One gram of ground tissue was placed in a ceramic crucible and ashed at 600°C in a furnace. The ashes were dissolved in 10 ml of 65% nitric acid and placed in a clean falcon tube of 50 ml and diluted to 25 ml using deionized water. The measurements of cadmium, lead, and nickel solutions were done at the KAS CRSL using atomic absorption spectrophotometer, Thermo Elemental model.

B. *Origanum* Samples Collected From Different Regions in Lebanon

Origanum plants and the soil around roots were collected together from different regions in Lebanon for analysis of cadmium, lead, and nickel. The samples from each region were collected from *Origanum* planted fields where soils looked to be different in physical properties (texture, color…etc). Table 14 shows the locations of the collected samples: South (6 samples), Beqaa (2 samples), and Jbeil (2 samples). Figure 3 shows the cites of the soil and *Origanum* samples collected from different regions in Lebanon.

Table 14. Location of the Collected *Origanum* Samples in Lebanon

Sample Number	Area	Leaves/Mixed
1	Zawtar	leaves
2	Zawtar	leaves
3	Zawtar	leaves
4	Adsheet	leaves
5	Sour	leaves
6	Sour	leaves
7	Tal Thnoub	leaves
8	Terbol	leaves
9	Jbeil	leaves
10	Jbeil	leaves

C. *Origanum* Samples Collected From Different Sale Outlets in Lebanon

Origanum samples were collected from different sale outlets in Lebanon for analysis of cadmium, lead, and nickel levels. Table 15 shows the samples that were purchased from the stores in different cities in Lebanon, mainly Beirut. These samples were either mixture samples (sesame, sumac, and salt) or fresh leaves. Figure 3 shows the sites of *Origanum* samples collected from sale outlets in Lebanon.

Table 15. Location of the Collected *Origanum* Samples from Different Sale Outlets in Lebanon

Sample Number	Area	Leaves/Mixture
11	Beirut	Leaves
12	Beirut	Leaves
13	Beirut	Leaves
14	Beirut	Leaves
15	Zawtar	Mixture
16	Beirut	Mixture
17	Beirut	Mixture
18	Beirut	Mixture
19	Beirut	Mixture
20	Beirut	Mixture
21	Ras el Maten	Mixture
22	Safita	Mixture
23	Batroun	Mixture
24	Chhim	Mixture

Figure 3. Map showing the sites of soil and *Origanum* samples collected from different regions and sale outlets in Lebanon

D. Soil Analysis

The physical and chemical properties of the soil used in the pot experiment and those collected from different regions in Lebanon were analyzed according to Bashour and Sayegh (2007).

1. *Physical Properties*

a. <u>Soil Moisture</u>

Soil moisture was determined using an oven. The soil samples were placed at 105°C for 24 hours. The difference between the air-dry and oven-dry samples was expressed in percentage of oven dry weight.

b. <u>Soil Texture</u>

Soil texture was determined using the mechanical analysis method. This method is also known as the Bouyoucos method. It is based on stokes' law which states that particles will fall at a velocity which are controlled not only by the radius of the particle, but also by the force due to gravity. Fifty grams of soil were blended with 50 ml of the dispersing agent sodium hexametaphosphate solution (calgon) for 5 minutes, placed in a 1,000 ml graduated cylinder, and made up to volume with distilled water. Then, the cylinder was closed with parafilm and shook well by turning it upside down several times and placed on the stand for 20 seconds. The hydrometer was then placed in the suspension and at 40 seconds, the hydrometer reading (first reading) was measured. The first hydrometer reading measures silt and clay. The temperature was also recorded for correcting the hydrometer reading. The second hydrometer reading was taken after 2 hours to determine the clay content. The temperature was also recorded for the correction of the second hydrometer reading. The percent sand, silt, and clay were calculated, the soil textural class was determined using the soil textural triangle.

2. *Chemical Properties*

a. Electrical Conductivity (EC)

The measurement of EC in soil analysis indicates the concentration of total soluble salts in the sample. The unit of measurement is mS/cm. The EC of the soil sample was measured using an EC meter. A 2:1 (water:soil) ratio was used for the EC measurement of the soil. The samples were shaken for 30 minutes on the mechanical shaker, filtered using a 150 mm filter paper, and EC was measured using EC meter,Eutech model.

b. Soil Reaction (pH)

The pH value indicates the acidity or alkalinity of a soil. The pH value of a solution is equal to the negative logarithm (base 10) of the hydrogen ions concentration in equivalent per liter. pH is very important in soil analysis because it affects the solubility of nutrients and microbial activity. pH was determined using a pH meter, Oakton model.

c. Calcium Carbonate ($CaCO_3$)

Calcium carbonate is a major component of the calcareous soils in Lebanon. The free calcium carbonate levels in soils determine the calcarousness of the soil. The amount and form of calcium carbonate and its distribution throughout the soil profile, affect soil physical and chemical characteristics. Calcium carbonate is measured in percentage and can be determined using the titration method. 100 ml of HCl 1 N were added to 5 g of soil, boiled for 5 minutes, and filtered. Later, 10 ml sample were titrated back with NaOH 0.5 N. phenolphthalein was used as an indicator. HCl endpoint is pink in color.

d. Cation Exchange Capacity (CEC)

The cation exchange capacity (CEC) is the ability of a soil to hold ions on its exchange complex. CEC measures the negative charges on soil surfaces and is expressed in cmol/Kg (meq/100g) soil. CEC is considered a storehouse for plant nutrients and prevents loss of nutrients by leaching. CEC experimental procedure is present in the laboratory manual of Sayegh and Bashour (2008).

e. Organic Matter (O.M.)

Organic matter is the organic part of the soil that is originated from plant and animal residues decomposed by soil microorganisms. It is mainly present in the top 25 cm of the soil profile. It is measured in percentage. OM was determined by the combustion method. Thirty grams of soil were put in a ceramic crucible to be oven dried at 105°C for 24 hours. Then, the crucible and oven-dry soil were transferred to a furnace at a temperature of 600°C for 3 hours. The difference in weights of the soil sample before and after ashing was the OM content.

f. Available Phosphorus (P)

Phosphorus is one of the major macronutrients in plant nutrition. It is considered the second most important macronutrient. Determining available phosphorus was done using Olsen's method. Five grams of soil were extracted by 100 ml of $NaHCO_3$ (1M), shaken on the mechanical shaker for 30 minutes and filtered. 10 ml of the filtrate were then transferred to a 50 ml volumetric flask. eight milliliters of ammonium molybdate-ascorbic acid solution were added to the sample and made up to volume (50 ml) with distilled water. phosphorus standard curve was prepared at concentrations 0, 2, 5, 10, and 15 mg P/L (ppm). The quantities of available phosphorus in the soil samples were estimated by a blue color forming, absorbance at 880 nm wavelength using a spectrophotometer, Shimadzu model.

g. Available Potassium (K)

Available K was extracted from the soil by adding 50 ml of ammonium acetate (1M) to 5 g of soil, shaken for 30 minutes on the mechanical shaker, filtered in a 150 mm filter paper (sartorius stedim, grade 390), and stored in a clean 50 ml falcon tube. The amount of available K in the extract was measured by flame photometer, BWB Technologies model.

h. Available Nutrients

Available nutrients (Fe, Zn, Cu, and Mn) and available heavy metals (Cd, Pb,and Ni) were extracted by DTPA-TEA Solution (Lindsay and Norvell method). Twenty grams of soil were extracted with 80 ml of DTPA-TEA solution and filtered using a 110mm filter paper (Whatman; number 42). The filtrate was then stored in a 50 ml falcon tube. All the 7 elements in the solution were measured by atomic absorption spectrophotometer, Thermo Elemental model .

i. Statistical Analysis

The design of the pot experiment was a CRD (Completely Randomized Design). Basically, the study is composed of three independent experiments where each heavy metal (Cd, Pb, and Ni) is the fixed or independent variable and the concentration for each of the heavy metals (Cd, Pb, and Ni) is the dependant variable. The program used for the statistical analysis is the SPSS statistical analysis program, version 23.0.

CHAPTER 4

RESULTS AND DISCUSSION

In this chapter, results of the soil analysis for the pot experiment and the selected soil samples will be presented and discussed. Moreover, the concentrations of cadmium, lead, and nickel in *Origanum syriacum* shoots will also be presented and discussed; in addition, these results will be compared with the limits set by different international organizations and governmental authorities.

A. Soil Analysis for Pot Experiment

Soil analysis is the basic step needed to be taken in any agricultural procedure. It is essential and is needed to be performed before any study conducted because it specifies the type of the soil being used. Table 16 shows the results of the physical and chemical properties of the soil used in the pot experiment.

Table 16. Physical and Chemical Characteristics of the Soil Used in the Pot Experiment.

Characteristics	Results
Textural class	Sand: 23.10%, Silt: 30.80%, Clay: 46.10%
	Clay
Electrical conductivity (EC)	0.14 mS/cm
Soil reaction (pH)	7.75
Free $CaCO_3$	26%
Organic matter	4.54%
CEC	19.3 cmol/Kg
Available Phosphorus	2.1 mg/Kg
Available Potassium	480 mg/Kg
Available Micronutrients (mg/Kg)[2]	
Fe	0.824
Zn	0.397
Cu	0.316
Mn	0.273
Available Heavy metals (mg/Kg)[3]	
Cd	0.043
Pb	ND
Ni	0.209

Note: ND: not detected

[2] DTPA Extraction using Lindsay and Norvell method, 1978
[3] DTPA Extraction using Lindsay and Norvell method, 1978

1. Soil Texture

Soil texture is considered one of the most important soil physical properties because it affects several factors such as water storage and infiltration rates, aeration, and soil fertility. There are three soil separates: sand, silt, and clay. Each soil separate has different characteristics from one another. Sand is known to have the greatest particle size (0.1-2 mm) while clay has the smallest particle size (< 0.002 mm); silt is the fraction with particle size in between sand and clay. Below is Table 17 that shows the soil separates and their diameter ranges.

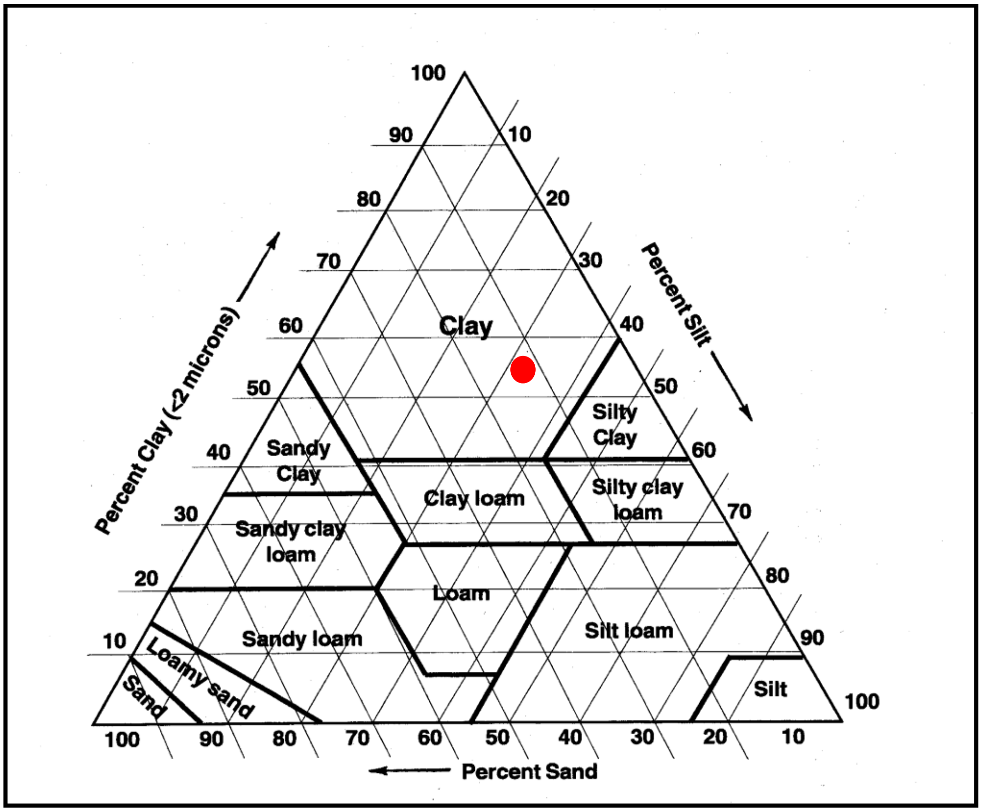
Table 17. Soil Separates and Their Diameter Ranges.

Soil Separate Name	Diameter Range (mm)	Familiar Size Comparison
Very Coarse Sand	2.0-1.0	Thickness of a coin
Coarse Sand	1.0-0.5	
Medium Sand	0.5-0.25	Sugar or salt crystal
Fine Sand	0.25-0.10	
Very Fine Sand	0.10-0.05	Thicknes of a book page
Silt	0.05-0.002	
Clay	<0.002	Smaller than bacteria

Source: (Gardiner and Miller, 2008)

The percentage of sand, silt, and clay in Table 16 and Figure 4 (Soil Textural Triangle) show that the soil sample has a clay texture.

Figure 4. Soil Textural Triangle Showing the Different Textural classes



Source: (Sayegh and Bashour, 2007)

Clay soils have the ability to store water because of their small particle size. They have low infiltration rates. Soil nutrients do not flush down the soil profile, therefore they are fertile soils. However, when clay soils dry, they become very hard and difficult to work with.

2. *Electrical Conductivity (EC) and Soil Reaction (pH)*

The Electrical conductivity (EC) of the soil show that it is not saline. Saline soils have an EC of 4 dS/m and more. Table 18 shows soil salinity classes and their effect on plant growth.

Table 18. Soil Salinity Classes and Crop Growth

Soil Salinity Class	Conductivity of the Saturation Extract (dS/m)	Effect on Crop Plants
Non Saline	0 - 2	Salinity effects negligible
Slightly Saline	2 - 4	Yields of sensitive crops may be restricted
Moderately Saline	4 - 8	Yields of many crops are restricted
Strongly Saline	8 - 16	Only tolerant crops yield satisfactorily
Very Strongly Saline	> 16	Only a few very tolerant crops yield satisfactorily

Source: (FAO, 1988)

The pH or soil reaction of the soil sample is slightly basic (slightly alkaline). Most crops grow in soils with pH between 5.5-8.5 because most of the nutrients required by the crops become available in this pH range and microorganisms are usually active in this range. Table 19 shows the pH classes and their ranges.

Table 19. Soil pH Classes and Their Ranges

pH Class	Ranges
Extremely Acid	3.5 – 4.4
Very Strongly Acid	4.5 – 5.0
Strongly Acid	5.1 – 5.5
Moderately Acid	5.6 – 6.0
Slightly Acid	6.1 – 6.5
Neutral	6.6 – 7.3
Slightly Alkaline	7.4 – 7.8
Moderately Alkaline	7.9 – 8.4
Strongly Alkaline	8.5 – 9.0

Source: **(**Gardiner and Miller, 2008)

3. *Free Calcium Carbonate (CaCO3)*

Calcium carbonate is the component of limestone. It is used to raise the pH of acidic soils in order to decrease the availability of toxic minerals such as Al and Mn. The soil sample was brought from AREC (Bekaa) and contains 26% of free calcium carbonate. It is highly calcareous, similar to many Lebanese soils which contain various levels of free $CaCO_3$.

4. *Organic Matter (O.M.)*

Organic matter is mainly present in the top 25 cm of the soil surfaces. It is originated from decomposing material of plants and animals. The O.M. occupies only 1-5% of the soil and it influences soil properties. It increases soil aeration and contributes to soil CEC. It is also a carbon source for the beneficial microorganisms such as N-fixing bacteria. The soil sample used in this study contains 4.54% O.M. which is considered a high level if compared to other cultivated soils in arid and semi-arid regions.

5. *Cation Exchange Capacity (CEC)*

Cation exchange capacity is the total number of exchangeable cations that the soil can adsorb expressed in centimole per kilogram of soil. The CEC of the soil sample is 19.3 cmol/Kg of soil and is considered medium. Clay soils and organic matter have high CEC. High CEC aids in reducing the loss of many plant nutrients in leaching.

6. Available Nutrients

Phosphorus and potassium are two of the macronutrients required by plants. They are needed in high quantities by the plants. The level of available phosphorus of the soil sample is low while potassium is sufficient. Moreover, iron, zinc, copper, and manganese are the micronutrients required by plants. They are needed in small quantities. Iron and manganese in the soil sample are considered low while zinc and copper are acceptable.

The heavy metals cadmium, lead, and nickel of the soil sample are not high. Even though nickel is considered an essential plant nutrient, high quantities of nickel are unwanted.

In conclusion, the soil sample is considered clayey, non-saline, slightly alkaline, highly calcareous, medium to high O.M. content, medium cation exchange capacity, low available phosphorus, sufficient available potassium, low available iron and manganese, and sufficient available zinc and copper.

B. Soil Analysis of the Selected Soil Samples in Lebanon

Soil analysis for the selected soil samples that were brought from different regions in Lebanon was measured. A total of 10 soil samples were collected from the South (Tyre, Zawtar, and Adsheet), North (Jbeil), and Beqaa (Tal Thnoub and Terbol).

Table 20 shows the physical and chemical properties of the selected soil samples. The soil physical property measured is texture. The soil chemical properties measured are electrical conductivity, pH, free calcium carbonate, available micronutrients (Fe, Zn, Cu, and Mn), and available heavy metals (Cd, Pb, and Ni). Available micronutrients and heavy metals were extracted using Lindsay and Norvell method.

Table 20. Physical and Chemical Characteristics of Soil Samples Collected from Different Regions in Lebanon

Soil Sample	Textural Class	EC (µS/cm³)	pH	$CaCO_3$ (%)	Available Micronutrients (mg/Kg)				Available Heavy Metals (mg/Kg)		
					Fe	Zn	Cu	Mn	Cd	Pb	Ni
1 (Zawtar)	**Clayey** (27.42%, 31.90%, 40.68%)[4]	94	7.62	9	0.71	0.81	0.80	0.19	0.13	ND	0.08
2 (Zawtar)	**Silt loam** (38.1%, 50.36%, 11.54)	163	7.80	17	0.46	1.26	0.80	0.05	0.09	ND	0.06
3 (Zawtar)	**Clay loam** (49.03%, 25.60%, 25.37%)	79	7.40	3	0.52	1.33	0.77	0.12	0.32	0.09	0.12
4 (Adsheet)	**Clayey** (15.78%, 35.62%, 48.60%)	821	7.49	60	0.50	0.79	0.56	ND	0.03	ND	0.11
5 (Sour)	**Clayey** (5%, 20%, 75%)	345	7.65	67	0.70	0.74	0.37	2.81	0.06	0.15	0.11
6 (Sour)	**Clayey** (10%, 20%, 70%)	307	7.65	43	0.94	1.14	0.59	2.22	0.04	0.04	0.13
7 (Tal Thnoub)	**Clayey** (20%, 30%, 50%)	417	7.59	61	5.60	7.18	3.03	46.72	0.27	0.11	0.53
8 (Terbol)	**Clayey** (15%, 25%, 60%)	511	7.53	14	3.06	2.33	1.74	4.58	0.06	ND	0.19
9 (Jbeil)	**Silty clay loam** (17.87%, 63.13%, 19%)	451	7.58	37	0.70	0.55	0.48	0.96	0.02	ND	0.14
10 (Jbeil)	**Silty clay** (12.23%, 40.80%, 46.97%)	537	7.37	21	0.55	2.16	1.68	2.24	0.02	0.06	0.14

Note: ND: not detected

[4] The percentage of sand, silt, and clay respectively.

Table 20 shows that most of the samples were clayey while some were silt loam, clay loam, and silt clay loam. The EC results ranged between 94-537 µS/cm which means that all the samples were non-saline. The pH values indicate that the soil samples were slightly alkaline. As for calcium carbonate ($CaCO_3$) content, samples 4, 5, 7, and 9 were highly calcareous in relation to the rest of the samples. Available micronutrients (Fe, Zn, Cu, and Mn) were measured and results show that iron and manganese were deficient in the soil samples.

In conclusion, the results of the selected soil samples varied in texture. Their texture were clayey, silty loam, clayey loam, clayey (samples 4-8), silty clay loam, and silty clay respectively. All samples were non-saline and slightly alkaline. The percentage of calcium carbonate ranged between low to extremely high and the highest calcium carbonate levels were 67 and 61 in samples 5 and 7 respectively. The amount of available iron for all samples was low except for sample 7. The amount of available zinc for all samples was sufficient but sample 7 contained high levels. Available copper was a little high for all samples however, sample 7 contained the highest copper level relative to the rest of the samples. Available manganese was low in samples 1-4 and 9. Samples 5,6 and 8 were a little higher in available manganese. Samples 7, on the other hand, had extremely high available manganese levels. The levels of available heavy metals (Cd, Pb, and Ni) ranged between ND to low in all samples.

C. The Pot Experiment

In this section, the concentrations of cadmium, lead, and nickel in *Origanum syriacum* shoots of the pot experiment will be presented in tables 21, 22 and 23. These tables will show the treatments and quantities absorbed by the *Origanum* shoots after the experiment has been completed. These tables will also be thoroughly discussed and interpreted.

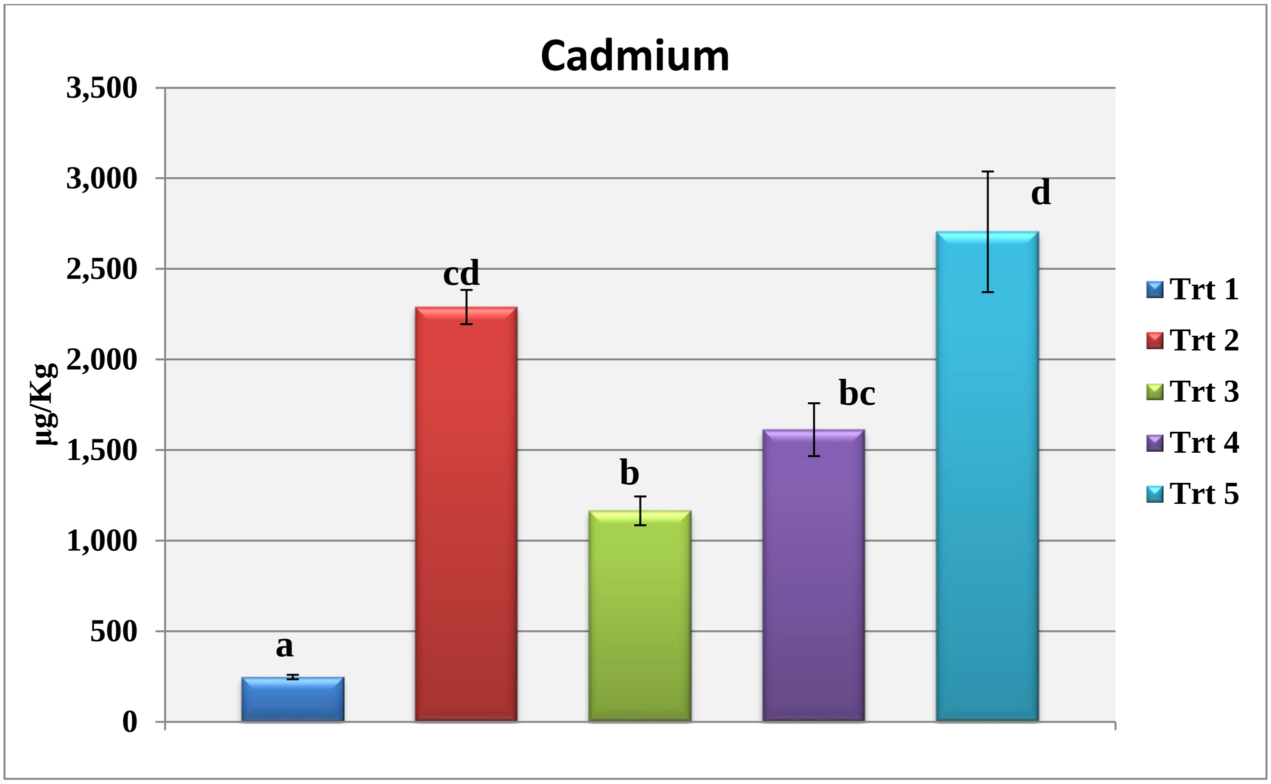
1. Cadmium

The results of the pot experiment on soil that received cadmium at concentrations ranging from 10-100 mg Cd/Kg soil are present in Table 21 and Figure 5.

Table 21. Concentration of Cadmium in *Origanum* Shoots at Different Cadmium Levels in Soil

Treatment (mg/Kg soil)	Replicate Number	Cadmium in Tissue (µg/Kg)	Average (µg/Kg)
Control	1	225	246.67[a]
Control	2	265	
Control	3	250	
10	1	2,125	2,289.17[cd]
10	2	2,450	
10	3	2,293	
20	1	1,025	1164.17[b]
20	2	1,300	
20	3	1168	
50	1	1,862	1,611.36[bc]
50	2	1,357	
50	3	1615	
100	1	3,279	2704.77[d]
100	2	2,127	
100	3	2708	

Figure 5. Concentration of Cadmium in *Origanum syriacum* Tissue



The data in Table 21 and Figure 5 show that increasing cadmium concentration in the soil lead to higher levels of cadmium in the tissue of *Origanum* tissue (stems + leaves). There was a significant difference at 0.05 level between the control and all the treatments but the differences in cadmium concentrations between the treatments were non significant. Results imply that once cadmium is present in the soil at a concentration of 10 mg/Kg (Trt 2) or more, *Origanum* shoots will absorb it in significantly increased concentrations. Treatment 5 showed the highest concentration of cadmium in *Origanum* shoots. As for treatments 3 and 4, the concentrations were not as high as treatment 2. This could be due to a defense mechanism the plants were trying to generate to stop the absorption of cadmium. Lydakis-Simantiris et al. (2012) support this result in their article "Cultivation of Medicinal and Aromatic Plants in Heavy Metal- Contaminated Soils". The higher the concentration of cadmium in the soil, the more the plant will absorb it. In their study, Lydakis-Simantiris et al. planted *Thymus vulgaris* rather than *Origanum syriacum*. They used 5 treatments of cadmium in the pot experiment and they are 0 (control), 1 mg/Kg, 3 mg/Kg, 10 mg/Kg, and 30 mg/Kg. Their results showed that the plant didn't absorb much cadmium in the first 4 treatments (0.34 ppm, 0.57 ppm, 0.65 ppm, and 0.87 ppm respectively), however in the last treatment, the absorption was highly significant (8.65 ppm). However, in this study, higher concentrations of cadmium were applied. The results showed that the absorption of cadmium in *Origanum* plants of treatments 2, 3, and 4 (10 ppm, 20 ppm, and 50 ppm) were somehow similar but when the concentration reaches 100 ppm, the absorption of cadmium in *Origanum* plants was the highest. Therefore, it can be stated that a contamination of cadmium with 10 mg/Kg or more will lead to a higher contamination in the tissue of *Origanum* plants. This means that soil contamination with cadmium is dangerous and can be harmful because it causes significant increase of cadmium in the tissue.

The results of Zheljazkov et al. (2008) also agree with the results of this study. They tested 5 medicinal species including *Origanum spp*. on 4 different soil plots 0.5 km, 3 km, 6 km, and 9 km (control) from a smelter. Cadmium levels in *Origanum* tissues were highest in the soil closest to the smelter and lowest in that of control (9 km).

The concentration of cadmium in the treated soils exceed the acceptable limits set by WHO, Australian government, and CODEX which is 0.1-0.3mg/Kg (WHO, 2007; CODEX, 2009). According to JECFA, the calculated daily intake for a 70 Kg adult is 70 µg (AHPA, 2009; Smolders, 2001). Ezeabara et al. (2014) in their article "Heavy Metal

Contamination of Herbal Drugs: Implication for Human Health-A Review" have presented a safe intake level of 15-50 µg/ day for adults and 2-25 µg/ day for children.

Calculating how much a person can consume *Origanum syriacum* if the concentration of cadmium in *Origanum* was 2705µg/Kg (highest level in the results) and the daily intake of a 70 Kg adult is 70 µg/Kg, according to JECFA, a person can have about 26 g of *Origanum* per day. This is considered a small amount and some people do consume more than this amount on a daily basis. However, by consuming one manoushe per day, this amount is considered acceptable since only about 12 g of *Origanum* are needed to prepare one manoushe. Moreover, calculating how much a person can consume *Origanum syriacum* if the concentration of cadmium in *Origanum* was 247µg/Kg (control treatment), the amount that can be consumed is about 10 times the above quantity (more than 100g/day) and there is no harm from consuming *Origanum* produced in fields that are not contaminated with cadmium.

It is very important for people not to exceed the daily intake of cadmium because high cadmium levels cause severe problems such as lung damage when inhaled, stomach irritation, vomiting, and maybe death when highly ingested (ATSDR, 2012). Also, kidney failure occurs when minor quantities are ingested over a long period of time (Wuana and Okieimen, 2011).

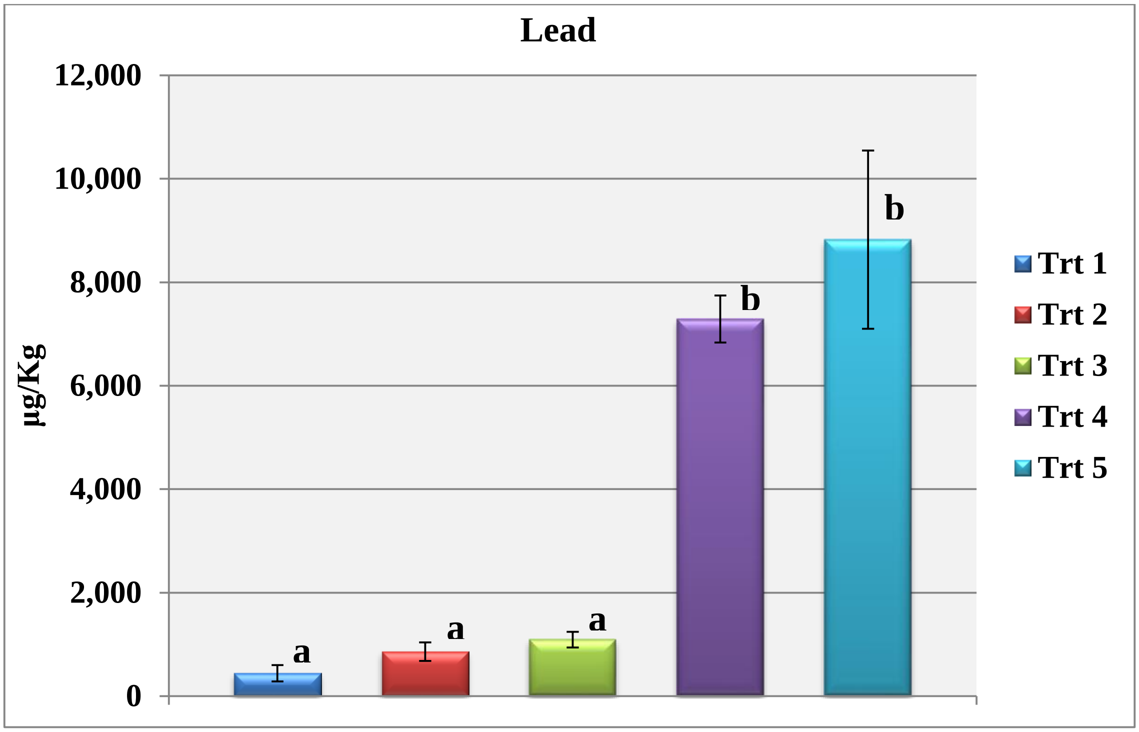
2. Lead

The results of the pot experiment on soil that received cadmium at concentrations ranging from 100-1,000 mg Pb/Kg soil are present in Table 22 and Figure 6.

Table 22. Concentration of Lead in *Origanum* Shoots at Different Lead Levels in Soil

Treatment (mg/Kg soil)	Replicate Number	Lead in Tissue (µg/Kg)	Average (µg/Kg)
Control	1	731.99	
Control	2	400.98	441.8[a]
Control	3	192.38	
100	1	1,211.51	
100	2	631.00	860.7[a]
100	3	739.58	
200	1	942.38	
200	2	1,391.08	1,089.1[a]
200	3	933.90	
500	1	6,503.70	
500	2	7,290.02	7290.0[b]
500	3	8,076.34	
1,000	1	6,996.13	
1,000	2	7,203.50	8822.6[b]
1,000	3	12,268.10	

Figure 6. Concentration of Lead in *Origanum Syriacum* Tissue



The results of Table 22 and Figure 6 show that the increase in concentrations of lead in soil led to the increase of lead level in *Origanum* top parts. The higher the lead concentration in the soil, the higher the lead concentration in the *Origanum* top part. The results, as presented in Figure 6, show the positive increase in lead concentration between the treatments. There was significant difference at 0.05 level between the control and treatments 4 and 5 but the increase between the control and treatments 2 and 3 was not significant. It is important to note that although the concentration of lead in the soil was 10 times that of cadmium, the increase of cadmium in *Origanum* tissue was similar or higher than that in lead.

Lydakis-Simantiris et al. (2012) results agree with our results. They reported that all treatments were significantly higher than the control, but there was no significant difference between the treatments, other than the control. The lead concentrations used in their study were 0 (control), 60 mg/Kg, 180 mg/Kg, 600 mg/Kg, and 1800 mg/Kg. These results do not agree with those shown in Table 24. The reasons could be due to the differences in plant varieties and in soil properties; factors such as calcium carbonate content, pH, texture, and organic matter content.

The results of Zheljazkov et al. (2008) agree with the results of this study. Similar to cadmium, lead levels in *Origanum* tissues were highest in the soil closest to the smelter (higher lead levels in the soil) and lowest in that of control.

According to the WHO, the maximum limit for lead in herbal products is 10 mg/Kg (WHO, 2007). JECFA, on the other hand, has set a daily limit of 250 µg for a 70 Kg adult (AHPA, 2009). CODEX has set a maximum level of 0.3 mg/Kg for leafy vegetables. Ezeabara et al. (2014) have presented a safe intake level of 20-280 µg/ day for adults and 10-275 µg/ day for children. The results of the pot experiment are all below the maximum limits of the WHO, but very high according to JECFA, Commission Regulation, CODEX, and Ezeabara et al.

Calculating how much a person can consume *Origanum* syriacum if the concentration of lead was 2,940 µg/Kg (highest level in the results) and the daily intake of a 70 Kg adult is 250 µg/Kg, a person can have about 85 g per day. This is considered an amount that most people do not consume per day. However, those who consume a lot of *Origanum* on a daily basis should limit their quantity because it leads to several health problems. Moreover, if the quantity of *Origanum* consumed from the control treatment is calculated, the result would show that a person can consume about 10 times (about 500g/day) that of treatment 5 (highest concentration). This implies that there is no danger from consuming *Origanum* grown in Lebanese fields that are not contaminated with lead, as is the case in Lebanon.

Lead mainly targets the nervous system but can also affect the kidneys, bones, and the brains (Patrick, 2006; IARC, 2006; EFSA, 2012). It can also cause high blood pressure, anemia, and maybe death. Lead has also been found to cause miscarriage to pregnant women and low sperm production to men (ATSDR, 2007).

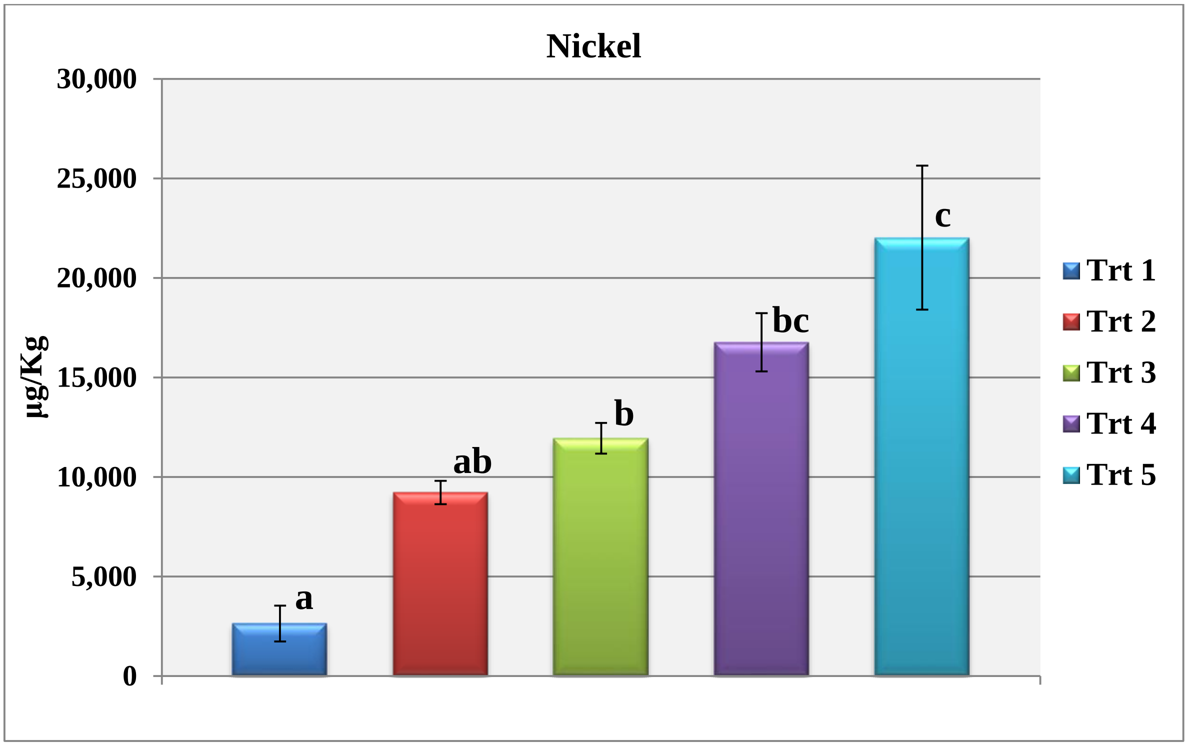
3. Nickel

The results of the pot experiment on soil that received cadmium at concentrations ranging from 50-500 mg Ni/Kg soil are present in Table 23 and Figure 7.

Table 23. Concentration of Nickel in *Origanum* Shoots at Different Nickel Levels in Soil

Concentration (mg/Kg)	Replicate	Nickel (μg/Kg)	Average (μg/Kg)
Control	1	1,025	2,641.1[a]
Control	2	2,775	
Control	3	4,123	
50	1	9,650	9,229.2[ab]
50	2	8,075	
50	3	9,963	
100	1	10,450	11,941.7[b]
100	2	12,350	
100	3	13,025	
200	1	19,650	16,764.7[bc]
200	2	15,732	
200	3	14,912	
500	1	17,740	22,022.2[c]
500	2	29,215	
500	3	19,112	

Figure 7. Concentrations of Nickel in *Origanum* Tissue



The data in Table 23 and Figure 7 show that the increase in the concentrations of nickel in soil led to increase in nickel concentration in *Origanum* top parts. Similar to cadmium and lead, the higher the nickel concentration in the soil, the higher the concentration in the *Origanum* top part. As shown in figure 7, there is a significant difference between the control and the other treatments. There was no significant difference at 0.05 level between treatments 2, 3, and 4; however there was a significant difference between treatments 1-4 and treatment 5. The results reported by Lydakis-Simantiris et al. (2012) agree with our results as they conclude that nickel concentration in tissue of *Origanum* spp was the highest when the concentration of nickel in the soil was at its peak.

According to the EFSA (2015), an oral intake of 1.1 µg/Kg body weight was found to cause contact dermatitis. However, those who are not sensitive to nickel can tolerate a daily intake of 2.8 µg/Kg bw. ATSDR, on the other hand, has set a similar daily nickel intake of 101-162 µg/day for adults.

Calculating how much a person can consume *Origanum syriacum* if the concentration of lead was 22,000 µg/Kg (highest level in the results) and the daily intake of a healthy (not sensitive) 70 Kg adult is 196 µg/Kg, a person can have about 9 g per day. This amount is considered very little since about 13g of *Origanum* are used in one man'oushe. Therefore, people should limit their *Origanum syriacum* intake if it contains high concentrations of nickel. However, at a level of 2,000 µg/Kg (control and samples collected from lebanon), the tolerable amounts that can be consumed are about 10 times this quantity, an amount that is not consumed usually.

Nickel's main target is the respiratory tract and the skin. The contact or consumption of high levels of nickel usually shows skin diseases such as skin rash, eczema, and contact dermatitis (ATSDR, 2007); when inhaled, nickel targets the respiratory tract (ATSDR, 2005; Cempel and Nikel, 2005). If nickel particles are in a soluble form, they may easily move from the lungs to the rest of the body, and if they are not soluble, then they stay in the lungs for a long time. (ATSDR, 2005) Nickel mainly moves to the kidneys after it enters the body, and then leaves in the urine. Nickel compounds are also considered carcinogenic to humans according to the International Agency for Research on Cancer (IARC, 2012). Nickel has been found to interfere with the physiological process of several elements needed by the body such as iron, copper, manganese, zinc, calcium, and magnesium (Punamiya et al., 2008; Cempel and Nikel, 2005).

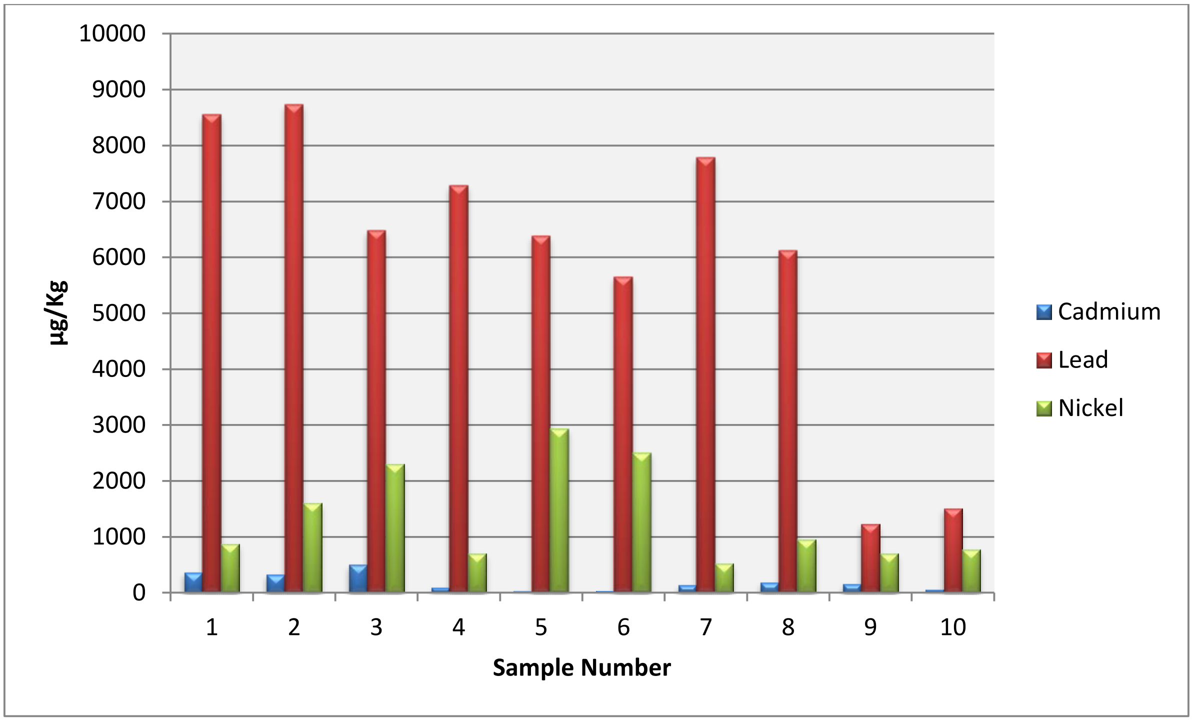
D. Cadmium, Lead, and Nickel Concentrations in *Origanum* Samples Collected in Lebanon

In this section, results of analysis for the *Origanum* samples collected from different fields in Lebanon will be discussed. These samples were collected according to regions and according to different physical properties of the soils within regions. These samples were collected from South (Zawtar and Tyre), Beqaa (Tal Thnoub and Terbol), and North (Jbeil).

Table 24. *Origanum syriacum* Collected from Different Regions in Lebanon

Sample Number	Area	Leaves or Mixture	Cadmium (µg/Kg)	Lead (µg/Kg)	Nickel (µg/Kg)
1	Zawtar	Leaves	367	8,550	875
2	Zawtar	Leaves	326	8,729	1,600
3	Zawtar	Leaves	501	6,478	2,300
4	Adsheet	Leaves	89	7,284	700
5	Tyre	Leaves	30	6,382	2,925
6	Tyre	Leaves	39	5,649	2,500
7	Tal Thnoub	Leaves	135	7,787	525
8	Terbol	Leaves	185	6,116	950
9	Jbeil	Leaves	157	1,233	700
10	Jbeil	Leaves	54	1,509	775
Maximum Limits (WHO)			**300**	**10,000**	**10,000**

Figure 8. Cadmium, Lead, and Nickel in *Origanum syriacum* Collected From Different Fields in Lebanon



Tables 24 and Figure 8 show the concentrations of cadmium, Lead, and nickel in *Origanum syriacum* samples collected from different regions in Lebanon. Six samples were collected from South Lebanon (3 from Zawtar, a town famous for za'atar production, 1 from Adsheet, a town close to Zawtar, and 2 from Tyre). Two samples were brought from Jbeil, North Lebanon, and 2 samples from Beqaa (Tal Thnoub and Terbol).

According to the WHO, the maximum limit for cadmium is 300µg/Kg (0.3 mg/Kg) (WHO, 2007). A safe intake of cadmium for adults is 70µg/Kg for a 70 Kg adult (1µg/Kg bw). The results show that the majority of the samples contain cadmium in concentrations less than 0.3 mg/Kg. There is only one sample that is marginally higher (Zawtar 3).

Lead concentrations in *Origanum* shoots of the 10 samples ranged between 1,200-8,700 µg/Kg (1.2-8.7 mg/Kg). These samples were within the maximum limit set by the WHO (10,000 µg/Kg).

Nickel is considered an essential element to plants and is present in many plant enzymes. It is a micronutrient and is needed in very low quantities by plants. Nickel does not only enter plants from the roots, but through plant leaves as well (Seregin and Kozhevnikova, 2006). It serves as a protector against pathogens and herbivores (He et al., 2012). Even though nickel is essential to plants, studies found no deficiency symptoms in the human body (Cempel and Nikel, 2005).

Nickel concentrations in the 10 samples ranged between 525-2925 µg/Kg (0.53-2.93 mg/Kg). The tolerable daily intake of nickel set by the EFSA (2015) for a normal person is 2.8 µg Ni/Kg body weight. However, for people who are sensitive to nickel, a daily intake of 1.1µg Ni/Kg body weight is considered the limit. Thus, the amount of *Origanum* that can be consumed by an adult is very high (several Kgs) to reach the daily allowable intake.

The best samples to choose between the above 10 samples would be the ones with the lowest concentrations of cadmium, lead, and nickel altogether and they are samples 9 and 10 brought from Jbeil. This matter is very important to those who consume high amounts of *Origanum syriacum* per day.

E. Cadmium, Lead, and Nickel Concentrations in *Origanum* Samples Collected from Sale Outlets in Lebanon

In this section, results of analysis for the *Origanum* samples collected from different stores in Lebanon will be discussed. These samples were collected from Beirut, Zawtar, Ras El Maten, Batroun, Chhim, and Safita. The samples collected were either fresh leaves or mixtures of dried *Origanum* leaves, sesame, sumac, and salt.

Table 25. *Origanum syriacum* collected from different sale outlets in Lebanon

Sample Number	Area	Leaves or Mixture	Cadmium (µg/Kg)	Lead (µg/Kg)	Nickel (µg/Kg)
11	Beirut	Leaves	94	6,978	625
12	Beirut	Leaves	70	6,001	575
13	Beirut	Leaves	18	1,719	950
14	Beirut	Leaves	33	4,988	600
15	Zawtar	Mixture	157	10,429	825
16	Beirut	Mixture	190	7,556	775
17	Beirut	Mixture	82	26,329	775
18	Beirut	Mixture	118	7,023	575
19	Beirut	Mixture	83	6,273	700
20	Beirut	Mixture	385	7,055	825
21	Ras el Maten	Mixture	466	7,366	900
22	Safita	Mixture	237	6,202	1,075
23	Batroun	Mixture	127	1,583	950
24	Chhim	Mixture	218	5,644	875
Maximum Limits (WHO)			**300**	**10,000**	**10,000**

Figure 9. Cadmium, Lead, and Nickel in *Origanum Syriacum* Collected from Different Sale Outlets in Lebanon

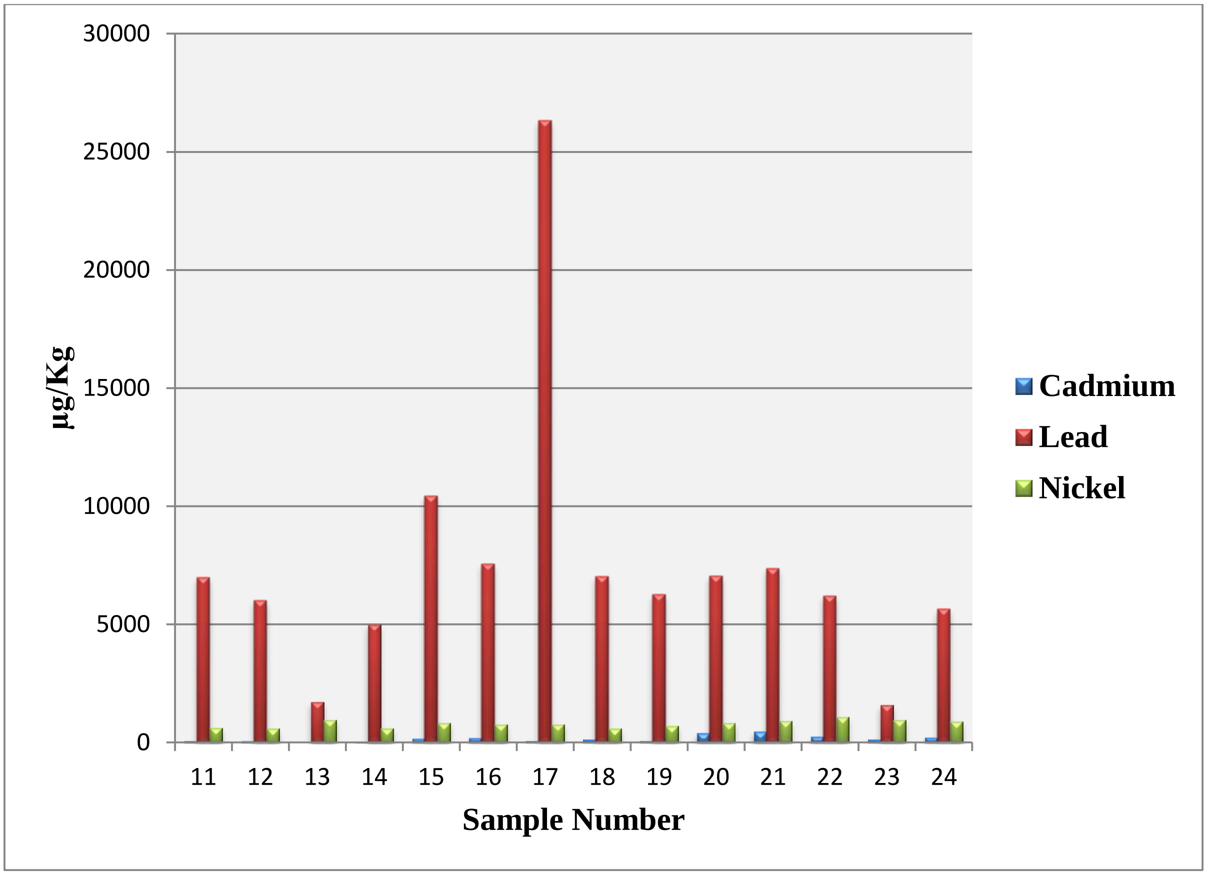


Table 25 and figure 9 show the concentrations of cadmium, Lead, and nickel in *Origanum syriacum* samples collected from different stores in Lebanon. These samples were mainly collected from several stores in Beirut; however, some samples were collected from stores outside Beirut such as Ras el Matn, Batroun, and Chhim. Only one sample was collected from Safita, Syria.

According to the WHO, the maximum limit for cadmium is 300µg/Kg (0.3 mg/Kg) (WHO, 2007). A safe intake of cadmium for adults is 70µg/Kg for a 70 Kg adult (1µg/Kg bw). The results show that the majority of the samples contain cadmium in concentrations less than 0.3 mg/Kg. There is only one sample that is marginally higher (Ras El Matn).

Lead concentration in *Origanum* shoots of the 14 samples ranged between 1,500-26,300 µg/Kg (1.5-26.3 mg/Kg). The highest lead concentration was found in the sample that was purchased in Beirut (26,329µg/Kg). This value is about twice the acceptable limit set by the WHO, which is 10,000µg/Kg. The rest of the samples were within the maximum limit set by the WHO.

Nickel concentrations in the 14 samples ranged between 575-1,075 µg/Kg (0.58-1.1 mg/Kg). The tolerable daily intake of nickel set by the EFSA (2015) for a normal person is 2.8 µg Ni/Kg body weight. However, for people who are sensitive to nickel, a daily intake of 1.1µg Ni/Kg body weight is considered the limit. Thus, the amount of *Origanum* that can be consumed by an adult is very high (several Kg) to reach the daily allowable intake.

The samples selected to be analyzed for cadmium, lead, and nickel levels were either bought as fresh leaves or mixtures (*Origanum* leaves, sesame, sumac, and salt). Therefore, heavy metal contamination might not be coming from the *Origanum* leaves, but it could be due to the other ingredients that come with the *Origanum* mixture.

It is advised to always try to consume the best quality of za'atar with the lowest amount of contamination with heavy metals. The less the heavy metal consumption, the less a person would be prone to health problems. The best samples to choose between the above 14 samples would be the ones with the lowest concentrations of cadmium, lead, and nickel altogether and they are samples 13 and 23 collected from Beirut and Batroun respectively. This matter is very important to those who consume high amounts of *Origanum syriacum* per day.

To summarize, none of the samples collected from the sale outlets in Lebanon contain higher levels than the maximum limits set by the WHO or other international organizations; except one mixture sample (purchased in Beirut) contained double the acceptable limits. The source of the lead in this sample may come from the mixture or the equipment used in grinding and mixing the materials.

CHAPTER 5

SUMMARY, CONCLUSION, AND RECOMMENDATIONS

Heavy metals have become one of the main factors that cause risks to humans and the ecosystem in general. Even though heavy metals are present naturally in soils, they are becoming pollutants in several soils due to several human activities. Human activities such as industrial, agricultural, medical, technological, or domestic have brought heavy metals contamination to soils to become a public health concern. Cadmium (Cd), lead (Pb), and nickel (Ni) are among the top hazardous heavy metals (Tchounwou et al., 2012). These heavy metals are taken by plants and are considered dangerous because they have no function in the human body and they have caused serious health issues when taken in high quantities.

Origanum syriacum or what is known as za'atar (زعتر) is used widely in Lebanon and is one of the most important staple herbs in this region because it is cheap, tasty, and found in most of our kitchens. It is consumed in the Mediterranean countries almost on a daily basis. The basic mixture of *Origanum* is *Origanum* leaves, sesame seeds, sumac (Rhus), and salt. *Origanum* is mostly consumed in man'oushe (منقوشه), the famous Mediterranean pastry. *Origanum* is also used for medical treatments. It has been reported that it treats gastrointestinal problems, respiratory diseases, hemorrhoids, sexual diseases, pains of animals' bites and poison, and Candida albicans, a yeast infection (Lukas et al., 2009; Zeina Kassaify et al., 2008).

Origanum has two very important purposes, consumption and medicinal, yet it can be dangerous if it absorbs heavy metals. If *Origanum* absorbs heavy metals, then *Origanum* consumption and use should be reduced to limit or inhibit heavy metal toxicity.

The three heavy metals that were included in the study are cadmium, lead, and nickel. High levels of cadmium have caused chronic effects on people. Consuming small amounts of cadmium over a long period of time has caused kidney failure, liver problems, stomach irritation, vomiting, and maybe death if highly ingested. It can also damage the lungs if highly inhaled (ATSDR, 2012). On the other hand, lead may target the nervous system, brains and kidneys, causes high blood pressure, anemia, and maybe death. Nickel, an essential plant micronutrient, is also a heavy metal that has shown to cause toxicity symptoms, but not as hazardous as cadmium and lead. It is usually present in the human body at low concentrations. A healthy human body possesses about 0.2 µg/L in the blood and 1-3 µg/L in urine (Cempel and Nikel, 2005). Nickel mainly causes skin irritations such as skin rash, eczema, and contact dermatitis (ATSDR, 2007). Nickel has also been found to target the respiratory tract when inhaled and the kidneys when it is ingested (Cempel and Nikel, 2005).

A pot experiment was conducted at the greenhouse area at AUB, Beirut, to examine whether heavy metals in the soil were absorbed by *Origanum* plants and in what concentration. The pots were then divided into three sets. First set was for cadmium (Cd), 2^{nd} for lead (Pb), and the 3^{rd} for nickel (Ni). Each set encompassed a total of 15 pots that were divided into 5 rows of 5 different concentrations and replicated 3 times. Moreover, different *Origanum* samples have also been collected from different fields in Lebanon to be tested for Cadmium, lead, and Nickel levels. Also, samples from the market were collected and analyzed for cadmium, lead, and nickel concentrations.

Results show that as cadmium, lead, or nickel concentrations increase in the soil, their levels increase in the *Origanum* shoots. There is a positive relationship between the heavy metal concentrations in soil and that in *Origanum* vegetative part. A significant difference was detected in the 3 heavy metals. These results agreed with a study conducted in Greece by Lydakis-Simantiris et al. (2012).

None of the *Origanum* samples collected from various fields in Lebanon was high in cadmium, lead, and nickel; however, two samples from the za'atar mixtures purchased from stores in Lebanon were higher than the maximum limits of WHO.

Knowing that the environmental and growth conditions in the greenhouse is very different from the open field and that the *Origanum* plant is a perennial plant that stays in the field for several years, the results of the pot experiment should be taken as an indicators for the subject. The following can be recommended:

- To conduct the research on *Origanum* in the field and harvest it for several years.
- To study the effect of heavy metals on the production of *Origanum syriacum*.
- To measure other types of hazardous heavy metals such as arsenic, chromium, and mercury.
- To monitor the *Origanum* mixtures in the market for the health of the general public

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