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Upadhyaya, Bandana. ***Determination of Cadmium Uptake by Parsley***

Abstract

In 2011, Centers for Disease Control and Prevention lists cadmium (Cd) as the 7th most hazardous substance. It is a carcinogen and is considered toxic to kidney, lung and liver. Cd is mostly released to the air, land, and water by human activities. The two major sources of Cd in the environment are, mining of metals such as zinc, and the burning of waste materials containing cadmium. Increasing cadmium content of the soil can result in an increase in the uptake of Cd by plants. Consumption of these plants is one of the pathways of human exposure to cadmium.

This research study reports the concentrations of Cd taken up by parsley grown in acidic, basic and neutral soils contaminated with CdO or $Cd(NO_3)_2$. The results of the study showed that the pH of the soil does not affect the amount of Cd absorbed by parsley. In plants contaminated with CdO, 37.47 mg to 75.27 mg of Cd per kg sample, and 208.06 mg to 495.66 mg of Cd per kg sample were isolated from the shoots and roots, respectively. In plants contaminated with $Cd(NO_3)_2$, 85.26 mg to 169.58 mg of Cd per kg sample, and 719.22 mg to 2795.43 mg of Cd per kg sample were isolated from the shoots and roots, respectively.

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Chapter I: Introduction

From the stand point of occupational or residential exposure, there are 35 metals of concern, 23 of these are heavy elements or "heavy metals": antimony, arsenic, bismuth, cadmium, cerium, chromium, cobalt, copper, gallium, gold, iron, lead, manganese, mercury, nickel, platinum, silver, tellurium, thallium, tin, uranium, vanadium, and zinc (Glanze, 1996). Several of these heavy metals and their compounds are considered toxic substances that adversely affect human health (Bruins, et. al., 2000). Some of these metals, in very small quantity, are required to support life not only in animals but also in plants (Ji & Silver, 1995). Manganese, zinc and nickel have been shown to play a role in plant metabolism (Welch, 1995). Manganese and zinc are involved in enzyme activation, while nickel is needed for urea metabolism and nitrogen fixation**.** Chromium is an essential nutrient that helps the body regulate metabolism of insulin, glucose and blood lipids (Anderson, 2000). In larger amounts, these metals become toxic to animals and to aerobic and anaerobic processes (Silver, 1996). Toxic metals build up in biological systems and become a significant health hazard (Diskshith & Diwan, 2003). One of the most toxic heavy metals is cadmium (Vig, et. al., 2003).

In 2011, the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) list indicates that cadmium is the 7th most hazardous substance (ATSDR, 2011). The CERCLA list names substances that have been shown to pose the most significant potential threat to human health due to its known or suspected toxicity. Cadmium is a human carcinogen. It is a toxic metal and it affects the cardiovascular, developmental, gastrointestinal, neurological, renal, reproductive, respiratory functions of the body (ATSDR, 2011).

Research studies on Cadmium among soil and plant researchers increased after the diagnosis of cadmium related disease known as *Itai-itai*. *Itai-itai* first appeared around 1912 and

was officially recognized in 1968 as the first disease induced by environmental pollution in Japan (Watanabe, et. al., 2004). The symptoms of *Itai-itai* include severe osteoporosis and osteomalacia with simultaneous severe renal dysfunction. It was determined that *Itai-itai* was primarily caused by consumption of cadmium contaminated rice grown along the Jinzu River (Cai, et. al., 1990). Mining activities had polluted the river with cadmium. The river was used for irrigation of the rice fields. The rice absorbed cadmium and cadmium accumulated in the older populations consuming contaminated rice (Watanabe, et. al., 2004).

Cadmium is relatively rare, it occurs in the environment mostly with zinc ores and to a lesser extent with lead and copper ores (Elinder, 1985). It is emitted into the atmosphere mainly from volcanic activities and from processes involved in the production of Zn metal (WHO, 2010). Waste incineration, production of Ni-Cadmium batteries and fossil fuel combustion also contribute to cadmium emissions (Robertson, et. al, 2001). In landfills the largest sources of cadmium are smelters, iron and steel plants, electroplating wastes and battery production (ATSDR, 2008).

Ingestion via food, especially plant-based food, is the major route by which cadmium, and many other toxic heavy metals, enters the human body from the environment (Vig, et. al, 2003). Food contains cadmium as a result of uptake from the soil by plants and bioaccumulation in terrestrial and aquatic animals (McLaughlin & Singh, 1999). According to World Health Organization (WHO, 2010) the tolerable weekly intake for cadmium is 7 μg/kg of body weight. For 150 lb body weight, this is equivalent to 477 mg of cadmium a week. The WHO reported that ingestion of heavy metals through food has been increasing since 1994, and heavy metals like cadmium, which accumulate in the body over time, are of particular concern.

Statement of the Problem

Cadmium uptake has been studied by other researchers using different plants such as corn, rice, beans, and spinach (Guo-Yan, et. al., 1995). To my knowledge, no research has been conducted on cadmium uptake by herbs such as parsley. This research project investigates the uptake of cadmium in parsley plant, *Petroselinum crispum*. Parsley is the world's most popular herb and it is a good source of flavanoids (anti-oxidant compounds) and folic acid (USDA National Nutrient Database, 2009).

Objectives of the Study

The main objective of this study is to determine the concentration of cadmium taken up by parsley grown in the greenhouse. The specific aims of the study are:

1. To determine the concentration of cadmium in the shoots and roots of parsley using atomic absorption spectrophotometer.
2. To determine the effects of acidic, basic and neutral soil on the amount of cadmium taken up by parsley.
3. To determine the effects of two chemical forms of cadmium (cadmium oxide and cadmium nitrate) on the amount of cadmium taken up by parsley.
4. To determine the effect of using two different concentrations of cadmium oxide and cadmium nitrate on the amount of cadmium taken up by parsley.

Chapter II: Literature Review

In recent years, urban gardening and farming are experiencing resurgence in North America. Urban gardeners and farmers use vacant lots, parks, roof tops, ponds, rivers and estuaries to produce significant amounts of food, mostly vegetables (Brown & Carter, 2003). In the United States, 35 % of vegetables, fruit, livestock, poultry and fish are produced by farms located within the metropolitan areas (Kaufman & Bailkey, 2004). The major threat to urban gardening is the presence of toxic levels of heavy metals in the soil **(**Bellows, et. al., 2003). These toxic metals may enter the food chain when taken up by edible plants as they acquire nutrients from the soil or surrounding environment (Heinegg, et. al., 2009). Additional exposures to these toxic metals from soil include inhalation of soil dust and direct ingestion by children (Dupler, 2001). The presence of heavy metals in agricultural soil or garden soil is a major concern, particularly with increasing urban gardening and farming activities (Brown & Carter, 2003; Kaufman & Bailkey, 2004).

Soil

Soils are the complex mixtures of minerals, and organic matter on the immediate surface of the earth that interact continuously in response to natural and imposed biological, chemical, and physical forces. Soil acts as a natural medium for the growth of land plants (USDA-NRCS, 1999). Previous studies show that soil properties such as soil pH, clay content and organic matter influence the solubility of cadmium and therefore its availability to plants (Eriksson, 1990; Eriksson et. al., 1996). The acidity or alkalinity of soil is one of the most important factors influencing uptake of heavy metal like cadmium by plants (Grey, et. al., 1998). At low or acidic pH, cadmium is exchanged from its binding sites on soil particles by hydrogen and aluminum ions and dissolved in the soil solution (Garcia-Miragaya & Page, 1978). The solubility reduces

as pH rises from acid to neutral, which enhances the adsorption of cadmium on organic matter and clay minerals (Eriksson, 1990; McBride, 1995). Clay particles due to their negatively charged surfaces can absorb Cd^{2+} to a higher level (Garcia-Miragaya & Page, 1978). Meanwhile, the phenol and carboxylic groups of organic matter have been shown to be more effective in adsorbing cadmium, and thereby making it unavailable to plants (Eriksson, 1990). Thus, low soil pH (pH < 7), can enhance the availability of toxic metals and also affect the activity of microbes in soil which in turn can affect plant growth (Chaney and Hornick, 1978).

Heavy Metals in Soil

Sources of toxic heavy metals in the environment are from both geological and anthropogenic sources (Diskshith & Diwan, 2003). Soil can be contaminated with heavy metals from natural sources which include weathering of soil, sediments and rocks, sandstorms, major forest fires and volcanic eruptions (Waldron, 1980). Anthropogenic sources of toxic metals include mining, smelting, coal and petroleum processing. Municipal sewage discharges, household waste incineration, solid waste disposal sites, phosphate fertilizers, lead-arsenic pesticides leaching, chipping or peeling of paint from old structures are anthropogenic sources of heavy metals in the environment. (Salt, et. al., 1995).

Heavy metals can be absorbed by plants from soil via root to shoot uptake. The fraction of heavy metals which can be readily mobilized in the soil environment and taken up by plant roots is considered the bioavailability fraction (Ernst, 1996). The term “bioavailability” has been defined as the extent to which a chemical can be absorbed by a living organism and reach the systemic circulation (Kelley, et. al., 2002). Hence, total concentration of metal in soil is not equal to metal bioavailability. The bioavailability of heavy metals to plants depends on a number of physical and chemical factors in soil including soil properties for example pH, organic

matter content, redox potential, and cation exchange capacity (CEC). Other soil properties such as sulfate (SO_4^{2-}), carbonate (CO_3^{2-}), hydroxide (OH^-) content, soil texture, clay content and the relative amount of other substances such as iron, calcium, magnesium, etc can affect the bioavailability of heavy metals to plants (Mwegoha & Kihampa, 2010). In addition, characteristics of plants and the chemical form of the substance can affect metal absorption by plants (EPA, 2004).

Research on Cadmium and its Absorption

Previous studies indicate that cadmium ion (Cd^{2+}) absorption can occur through non-specific movement, such as leakage, through a Mg^{2+} or Ca^{2+} plasma membrane channel (Nies, 1995). Another pathway is through the transport in the bacterium *Alcaligenes eutrophus* where Cd^{2+} enters the cell through a Mg^{2+} uptake system (Nies & Silver, 1989). The evidence from bacterial and animal cell studies suggests that absorption by both transition metal carrier transporters (i.e., membrane spanning transport proteins that transfer transition metals from the apoplasm to the cytosol) and divalent cation channels are likely to play essential roles in cadmium uptake by plant roots (McLaughlin & Singh, 1999).

Guo-Yan, et. al. (1995) and Guo, et. al. (1995) have reported on the variation in cadmium uptake, distribution and binding among various plant species. According to their studies, there are large genetic differences between plant species in the ability to mobilize cadmium from root - - to - shoot transport. The studies concluded that corn had a greater capacity to translocate cadmium from root – to – shoot transport than other species (such as bean, rice, flax and spinach). Table 1 lists the biotic and non-biotic factors that affect cadmium uptake by plants (McLaughlin & Singh, 1999).

Table 1

Factors Affecting Cadmium Uptake by Plants

Biotic	**Non-Biotic**
Plant species	Soil pH
Crop cultivar	Clay content
Plant tissue	Carbonates
Leaf age	Metal oxides (iron and manganese)
Root activity	Redox potential
Rooting pattern	Organic matter (type and content)
Rhizosphere and root associated microorganisms (such as mycorrhizal fungi)	Complexing ligands
	Soluble salts
	Soil management practices: phosphate fertilizers, manures, lime, sewage sludge, and irrigation

Hatch, et. al. (1988) studied the effect of pH on cadmium absorption and distribution in four plant species (cocksfoot, perennial ryegrass, lettuce, and water cress). They used a system of flowing solution culture with cadmium added at 0.018 mmol/m^3 and the pH of the solution was controlled at 5.0, 5.5, 6.0 or 7.0. The total cadmium content increased by factor of 4, 8, 10 and 10, respectively, in cocksfoot, rye grass, lettuce and watercress when the pH of the soil was increased from 5.0 to 7.0. In the same study the concentration of cadmium transported to the shoot in the edible leafy plants (spinach and watercress) was more than 3 times higher than the two grasses (cocksfoot and ryegrass). The concentration of cadmium in dry shoots increased in both spinach and water cress with an increase in pH from 5.0 to 7.0. Hence, the study showed that cadmium absorption increases with an increase in solution pH (5.0 to 7.0) and there is a risk of cadmium transfer from edible part of the plant to the food chain (Hatch, et. al., 1988). Most previous studies show that roots contain the highest amount of cadmium; the gradient of cadmium concentration in plants declines in the order: roots > shoots > grains or seeds (Wagner, 1993).

Detrimental Health Effects of Cadmium

The two major adverse health effects caused by cadmium exposure can be distinguished as acute and chronic (USEPA, 1981). Acute cadmium exposure can be the result of high doses of cadmium inhalation in an occupational setting (Wisconsin DHS, 2010). Cadmium absorbed via inhalation or ingestion is transported through the human body by blood. The absorbed cadmium is carried into the liver and incorporated into the metallothionein (metal binding protein) and then carried to the kidney where it is deposited as cadmium-thionein (USEPA, 1981). Hence, nephrotoxic effects of cadmium are the result of cadmium's mode of transport to the kidney and deposition in the kidney. Long term lower level exposures of cadmium via air or

food can induce renal tubular dysfunction as a result of cadmium accumulation in the kidney and osteoporosis (brittleness of the bones). Other organs where cadmium accumulates are lungs, pancreas, spleen, endocrine organs and testes (Roberts, 1999).

Parsley

Parsley is an herb that is native to the Mediterranean region of southern Europe (Ensminger, et. al., 1983). Parsley has been used as garnish since the civilization of ancient Romans. Initially, it was used for medicinal purposes but started to gain popularity as a seasoning sometime in the middle ages in Europe. There are three different kinds of parsley: curly leaf, flat leaf and turnip-rooted or Hamburg parsley (Herb Gardening, 2011).

Petroselinum crispum, also known as plain Italian dark green parsley, is used in culinary preparation and has a strong distinctive flavor. Parsley can be germinated from seeds in about 15 to 20 days or can be grown from plugs. Parsley grows best in a location with full sunlight and relatively rich, moist well drained soil with a soil pH between 4.90 to 8.30 (Simon, et. al., 1984). The life period of parsley is 11 weeks.

Italian Parsley is a biennial plant with flat dark green leaves (Figure 1) which are a rich source of vitamins and make an excellent garnish. Table 2 lists the major nutrients present in two tablespoons or 7.50 grams of fresh parsley (USDA National Nutrient Database, 2009).

Figure 1. Italian Parsley

Table 2

Major Nutrients in Fresh Parsley

Nutrient	Amount	Daily Value (%)	Nutrient Density	World Healthiest Foods Rating
Vitamin K	123.00 mcg	153.8	1025.0	Excellent
Vitamin C	9.97 mg	16.6	110.8	Excellent
Vitamin A	631.80 IU	12.6	84.2	Excellent
Folate	11.40 mcg	2.9	19.0	Good
Iron	0.46 mg	2.6	17.0	Good

Atomic Absorption Spectroscopy (AAS)

Metal ion concentrations can be quantitatively determined using the atomic absorption spectrophotometer (AAS). AAS has been used by many researchers to measure the amount of

metals in water, sediments, soils or rocks. It is widely used in analytical laboratories to determine the concentration of metals in the environment. When metals absorb light they are converted into what is referred to as an excited state. Metals absorb light at a specific wavelength. Analyzing samples for any specific element requires a lamp chosen that produces a wavelength of light that is absorbed by that element. By setting the AAS to a specific wavelength, the quantity of the metal can be measured.

In this study, the spectrophotometer used to analyze the samples of soil and plant for cadmium detection, is a flame atomic absorption spectrophotometer (Thermo Elemental Solaar S4 AA). The flame atomic absorption spectrophotometer has four basic components: interchangeable hollow cathode lamp that emits light with element specific wavelengths, a sample aspirator, a flame apparatus for volatilizing the sample, and a photon detector. In flame atomic absorption spectrophotometer, the samples are analyzed in solution form. Sample solutions are aspirated into the flame. A beam of ultra violet (UV) light is focused through a flame and into a detector, the source of light is a hollow cathode lamp set to produce a specific wavelength. When the flame burns the samples, the atoms jump from the ground state to the excited state. In the excited state, the atoms absorb the UV light from the beam. The detector measures the amount of light that passes via the flame. The less light that the detector finds, the more light the atoms absorbed. Hence, a metal of higher concentration will absorb more light.

Chapter III: Methodology

This research project was conducted in Department of Chemistry, Research Laboratories (Jarvis Hall Science Wing). The parsley plants were grown in the tropical section of the Green House, Department of Biology.

Reagents and Materials

Table 3 lists the reagents used in this study.

Table 3

List of reagents used in the study

Name of reagent	Manufacturer
Acetone	Fisher Scientific, Fair Lawn, NJ
Ammonium sulfate	Fisher Scientific, Fair Lawn, NJ
Cadmium oxide	General Chemical Company, New York, NY
Cadmium Nitrate	Mallinckrodt, Phillipsburg, NJ
Calcium carbonate	Fisher Scientific, Fair Lawn, NJ
Concentrated Hydrochloric Acid	Fisher Scientific, Fair Lawn, NJ
Concentrated Nitric Acid	Fisher Scientific, Fair Lawn, NJ
Concentrated Sulfuric Acid	VWR Scientific Products, West Chester, PA
30% Hydrogen Peroxide	Fisher Scientific, Fair Lawn, NJ
2- mercapthoethanol (98%)	Aldrich Chemical Company, Milwaukee, WI
Trichloroacetic acid	J. T. Baker, Phillipsburg, NJ
TRIS	Fisher Scientific, Fair Lawn, NJ

Soil: The soil used for growing parsley was Berger Mix BM2 Germination Mix (Item Number: 73020403) purchased from J R Johnson supply (Roseville, MN).

Parsley seeds: Parsley Plain Italian Dark Green Untreated seeds (Item Number: SKU 00639-00-02) from Harris Seeds Garden Trends Inc. (Rochester, NY), were used in this study.

Pots: Self watering pots were purchased from Fleet Farm (Menomonie, WI). Each pot had a lower chamber for holding water to maintain the moisture of the soil (see Figure 2).

Figure 2. Self watering pot

Instrumentation

An Atomic Absorption Spectrophotometer (S Series 710579 v1.18) with an air-acetylene flame equipped with cadmium cathode lamp was used for this study. The absorbance detector was interfaced with a Hewlett - Packard laptop equipped with Thermo Elemental SOLAAR S4 software. Spectrophotometer, flame and calibration conditions and parameters for the detection of cadmium in root, shoot, and soil samples are shown in Table 4.

Table 4

Flame Atomic Absorption Conditions for Cd Determination in Plant and Soil Samples

Conditions/parameters	Plant samples	Soil samples
Wavelength	228.8 nm	228.8 nm
Band-pass	0.5 nm	1.0 nm
Lamp current	50 %	50 %
Background correction	off	off
Signal type	continuous	continuous
Number of Replications	3	3
Measurement time	4 seconds	4 seconds
Flame type	Air – C_2H_2	Air – C_2H_2
Fuel flow	1.2 L/min	1.2 L/min
Nebulizer uptake	4 seconds	4 seconds
Burner height	7mm	7mm
Calibration mode	Normal	Normal
Line fit	Linear	Quadratic
Concentration unit	mg/L	mg/L
Acceptable fit	0.95	0.95

Growing Parsley

Parsley seeds were germinated in plastic trays with about 25 seeds per tray in the germination chamber. After a week, the plants were transferred to the greenhouse. The seeds were allowed to germinate for 15 to 20 days (see Figure 3). Following germination, the plants

were transplanted to self watering pots. Each pot contained about six plants. Parsley plants were grown in the tropical section of the greenhouse (see Figure 4). The temperature in this section was maintained at 75 ^{o}F; the humidity was maintained at 75 %.

Figure 3. Germinating parsley

Figure 4. Parsley pots in Greenhouse

Table 5

Content of pots

# of Pots	pH of soil	Amount of Cd added	Form
1 & 2	4.60-6.00	None	Control
3 & 4	6.60-7.30	None	Control
5 & 6	7.90-9.40	None	Control
7 & 8	5.60-6.00	210 mg	$Cd(NO_3)_2$
9 & 10	6.60-7.30	210 mg	$Cd(NO_3)_2$
11 & 12	7.90-8.40	210 mg	$Cd(NO_3)_2$
13 & 14	5.60-6.00	210 mg	CdO
15 & 16	6.60-7.30	210 mg	CdO
17 & 18	7.90-8.40	210 mg	CdO
19 & 20	5.60-6.00	630 mg	$Cd(NO_3)_2$
21 &22	6.60-7.30	630 mg	$Cd(NO_3)_2$
23 & 24	7.90-8.40	630 mg	$Cd(NO_3)_2$
25 & 26	5.60-6.00	630 mg	CdO
27 &28	6.60-7.30	630 mg	CdO
29 & 30	7.90-8.40	630 mg	CdO

Plant Treatment

A total of 30 pots were used to grow the plants (Table 5); 6 were control pots, these did not contain cadmium; and 24 were treatment pots. In the treated pots, cadmium solutions were added three times a week. The total amounts of cadmium added to the treated pots are shown in

Table 5. After eight weeks, the mature plants were harvested and rinsed with distilled water. The roots were separated from the shoots (see Figure 5). The separated plants were stored in the freezer until Atomic Absorption Spectroscopy studies.

Figure 5. Harvest of parsley

Soil Preparation, pH Adjustment and measurement, and soil treatment

The pH of the soil was measured using a pH probe (calibrated using buffers pH 4.00 and pH 10.00) connected to a Logger Pro interface, which was connected to a laptop computer. About 10 g of soil sample was weighed and 30 mL of distilled water was added to the soil and the mixture was mixed thoroughly. The soil was allowed to settle at the bottom and the pH of the supernatant liquid was measured.

The untreated soil has a pH of 6.60 to 7.30 and will be considered neutral. The pH of the soil was adjusted to an acidic pH (4.60-6.00) using ammonium sulfate; to a basic pH (7.90-8.40) using limestone or calcium carbonate. The pH of the soil was measured once a week.

The soil was contaminated using two concentrations (210 mg and 630 mg); each of cadmium oxide and cadmium nitrate solutions.

Determination of Cadmium in soil samples

The soil samples directly beneath the roots (0 to 20 cm depth) of the plants were used to determine the amount of cadmium in the soil. The procedures listed in the steps below were adopted from Lorentzen & Kingston, 1996 EPA method 3050B (see reference) with slight modification.

1. The soil samples were dried in the oven for 24 hours at 102 ^{o}C.
2. Using an analytical balance, one gram of dry soil sample was used from each pot. The mass of the soil sample was recorded up to 0.0001 g.
3. The soil sample was transferred into a digestion flask and 10 mL of nitric acid, 1:1 (v/v), was added to the flask.
4. Using a hot plate, the mixture was heated to ~95 ^{o}C for 15 minutes, without boiling.
5. After cooling to less than 70 ^{o}C, 5 mL of concentrated nitric acid (HNO_3) was added and the sample was refluxed for 30 minutes at ~95 ^{o}C. This step was repeated twice.
6. The mixture was evaporated to ~5 mL without boiling.
7. After cooling to less than 70 ^{o}C, 2 mL of distilled water was added followed by a slow addition of maximum 10 mL of 30 % hydrogen peroxide (H_2O_2).
8. The solution was heated to ~95 ^{o}C, without boiling, until no effervescence was observed.
9. After cooling to less than 70 ^{o}C, 5 mL of concentrated hydrochloric acid (HCL) and 10 mL of distilled water were added and the sample was refluxed for 15 minutes without boiling.

10. The solution was cooled to room temperature; the sample was filtered into 100 mL volumetric flask and diluted quantitatively to the mark using distilled water. The cadmium content of the solution was measured using the AAS.

Determination of Cadmium in Parsley

The procedure listed in the steps below was adopted from Intawongse & Dean, 2006 (see reference) with slight modification.

1. The plant samples were dried in an oven at 88 ^{o}C for 48 hours.

2. Using an analytical balance, one gram of the dried sample, was placed into a digestion tube.

3. To the sample in the tube, 10 mL of concentrated HNO_3 was added and the mixture was heated to 95 ^{o}C for approximately an hour.

4. After cooling, 5 mL of concentrated sulfuric acid (H_2SO_4) was added to the mixture; the mixture was heated to 120 ^{o}C until charring first appeared.

5. After cooling, 5 mL of concentrated HNO_3 was added to the tube; the tube was heated to 130 ^{o}C.

6. Additional amount of HNO_3 were added with continued heating until the sample appeared clear or a pale straw color. Figure 6 shows the digestion set-up.

7. After cooling, 1 ml of 30 % H_2O_2 was added to the tube and the sample was heated to 130 ^{o}C (maximum). This procedure was repeated until all the brown fumes ceased to appear.

8. After cooling, 10 mL of distilled water and 0.5 mL of concentrated HNO_3 were added to the tube; the sample was heated to 130 ^{o}C until the white fumes were emitted.

9. After cooling, 10 mL of distilled water and 1 mL of 30 % H_2O_2 were added, the mixture was heated to 130 ^oC (maximum) until white fumes were emitted.

10. Finally, the digested sample was cooled, filtered into a 50 mL volumetric flask, and then distilled water was added to dilute the solution to the mark (see Figure 7). The sample was analyzed for its cadmium content using AAS.

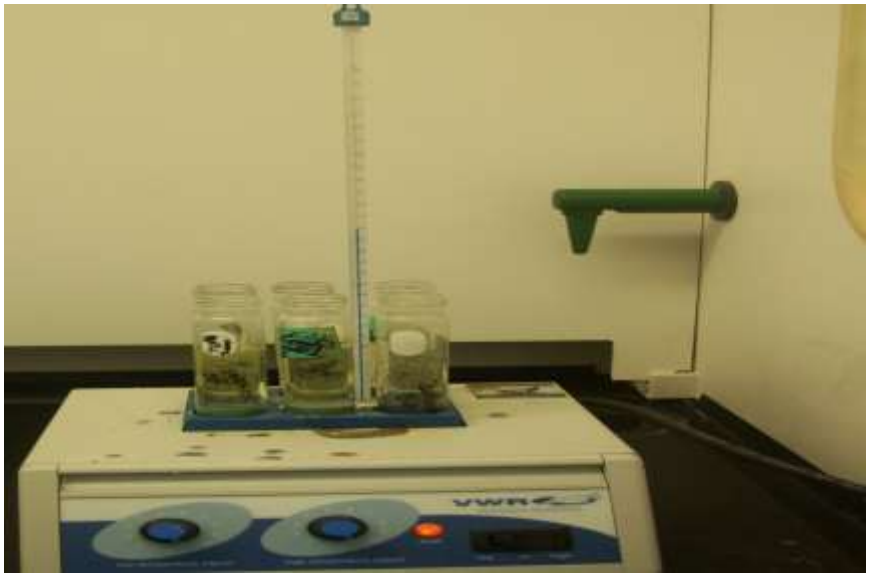

Figure 6. Digestion of parsley roots and shoots

Figure 7. Filtration set up for roots and shoots digested samples

Preparation of Cadmium Nitrate Stock Solution and Standard Solutions

Cadmium nitrate stock solution was prepared using 0.03016 grams pure solid cadmium nitrate (Mallinckrodt; Phillipsburg, NJ). The solid was quantitatively transferred into a 1-Liter volumetric flask, diluted to the mark with de-ionized water, and mixed well by inversion. The concentration of this cadmium nitrate stock solution was 30.16 parts per million (ppm).

The cadmium nitrate stock solution was used to prepare a series of diluted cadmium nitrate standard solutions. Precise volumes of the cadmium nitrate stock solution were transferred into 250 mL glass volumetric flasks and diluted to mark with de-ionized water. These diluted cadmium nitrate standards were mixed well by inversion and used for generating the standard plot used to determine the cadmium concentration in the roots and shoots samples. The calibration curve for the plant samples was determined using cadmium nitrate standard solutions with the following concentrations: 1.00 ppm, 2.50 ppm, 5.00 ppm, 6.00 ppm, 7.00 ppm, 8.00 ppm, 9.00 ppm, and 10.0 ppm.

The cadmium nitrate stock solution used in the analysis of cadmium in soil samples was prepared using 0.1000 g pure solid cadmium nitrate. The solid was quantitatively transferred into a 1-Liter volumetric flask, diluted to the mark with de-ionized water, and mixed well by inversion. The concentration of cadmium nitrate in this stock solution was 100.0 ppm.

The cadmium nitrate (100.0 ppm) stock solution was used to prepare a series of diluted cadmium nitrate standard solutions. Precise volumes of the cadmium nitrate stock solution were transferred into 250 mL glass volumetric flasks and diluted to the mark with de-ionized water. These diluted cadmium nitrate standards were mixed well by inversion and were used for generating the standard curve for the analysis of cadmium in soil samples. The calibration curve

was determined using cadmium nitrate standard solutions with the following concentrations: 1.00 ppm, 5.00 ppm, 10.0 ppm, 20.0 ppm, 40.0 ppm, and 60.0 ppm.

For all cadmium determination, the samples were run in the following sequence: blank solution, standard solutions (lower to higher concentration), control samples and treatment samples. After each sample the AAS was aspirated using distilled water. The data was collected using Thermo Elemental SOLAAR S4 software. The flame atomic absorption conditions were maintained as mentioned in Table 4.

Data Analysis

The data was analyzed by plotting graph of absorbance versus concentration. A set of known standards were run to determine the calibration curve. When the absorbance of the treatment sample was measured, its measurements were compared with the known calibration curve to determine the cadmium concentration. Linear regression equations with 99.86 and 98.55 correlations were used to determine the concentration of cadmium in the plant and soil samples, respectively (Figures 8 & 9).

An independent sample t-test was used to test for differences in the amount of cadmium in plant samples treated with cadmium oxide and cadmium nitrate. An F-test was performed to determine if the variances of the two mean values were significantly different. If the variances of the mean values were found to have significant difference, then an independent t-test for unequal variances was performed. If the variances of the mean values were found to have no significant difference, then an independent t-test for equal variances was performed.

A 2 x 2 Analysis of Variance (ANOVA) was used to test for differences in the average cadmium absorption between two chemical forms of cadmium (cadmium oxide and cadmium nitrate) and between two cadmium concentration levels (210 mg and 630 mg).

Chapter IV: Results

Standard Linear Regression Data

Tables 6 and 7 show various concentrations of cadmium nitrate standard solutions used to construct the standard curve for cadmium determination. The data listed in Tables 6 and 7 were used to generate the standard curves shown in Figures 8 and 9. These curves were created using Microsoft Office Excel 97-2003. The linear regression equation used to determine the concentration of cadmium in the roots and shoots samples was $y = 0.0260x + 0.0007$; the one used to determine the concentration of cadmium in soil samples was $y = 0.0165x + 0.0463$.

Table 6

Concentration of standard solutions and absorbance readings, for the analyses of Cd in shoots and roots samples

Standard Solution #	Cadmium Concentration (mg/L)	Absorbance Reading
Blank	0.00	0.00179
1	1.00	0.02469
2	2.50	0.07169
3	5.00	0.12608
4	6.00	0.15257
5	7.00	0.18299
6	8.00	0.20719
7	9.00	0.23759
8	10.0	0.26253

Table 7

Concentration of standard solutions and absorbance readings, for the analysis of Cd in soil samples

Standard Solution #	Cadmium Concentration (mg/L)	Absorbance Reading
Blank	0.00	0.00072
1	1.00	0.02135
2	5.00	0.11216
3	10.0	0.21718
4	20.0	0.42194
5	40.0	0.76323
6	60.0	0.98011

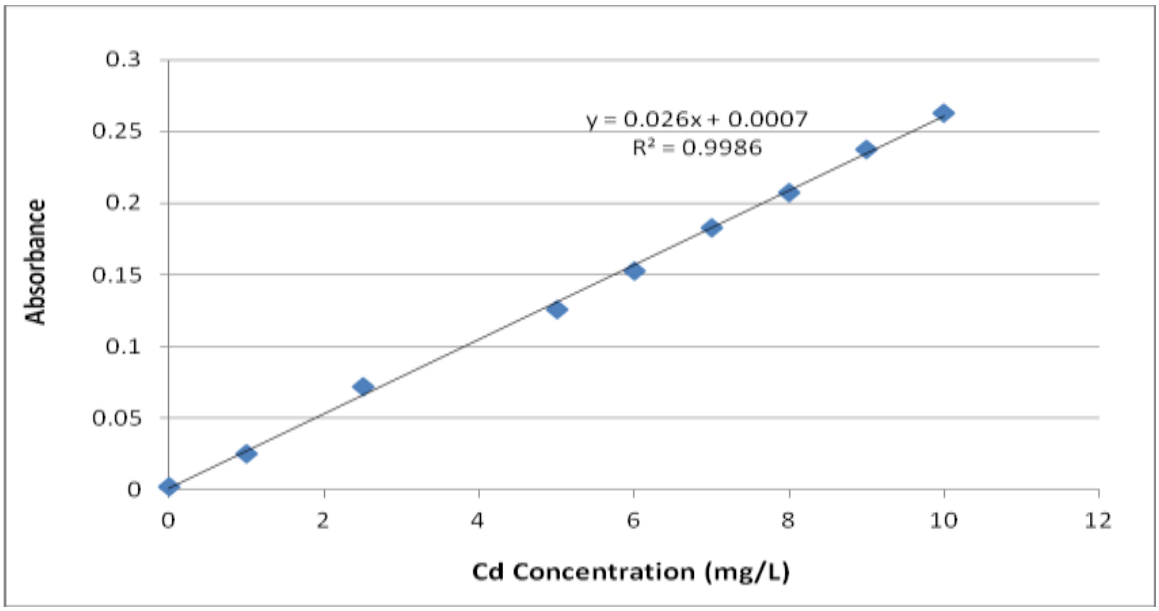

Figure 8. Standard curve used for Cd determination in shoots and roots samples

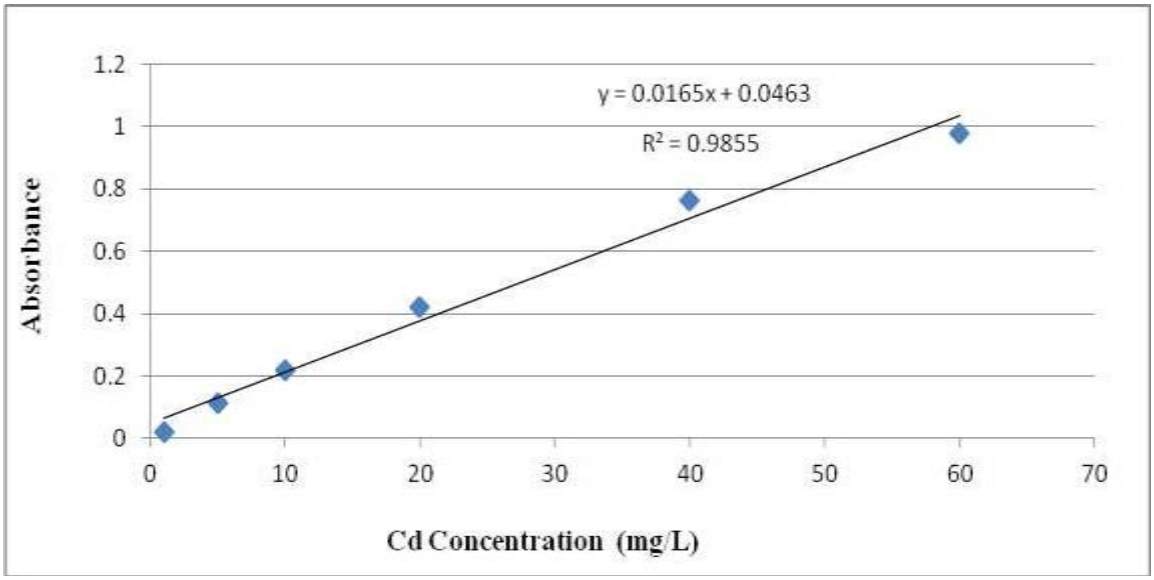

Figure 9. Standard curve used for Cd determination in soil samples

Concentration of Cadmium in Plant and Soil Samples

Tables 8 and 9 show the amount of cadmium in the shoots and roots, respectively, of parsley plants grown in soil with different pH values. The shoots from plants in the same pot

were analyzed as one sample. The roots from plants in the same pot were analyzed as one sample. Hence, there were a total of 30 shoot samples and 30 root samples from 30 pots to determine the effect of the pH of the soil on cadmium uptake two replicate pots were used. The average of the two replicate pot samples was calculated; this was the concentration (mean ± standard deviation) of cadmium in the shoots or roots of parsley.

Table 10 shows the amount of cadmium in soil samples at various soil pH levels. Soil samples from each pot were analyzed in three replicates. Hence, there were a total of 90 samples for the 30 pots of soil. The average of the three replicate soil samples was calculated for each of the two replicate pots, and the average of the two replicate pot samples was calculated; which was the concentration (mean ± standard deviation) of cadmium in the soil sample. Also, the remnant soils from panel below each pot were analyzed to observe any cadmium presence; and no cadmium was detected.

Table 8

Concentration of cadmium (in mg/kg dry sample) in the shoots of Parsley

Amount of Cd added	Form of Cd	Soil pH, Acidic	Soil pH, Neutral	Soil pH, Basic
Control	-	ND	ND	ND
210 mg	CdO	37.5 ± 0.5	39.8 ± 3.7	39.0 ± 8.6
630 mg	CdO	75.3 ± 6.5	68.7 ± 10.4	62.7 ± 9.3
210 mg	$Cd(NO_3)_2$	107 ± 9	129 ± 27	85.3 ± 6.4
630 mg	$Cd(NO_3)_2$	170 ± 39	135 ± 7	167 ± 50

Table 9

Concentration of cadmium (in mg/kg dry sample) in the roots of Parsley

Amount of Cd added	Form of Cd	Soil pH, Acidic	Soil pH, Neutral	Soil pH, Basic
Control	-	ND	ND	ND
210 mg	CdO	218 ± 35	319 ± 43	208 ± 84
630 mg	CdO	373 ± 49	496 ± 78	278 ± 48
210 mg	$Cd(NO_3)_2$	852 ± 59	719 ± 125	786 ± 132
630 mg	$Cd(NO_3)_2$	2600 ± 1535	2795 ± 1150	1321 ± 535

Table 10

Concentration of cadmium (in mg/kg dry sample) in soil samples

Amount of Cd added	Form of Cd	Soil pH, Acidic	Soil pH, Neutral	Soil pH, Basic
Control	-	ND	ND	ND
210 mg	CdO	1637 ± 104	1215 ± 100	1176 ± 289
630 mg	CdO	3461 ± 189	2694 ± 368	3262 ± 247
210 mg	$Cd(NO_3)_2$	1959 ± 152	2326 ± 205	2455 ± 152
630 mg	$Cd(NO_3)_2$	4990 ± 93	5017 ± 121	4582 ± 197

Soil pH measurement

Table 11 shows the initial and final pH of the soil. The initial pH was determined at week 0 and the final pH was determined after 7 weeks, when the plants were harvested.

Table 11

Soil pH determined at Week 0 and at harvest (Week 7)

	Acidic Soil		Neutral Soil		Basic Soil	
	Initial	Final	Initial	Final	Initial	Final
Control 1	5.68	5.58	7.01	7.81	7.99	8.01
Control 2	4.61	5.94	6.99	7.93	9.60	8.06
CdO 210 mg # 1	5.55	5.84	7.00	7.07	8.40	7.58
CdO 210 mg # 2	5.55	6.06	7.00	7.38	8.40	7.76
CdO 630 mg #1	5.61	6.09	7.30	7.46	8.01	7.58
CdO 630 mg #2	5.61	6.26	7.30	7.73	8.01	7.57
$Cd(NO_3)_2$ 210 mg #1	5.60	6.19	7.00	7.80	7.90	7.90
$Cd(NO_3)_2$ 210 mg #2	5.60	6.19	7.00	7.87	7.90	7.63
$Cd(NO_3)_2$ 630 mg #1	5.64	5.91	7.30	7.88	8.41	7.53
$Cd(NO_3)_2$ 630 mg #2	5.64	6.03	7.30	7.78	8.41	7.60

Chapter V: Discussion

Amount of Cadmium in the shoots and roots of Parsley

The average concentrations of cadmium in the shoots of parsley ranges from 37.5 ± 0.5 to 170 ± 39 mg of Cd per kg sample. In the root samples, the amount of cadmium detected ranges from 208 ± 84 to 2795 ± 1150 mg of Cd per kg sample. The amounts of cadmium in the roots are significantly higher than the amount of cadmium in the shoots of parsley ($p < 0.05$). These results are consistent with previous related studies (Wagner, 1993) which indicated that the roots of plants contain the highest amount of cadmium

Effect of the pH of the Soil on Cadmium Absorption

Figure 10 shows the average cadmium concentrations in the shoots of parsley grown in soil with an acidic, neutral and basic pH; Figure 11 shows the average cadmium concentrations in the roots of parsley grown in soil with an acidic, neutral and basic pH. These graphs were generated from the data listed in Tables 8 and 9.

The average amount of cadmium (in mg per kg sample) detected in the shoots of parsley grown in soil contaminated with 210 mg of cadmium oxide ranges from 37.5 ± 0.5 (acidic soil) to 39.8 ± 3.7 (neutral soil); in plants grown in soil contaminated with 630 mg of cadmium oxide, it ranges from 62.7 ± 9.3 (basic soil) to 75.3 ± 6.5 (acidic soil); in plants grown in soil contaminated with 210 mg cadmium nitrate, it ranges from 85.3 ± 6.4 (basic soil) to 129 ± 27 (neutral soil); in plants grown in soil contaminated with 630 mg cadmium nitrate, it ranges from 135 ± 7 (neutral soil) to 170 ± 39 (acidic soil).

The average amount of cadmium (in mg per kg sample) detected in the roots of parsley grown in soil contaminated with 210 mg of cadmium oxide ranges from 208 ± 84 (basic soil) to

319 ± 43 (neutral soil); in plants grown in soil contaminated with 630 mg of cadmium oxide, it ranges from 278 ± 48 (basic soil) to 496 ± 78 (neutral soil); in plants grown in soil contaminated with 210 mg cadmium nitrate, it ranges from 719 ± 125 (neutral soil) to 852 ± 59 (acidic soil); in plants grown in soil contaminated with 630 cadmium nitrate, it ranges from 1321 ± 535 (basic soil) to 2795 ± 1150 (neutral soil).

One way ANOVA was used to test for differences in the average amount of cadmium in the shoots and roots of parsley grown in acidic, neutral or basic soil. The results of these tests show that the concentrations of cadmium in the roots or shoots of parsley grown in an acidic, neutral or basic soil were not significantly different ($p > 0.05$). These results are not consistent with an earlier study (Hatch, et. al. 1988) that reported that the concentration of cadmium taken up by lettuce and watercress increases with increasing pH of the soil.

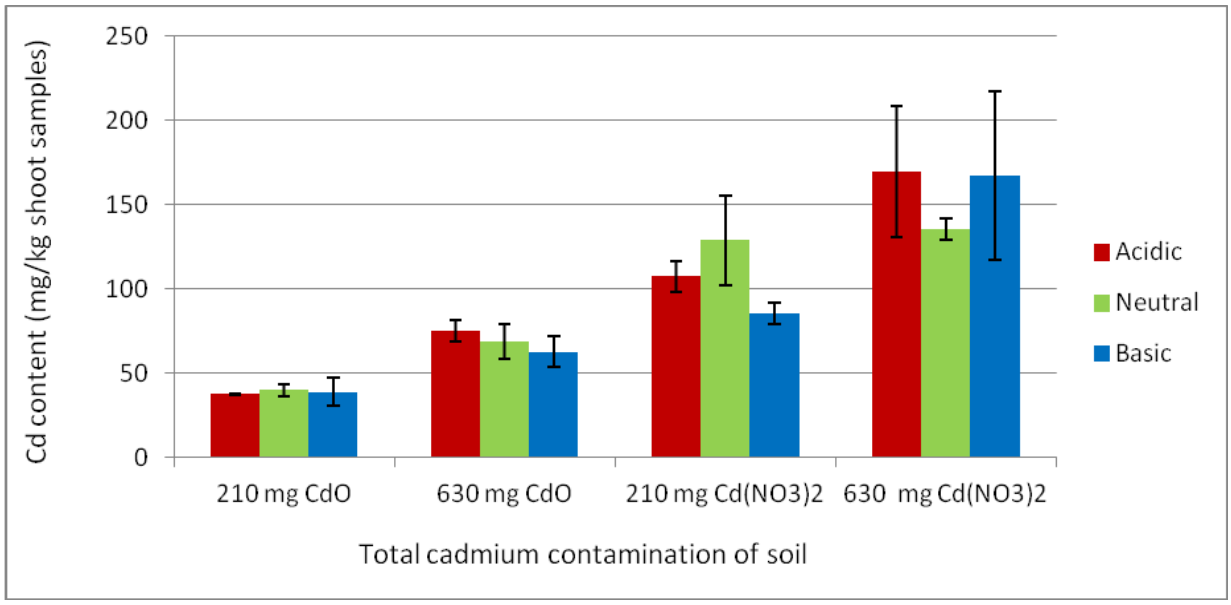

Figure 10. Average amount of cadmium in the shoots of parsley

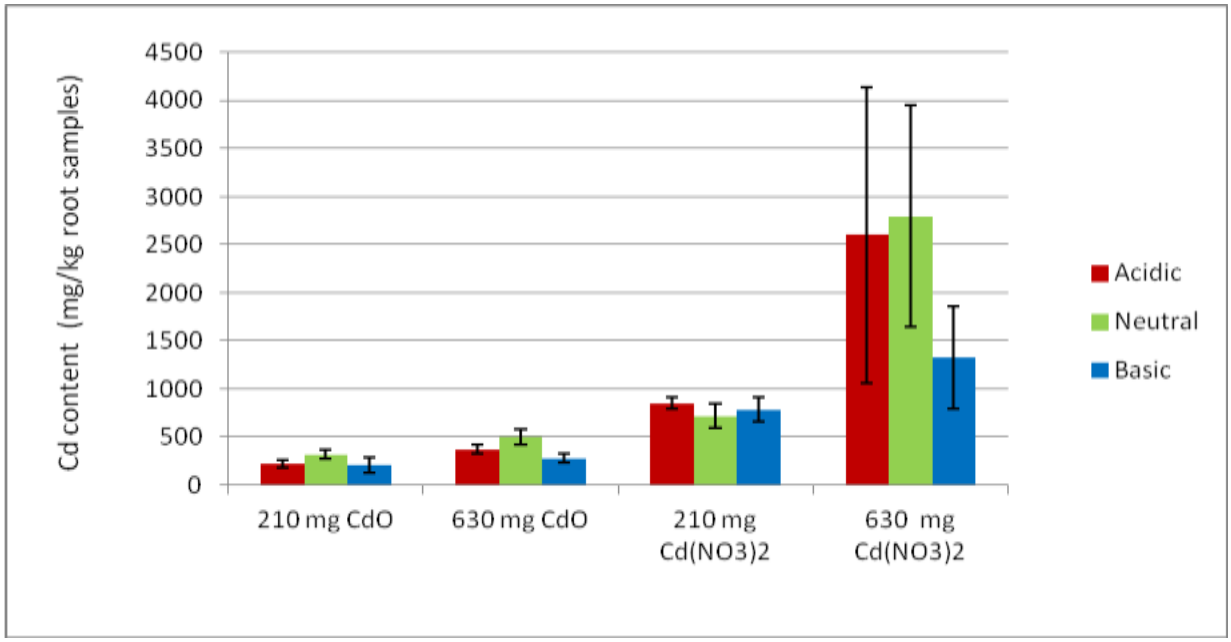

Figure 11. Average amount of cadmium in the roots of parsley

Comparison of Cd uptake using two different chemical forms of Cd: CdO and Cd $(NO_3)_2$

Tables 12 and 13 show the amount of cadmium from the shoots and roots, respectively, of parsley treated with cadmium oxide and cadmium nitrate. An independent samples t-test was used to test for differences in the average cadmium concentration in plants treated with cadmium oxide or cadmium nitrate. A separate independent samples t-test was conducted to determine differences in the average cadmium concentration in the shoots and roots of parsley.

The results of the t-tests show that in the shoots and roots of parsley the concentrations of cadmium were significantly higher ($p < 0.05$) in plants treated with Cd $(NO_3)_2$ than in plants treated with CdO. This can be attributed to differences in the solubility between CdO and Cd $(NO_3)_2$ in water; CdO is not soluble in water while Cd $(NO_3)_2$ is soluble in water.

Table 12

Concentration of cadmium (in mg/kg dry sample) in the shoots of Parsley

Soil pH	Treated with CdO	Treated with $Cd(NO_3)_2$	Amount of CdO or $Cd(NO_3)_2$
Acidic	37.5 ± 0.5	107 ± 9	210 mg
Neutral	39.8 ± 3.7	129 ± 27	210 mg
Basic	39.0 ± 8.6	85.3 ± 6.4	210 mg
Acidic	75.3 ± 6.5	170 ± 39	630 mg
Neutral	68.7 ± 10.4	135 ± 7	630 mg
Basic	62.7 ± 9.3	167 ± 50	630 mg

Table 13

Concentration of cadmium (in mg/kg dry sample) in the roots of Parsley

Soil pH	Treated with CdO	Treated with $Cd(NO_3)_2$	Amount of CdO or $Cd(NO_3)_2$
Acidic	218 ± 35	852 ± 59	210 mg
Neutral	319 ± 43	719 ± 125	210 mg
Basic	208 ± 84	786 ± 133	210 mg
Acidic	373 ± 49	2600 ± 1535	630 mg
Neutral	496 ± 78	2795 ± 1150	630 mg
Basic	278 ± 48	1321 ± 535	630 mg

Effect of concentration of cadmium in the soil on cadmium uptake

Tables 14 and 15 list the concentrations of cadmium in the shoots and roots of parsley, respectively, when two different concentrations (210 mg and 630 mg) for each chemical form of cadmium (cadmium oxide and cadmium nitrate) were added to the soil. A 2 x 2 ANOVA was used to test for differences in the average cadmium concentrations in plants treated with two chemical forms of cadmium (cadmium oxide and cadmium nitrate) and two cadmium concentration levels (210 mg and 630 mg). A separate 2 x 2 ANOVA was conducted to determine differences of cadmium concentrations in the shoots and roots of parsley.

The results of the statistical analyses showed that the concentrations of cadmium in the shoots and roots of parsley were significantly higher ($p < 0.05$) in plants treated with 630 mg of either

cadmium oxide or cadmium nitrate, compared to the plants treated with 210 mg of cadmium oxide or cadmium nitrate.

Table 14

Concentration of cadmium (in mg/kg dry sample) in the shoots of Parsley

Soil pH	210 mg Added to soil	630 mg Added to soil	Form of Cd
Acidic	37.5 ± 0.5	107 ± 9	CdO
Neutral	39.81 ± 3.74	129 ± 27	CdO
Basic	39.0 ± 8.6	85.3 ± 6.4	CdO
Acidic	75.3 ± 6.5	170 ± 39	$Cd(NO_3)_2$
Neutral	68.7 ± 10.4	135 ± 7	$Cd(NO_3)_2$
Basic	62.7 ± 9.3	167 ± 50	$Cd(NO_3)_2$

Table 15

Concentration of cadmium (in mg/kg dry sample) in the roots of Parsley

Soil pH	210 mg Added to soil	630 mg Added to soil	Form of Cd
Acidic	218 ± 35	373 ± 49	CdO
Neutral	319 ± 43	496 ± 78	CdO
Basic	208 ± 84	278 ± 48	CdO
Acidic	852 ± 59	2600 ± 1535	$Cd(NO_3)_2$
Neutral	719 ± 125	2795 ± 1150	$Cd(NO_3)_2$
Basic	786 ± 133	1321 ± 535	$Cd(NO_3)_2$

Conclusions

From the results of this study the following conclusions can be made:

- Parsley absorbs cadmium in soil contaminated with cadmium oxide and cadmium nitrate.
- The amount of cadmium absorbed by parsley is independent of the pH of the soil.
- The amount of cadmium taken up by parsley increases with increasing concentration of the cadmium in the soil.
- The amount of cadmium in the roots of parsley is significantly higher than in the shoots of the plants.
- The amount of cadmium taken up by parsley is significantly higher in plants grown in soil contaminated with cadmium nitrate than in plants grown in soil contaminated with cadmium oxide.

Recommendations

After reviewing and understanding what I have accomplished in this research project I would like to make the following recommendations for further studies:

- To determine the effect of the addition of fertilizers on the amount of cadmium taken up by parsley.
- To determine the effect of the organic matter in the soil on the amount of cadmium absorbed by the plant.
- To determine the cadmium uptake by other herbs such as dill, cilantro, basil, etc.

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