EFFECTS OF APPLICATION OF LHERZOLITE IN GROWTH OF *SPINACIA OLERACEA* L. AND SOIL MICROBES IN CADMIUM CONTAMINATED SOIL IN CHITTAGONG, BANGLADESH

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2015

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By

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This thesis is submitted to the faculty of the department of environmental sciences at Asian University for Women in partial fulfillment of the requirements for the degree of BACHELOR OF SCIENCE

Asian University for Women

Chittagong-4000, Bangladesh

2015

ACKNOWLEDGMENTS

First of all, all praise be to the almighty God for giving me the courage, strength and the spirit to continuously work on this thesis and make it a successful completion.

I am so grateful to Asian University for Women, Chittagong, Bangladesh for giving me the financial support and the opportunity to do this thesis and Chittagong University, Chittagong, Bangladesh for helping me with experimental materials procedures.

My sincere gratitude goes to my thesis advisors, DrAbulKashem and Dr. A.K.M. MoniruzzamanMollah for their endless patience, valuable guidance, immense knowledge and continuous encouragement extended to me since I started my thesis, throughout the lab experiments, writing the thesis, and until the very end of its completion.

Beside my thesis advisors, I would take this opportunity to thank the lab officers – Ms. Jahowa Islam and Fariha Natasha – for helping me during my lab experiments.

I am also thankful to my fellow mates – SavitriKumari, Mei Lan, GayatriKumari, WasanaBandara, Zahra jamilaIkra, LalrinawmiHrahsel, GangodawilegeNimanthi, NusratYasmin, SharminAkter, for helping me during my lab experiments and data analysis.

Last, but not the least, I am deeply indebted to my beloved parents and siblings for the unceasing motivation, constant support and attention.

My sense of gratitude goes to all those whoever supported me directly or indirectly in getting this thesis a successful one.

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ABSTRACT

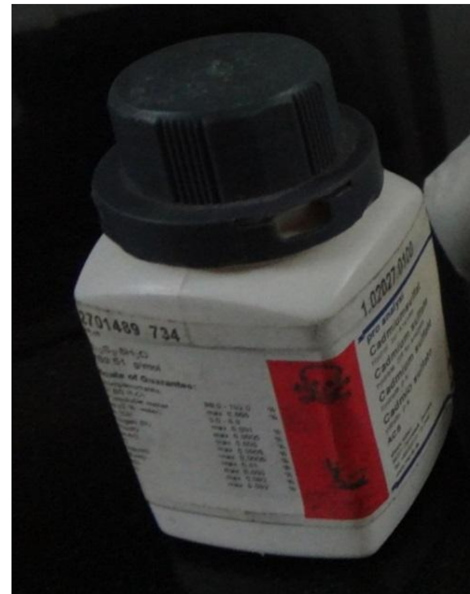
Cadmium (Cd) is one of the most toxic heavy metals for both plants and animals. Generally, availability of Cd in agricultural soils causes suppression in plant growth by increasing the concentration of Cd in plant parts. This experiment was designed to investigate the effect of Lherzolite on growth of *SpinaciaOleracea* L. and Microbial Population in Cd contaminated soils. Effect on microbial population was measure by measuring microbial biomass carbon (C_{mic}), microbial basal respiration, and fungal and bacterial population in soil. Effect on plant growth was done by measuring the dry weight of plant shoot and root and by quantifying the amount of Cd concentrated in plant tissues. It was hypothesized that application of lherzolite in sandy loam soil will decrease the bioavailability of cadmium and thereby result in an increase in plant growth. Results revealed that increased application of lherzolitedoes not display a significant increase in plant root weight; however, it significantly increases plant shoot weight and decreases the tissue cadmium concentration in plant shoots, whereas the change in microbial population and microbial biomass carbon does not have significant difference. The data suggests that lherzolite can be a good remediating chemical for Cd without any side effects on microbial population and its activities.

1. INTRODUCTION

1.1.Introduction to Cadmium (Cd) and its Effects in Living Beings

1.1.1. Introduction to Heavy Metals and Cadmium

Heavy metals are introduced to the soil by both natural and anthropogenic activities. Natural accumulation of heavy metals in soil is comparatively a slow and a gradual process, which does not attract serious attention. However, anthropogenic contribution for the input of heavy metals intensifies their concentration in soil, which causes serious problems (Begum et al., 2009). Cd is one of such toxic heavy metals withincreasing concerns. Cadmium is one of the most dangerous soil pollutants as they can easily penetrate into consumable plants through root absorption (Moreno et al., 2002). It is universally abundant, potentially hazardous, well-known non-essential (Kibria et al., 2010) heavy metal toxic not only to human beings but also to plants, animals, and microorganisms as well (Moreno et al., 2002).



Cadmium is added to the soil by both natural and anthropogenic processes. Natural processes include weathering of rocks and minerals. Anthropogenic activities include farm yard manure, waste incineration, metal working industries, urban traffic, atmospheric deposition, cement factories, and mainly application of sewage sludge and commercial fertilizers (Kibria et al., 2013). “Smelting and mining are among the largest and perhaps the oldest industrial sources of soil contamination by heavy metals,” mentions (Muhlbachova and Simon, 2003). A significant increase in Cd concentration in soils has been caused due to prolong usage of Cd containing phosphorous fertilizers (Kibria et al., 2010). Usage of synthetic chemicals in agricultural lands has brought an increasing awareness and concern over heavy metal contamination and their

effects on food chain, human health, and environmental problems in the recent past years. Whatever the cause is, heavy metal contamination in soil is a serious environmental problem to the present world.

1.1.2. Effects of Cadmium on Humans and Other Animals

Uptake of toxic heavy metal by food and forage plants from contaminated soils is one of the important ways those heavy metals enter into the food chain. This ultimately leads to accumulation of heavy metals in human body due to consumption (Kibria et al., 2006). Similar to many other heavy metals, Cd also poses a potential threat to the health of humans and other living beings as well (Kibria et al., 2010) as it enters the food chain and gets bio accumulated within an organism and bio magnified when it passes through the food web. The summery of effects of cadmium on human health is shown in Figure 2.

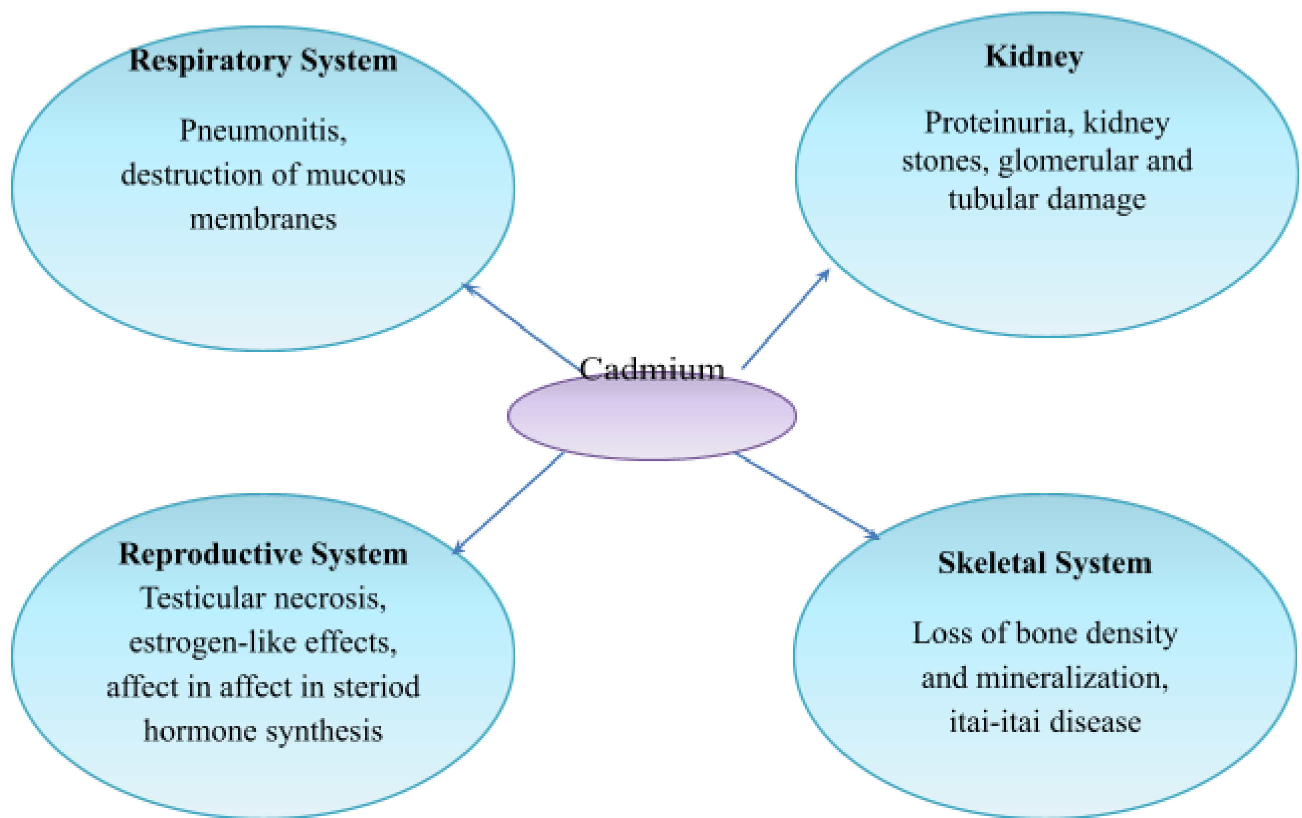


Figure 2: Effect of Cd on human health

As shown in Figure 2, cadmium can affect human kidneys, skeletal system reproductive system, and respiratory system as well (WHO, 2015). Hence, people suffer from renal tubular disease upon consumption of rice with excess concentrations of Cd (Kibria et al., 2010). It is also identified as a carcinogenic metal upon inhalation (WHO, 2015). Therefore, human exposure to these toxic heavy metals through dietary intake is of increasing concern and needs immediate attention. A limit of 0.2 mg Cd kg^{-1} in cereals and legumes for human consumption has been set by the The Codex Alimentarius Commission and international foods standards organization (Kibria et al., 2010).Thus, it is important to minimize the intake of Cd from plants, which occurs as a consequence of increasing Cd concentration in soil that is being up-taken by plant species.

1.1.3. Effect of Cadmium on Soil Microbial Populations

Heavy metals have been continuously introduced onto soil environment due to various anthropogenic activities during the recent decades (Shentu et al., 2008). Cadmium is one of the worst culprits and the most deleterious heavy metal pollutants, and is found to be ubiquitous in the environment (Min et al., 2004). Consequently, plant growth, microbial morphology and metabolism, denaturation of proteins and disturbance in cell functions, and disruption in cell membranes are all affected due to heavy metal contamination in soil (Shentu et al., 2008).

Cd from soil solution may easily be transported and accumulated in plant tissues, through which Cd enters the food chain and reaches the upper trophic levels (Min et al., 2004). However, effects of Cd is not only limited to animals and human health, but also shows adverse effects on microbial population, microbial size and composition, microbial biological activities (Min et al., 2004), and microbial- mediated activities (Muhlbachova and Tlustos, 2006). It is known that the total number of microbes, the varieties, and the density of their population highly depend on the

total content and the concentration of different forms of toxic heavy metals (Wyszkowska and Wyszkowski., 2002).

1.2.Relation among Soil Quality, Cadmium and Soil Biota

1.2.1. Soil Quality

There are different types of soils such as sandy loam, sandy clay loam, and clay loam. Among those different types of soil, sandy loam is the best of the agricultural worlds. However, that is also identified as the type of soil that can accumulate the highest percentage of Cd when it is contaminated with cadmium compounds (Kibria et al., 2006).

1.2.2. Solubility of Cadmium

Solubility of Cd is one of the important factors that determines the uptake of cadmium in plant tissues through root hair cellsand its availability to plants and soil microbes are influenced by chemical and physical properties of soils such as composition of the organic matter, texture, pH, and availability of water in soil (Kibria et al., 2010). Regardless of the total concentration of Cd, the available fraction of Cd in soil solution is more likely to correlate well with the toxicity parameters (Abdousalam, 2010). Moreover, the ability for cadmium to be absorb by roots, to accumulate them within plant tissues, and to tolerate the presence of Cd are all different parameters that widely differ among plant species and cultivars (Kibria et al., 2010). Depending on what form and what metals are available in the soil, microorganisms interact with metals in different ways: a lot of metals are important to microorganisms as electron accepters or as cofactors in certain enzymes, whereas some other metals are extremely toxic and harmful to microbial growth and activities (Abdousalam, 2010). It may also depend on the concentration of available heavy metals.

1.2.3. Soil Biota

Soil biota is one of the vital components thatmaintains soil quality (Shentu et al., 2008). Soil microorganisms play an important role in regulating the functions of soil ecosystem related to soil fertility and primary production as it takes part in biogeochemical cycles like decomposition of organic matter and nutrient cycling (Shentu et al., 2008). Research has shown that there exists a close relation between microbial biomass and soil fertility indices (Abdousalam, 2010). Soil microorganisms are the living portion of soil that is responsible for decomposition and degradation or transformation of toxic compounds, and decomposition of organic matter (Abdousalam, 2010). Hence, they greatly contribute for the improvement of soil structure and texture by metabolizing various organic compounds and elements like nitrogen, phosphorous, sulfur and other elements (Wyszkowska and Wyszkowski., 2002). Moreover, they can be considered as the early indicator of change in soil quality and organic matter because microbes are the labile fraction of soil organic matter (Abdousalam, 2010).

1.3.Soil Ecology and Importance of Microbial Population

Soil microorganisms are involved in multiple cyclic processes that directly influence soil quality. The supply of mineralized elements like nitrogen, carbon and phosphorous from soil organic matter, decomposition of animal, plant, and other residual organic matter, and maintenance of soil structure and function are all highly dependent on the proper functioning of the soil microbial ecosystem (Abdousalam, 2010). Microbial population, both bacterial and fungal population, changes rapidly as a response to change in soil physical and chemical properties. This leads to decreased soil microbial biomass carbon and microbial respiration. Soil respiration and soil microbial biomass carbon, which gives the amount of CO_2 evaluated per unit of biomass, are useful indicators of soil quality and soil contamination

1.3.1. Soil Microbial Biomass Carbon (C_{mic})

Soil microbial biomass carbon includes the living component of soil organic matter excluding plant roots and soil animals larger than 5 nm^3. Microbial biomass is a measure of the weight of the microorganisms in soil, which is mostly fungi, bacteria, and archaea. The extractable amount of microbial biomass carbon in soil is roughly about 5% of the organic carbon (Interpreting Microbial Biomass Carbon, 2015). There are different methods to measure microbial biomass, viz. direct microscopy method, component extraction method, initial respiratory response method, and fumigation extraction method.

Microbial biomass is directly affected by any heavy metal toxicity because metal toxicity decreases the efficiency of energy utilization in microbial metabolic processes, which leads to the requirement of a greater amount of carbon for maintenance, while decreasing the quantity of carbon incorporated into the microbial biomass (Shentu et al., 2008). Hence, measuring microbial biomass carbon (C_{mic}) is a good indicator of the quality of soil. Therefore, it also enables to assess the effect of lherzolite on soil ecology.

In this experiment, microbial biomass carbon was measured using fumigation extraction method (FE). During chloroform fumigation extraction, chloroform ($CHCl_3$) lyses and kills soil microbes, releasing the cytoplasmic content of the cells into the soil environment, and this can be quantified by back titration with chromate ions.

1.3.2. Soil Microbial Activity

Microbial activity can be measured by microbial basal respiration method. This can be done by measuring the CO_2 released or the O_2 up taken by microbes. Microbial respiration is influenced by soil moisture, temperature, and soil salinity. Moreover, it is also important to know

that basal respiration is a function of the size of the microbial biomass available in the soil. In addition to giving estimation about the total microbial activity, basal respiration reflects both the quality and the quantity of the carbon sources as well (Cheng et al., 2013).

There are two main methods to quantify basal respiration: electric conductivity method and alkali absorption method. The second method was exercised during the trial experiments because it is the most widely used technique performed to quantify the amount of CO_2 evolved when testing microbial basal respiration.

1.3.3. Soil Microbial Population

There are different method to estimate soil microbial population, viz. dilution plating technique, contact slide assay, and direct microscopy method. During this research experiment, microorganisms were estimated using dilution and plating technique with different culture media: nutrient agar (NA) was used to culture bacteria, and potato dextrose agar (PDA) was used to culture fungi. Before the experiments, all the glassware and other pieces of equipment were autoclaved properly.

1.4.Heavy Metal Remediation and Application of Lherzolite

1.4.1. Metal Remediation

Soils that are contaminated with heavy metals should be purified for effective and healthy microbial activities and plant cultivation. Therefore, strong efforts are being developed and new techniques have already been discovered to remediate heavy metal contaminated soils (Montinaro et al., 2012). Liming and organic matter supplementation are the two most famous techniques to decrease or eliminate the negative effect of heavy metals on soil quality, in plant growth and development, and on microbial population and activities (Wyszkowska and

Wyszkowski., 2002). However, new substances are being experimented to accelerate reclamation of soils contaminated with heavy metals. There are several methods of remediation: phytoremediation, chemo remediation, and bioremediation. This research study was mainly based on chemoremediation – by using a chemical known as lherzolite.

1.4.2. Introduction to Lherzolite

This research experiment basically relies on chemo remediation – using a chemical known as Lherzolie. This is a powdery chemical mined in Japan, and is commercially available in Japan as a raw material in cement producing companies and in Mg fertilizers; it is also used in concrete construction activiites (Kashem and Kawai, 2009). Lherzolite has a pH of 9.0, and is a mixture of SiO_2 (38.5%), CaO (2.6%), MgO (36%), Fe_2O_3 (5.9%), Al_2O_3 (1.9%), and Ni (0.17%) (Kashem and Kawai, 2009).

It is one of the chemicals that have been experimented in Japan to examine whether or not its application can remediate Cd contaminated soils. (Kashem et al. assessed the effect of lherzolite on the solubility of two different heavy metals: Cd and zinc (Zn) (2009). They concluded that the application of lherzolite lowered the availability of both Cd and Zn in contaminated soil leading to lower bioavailability of Cd and Zn, lower uptake byplant roots, lower toxicity, whichultimately resulted in increased plant growth.

Similar to other alkaline agents, lherzolite also increases soil pH, and provides a favorable condition to form oxides, metal carbonate precipitates, and other complexes that can reduce metal solubility and increases the sorption of heavy metals (Kashem and Kawai, 2009). Lherzolite provides a surface that would let adsorb Cd and binds on the surface. Hence, adsorption of Cd in lherzolite will decrease the amount of Cd in soil – which consequently decreases bioavailability of Cd in soil and increases overall plant growth.

Heavy metal pollution in soil is becoming a threatening environmental challenge, yet very limited information is in the literature regarding heavy metal induced changes microhabitat of soil microbes (Shentu et al., 2008). Hence, soil microbiological activities are usually considered as an early and sensitive indicator of soil contamination by heavy metals (Shentu et al., 2008). Soil contamination with heavy metals can be measured by microbial parameters such as number, weight, and activity of microorganisms (Wyszkowska and Wyszkowski., 2002). Soil microbial biomass carbon (C_{mic}), microbial activities such as endo or exo cellular enzyme activities and basal respiration, C and N mineralization are some of the microbial parameters that are exhibition considerable changes when associated with the sensitivity of heavy metal toxicity. However, only (C_{mic}), basal respiration, and fungal and bacterial population are taken as microbial parameters to examine the effect of Cd and lherzolite in this study.

There are three objectives in this study: one, to quantify the effect of Cd on plant growth and microbial parameters; two, to explore the effect of lherzolite on plant growth and microbial parameters and to explore if lherzoite can be treated as a good fertilizer; and three, to quantify the effect of application of lherzolite on Cd contaminated soil on plant growth and microbial parameters

It is hypothesized that application of lherzolite in sandy loam soil will decrease the bioavailability of cadmium; hence, it results in an increase in plant growth whereas the microbial population would more or less remain unaltered.

2. PROCEDURE

2.1.Preparation of Soil Samples

Sandy loam surface soil from an agricultural field closer to Chittagong University, Bangladesh, was collected and its physio-chemical soil quality parameters were measured using standard procedures.

Figure 4: Addition of Cadmium sulfate to the soil samples

1.5 kg of soil were added into each pot for the experiment. In order to add 0, 2.5, and 5 ppm Cd, $CdSO_4$ was first dissolved in water and added to the soil in solution form as shown in Figure 4. Lherzolite powder was added to the soil samples in its natural powdery form as show in Figure 5. 0%, 2.5%, and 5% oflherzolite was mixed well with soil. The soil samples were then left for two weeks for incubation. Water was added to the incubation soil samples upon requirements.

Figure 5: Soil samples with lherzolite added.

2.1.1. Treatments and Replications

The experiment was done with nine treatments and three replications for each treatment. After the incubation period was over, 6 to 10 healthy, uniform, commercially available *SpinaciaOleracea* L.seeds were introduced into each pot at equal distance and were left under shady environment for germination. After three weeks of period, when seeds were germinated enough, they were transferred to a sunny place.

The pots were arranged in randomized block design, and were rearranged periodically. Water was added to the pots on a regular basis – generally once in two days – and upon requirement to maintain the field capacity of the soils. Two doses of nitrogen fertilizer in the form of urea were also added at an amount of 50 mg N per kg of soil. Growth parameters such as approximate shoot height and number of leaves were recorded in a regular basis during its development as well as immediately before the harvest. After one month of plant development, excess plants grown from each pot were removed leaving only three selected healthy plants from each pot.

2.1.2. Harvesting

After 50 days since seeds were put into the pots, plants that were grown in all the different treatments were harvested separately and labeled properly. Figure 6 is an image of the all the plants of all the samples just before harvesting.

Figure 6: Pots with the plants just before harvesting

Immediately after the harvest, plant samples were taken to the laboratory. Each plant was rinsed and washed properly with water to remove additional adhesive soil particles. Plants were then separated into roots and shoots and labeled accordingly. Each plant from each sample was counted and recorded for the number of leaves, shoot height (from the bottom of the shoot to the end of the longest leaf), root length (from the top of the root until the end of the longest root), fresh shoot weight, and fresh root weight.

2.2.Preparation of Plant Samples for Tissue Cd Concentration

Plant shoots and roots were discarded, labeled, and put into different containers. These samples were then oven kept for oven dry at 65°C for 72 hours. After three days, the dry weight of each shoot and root sample was recorded. The shoots of all the samples were crushed very well using a motor and a pestle. They were then packed, and labeled separately for further experiments to analyze the tissue Cd concentration.

These samples were then sent to Chittagong University, Chittagong, Bangladesh for further experiments on tissue Cd concentration. They were then digested with nitric-perchloric acid with 3:1 ratio and filtered. The filtrate of each sample was taken for Cd analysis using atomic absorption spectrum (AAS) as in (Kashem and Kawai, 2009). The same procedure was followed for all the samples and the values were recorded accordingly.

2.3. Preparation of Soil Samples for the Analysis of Soil Microbial Parameters

Soil samples from each pot were collected separately immediately the next day after the harvest. Soil samples were air dried, sieved, packed, labeled properly, and stored in the refrigerator at 4°C until the experiments on relevant microbial parameters took place.

2.4.Analysis of Soil Microbial Parameters

2.4.1. Soil Microbial Biomass Carbon (C_{mic})

Two samples of 3.75 gram of 20% moist soil were taken and put into two separate beakers. One beaker containing soil along with another beaker containing 25ml chloroform with 3 or 4 boiling chips were introduced into the vacuum desiccator. The beaker was introduced into the fumigation changed was labeled as fumigated samples, while the other beaker containing the

soil sample which was kept out of the vacuum desiccator was considered as control or the un-fumigated sample.

The desiccator was evacuated for about 30 minutes – until the whole chloroform evaporated. Both the fumigated and the un-fumigated soil samples were incubated at 25°C for 24 hours. After 24 hours, both the fumigated and the un-fumigated soil samples were transferred into two conical flasks and 15ml of 0.5M K_2SO_4 was added and mixed well. They were then put into two test tubes and centrifuged at 300rpm for three minutes and filtered using Whatman filter paper.

After filtering, 8ml of each filtrate samples were taken into another beaker and 2ml of 0.4N, $K_2Cr_2O_7$, 10ml of concentrated H_2SO_4, and 5 ml of C. H_3PO_4 were all added and mixed well. It was then kept to reflux in water bath for about 30 minutes at room temperature. The beakers were taken back from the water bath after 30 minutes, diluted with 20 ml water, and the residual dichromate was back titrated with ferric ammonium sulfate in the presence if diphenyl amine indicator. Initial burate readings and final burate readings were all recorded for the calculation. Same procedure was followed for all the soil samples collected.

Soil microbial biomass carbon was calculated using the following equation:

Extracted organic carbon (µg per ml) = S*M*(D/A)*E*1000

Where, S = sample reading, M= normality of $K_2Cr_2O_7$, D= Volume of $K_2Cr_2O_7$added to the reaction mixture, A=Aliquot of the extract, and E=conversion of the oxidation number of chromium (Cr^{6+} to Cr^{3+})

Soil microbial biomass carbon (µg C_{mic} per gram of soil) = difference in the fumigated and the un-fumigated values / 0.38

All values were computerized and analyzed using Microsoft excel and Mini tab software.

2.4.2. Soil Microbial Basal Respiration

Two 10 g of 20% moist soil were taken into two separate beakers. An absorption tube with 7.5ml, 0.1M NaOH was hanged on the beaker to trap CO_2, along with another beaker containing water to maintain the moisture content inside the big beaker containing soil sample. The setup was kept for incubation for 72 hours in dark. After incubation, both the test tubes (from the experimental and the control) containing NaOH were taken out of the beakers. theNaOH from the test tubes was transferred into two different small beakers to and the EC values were measured using EC meter. Same experimental procedure was followed for the rest of samples as well. Results were recorded, and the analysis was done based on the standard EC curve that was established before the experiment itself.

2.4.3. Estimation of Soil Microbial Population

2.4.3.1.Preparation of Media for Bacterial and Fungal Growth

Nutrient agar (NA) plates were prepared to culture bacteria by adding 14grams of NA powder into 1 liter conical flask containing 500 ml distilled water and was mixed well. Similarly, 19.5 grams of potato dextrose agar (PDA) powder was taken and introduced to 1 liter conical flask containing 500 ml water, and mixed well. Both NA and PDA were autoclaved and poured into petri dishes under sterile conditions. They were then and allowed to solidify, and was kept in the refrigerator.

2.4.3.2.Serial Dilution of Soil Samples

During this research, serial dilution and plating was done to calculate microbial population. Four test tubes filled with 9 ml of sterile distilled water were kept in a test tube rack labeled as 10^{-2}, 10^{-3}, 10^{-4}, and $10^{-5.}$ 10 grams of soil was suspended in 95ml sterilized distilled

water in a conical flask and mixed well to prepare 10^{-1} dilution. 1 ml of this suspension was transferred to a test tube containing 9 ml sterile distilled water labeled as 10^{-2} to and mixed well to get 10^{-2} dilution. One ml of this was transferred to the test tube labeled as 10^{-3} dilution. This procedure was followed in a serial order to prepare the dilutions until 10^{-5}. All glass-wares were sterilized before the experiment.

2.4.3.3.Microbial Plating

30µl of 10^{-4} and 10^{-5} dilutions of cell suspension were inoculated on the surface of nutrient agar (NA) plates previously prepared to culture bacterial population. Similarly, 30µl of 10^{-3} and 10^{-4} dilutions of cell suspension were inoculated on the surface of potato dextrose agar (PDA) plates previously prepared to culture fungal population. They were evenly spread on the plates and labeled properly. Plating was done under the clean conditions and biohazard hood to avoid contamination. Inoculated plates were then kept in the incubator for 48 hours under 37°C for incubation. The same procedure was followed for all the soil samples. After 48 hours, plates were taken out, and counted for colony forming units (CFU) from each media of all the samples. Microbial population was calculated using the following formula:

N

$$umber\ of\ colonies\ per\ ml = \frac{number\ of\ colonies\ resulted\ from\ a\ particular\ dilution * power\ of\ the\ dilution}{volume\ of\ the\ dilution\ used\ for\ plating}$$

3. RESULTS

3.1.Fresh and Dry Weights of Shoots and Roots.

Figures 7 and 8 show the average fresh and dry weights of the shoots and roots respectively of all the samples experimented.

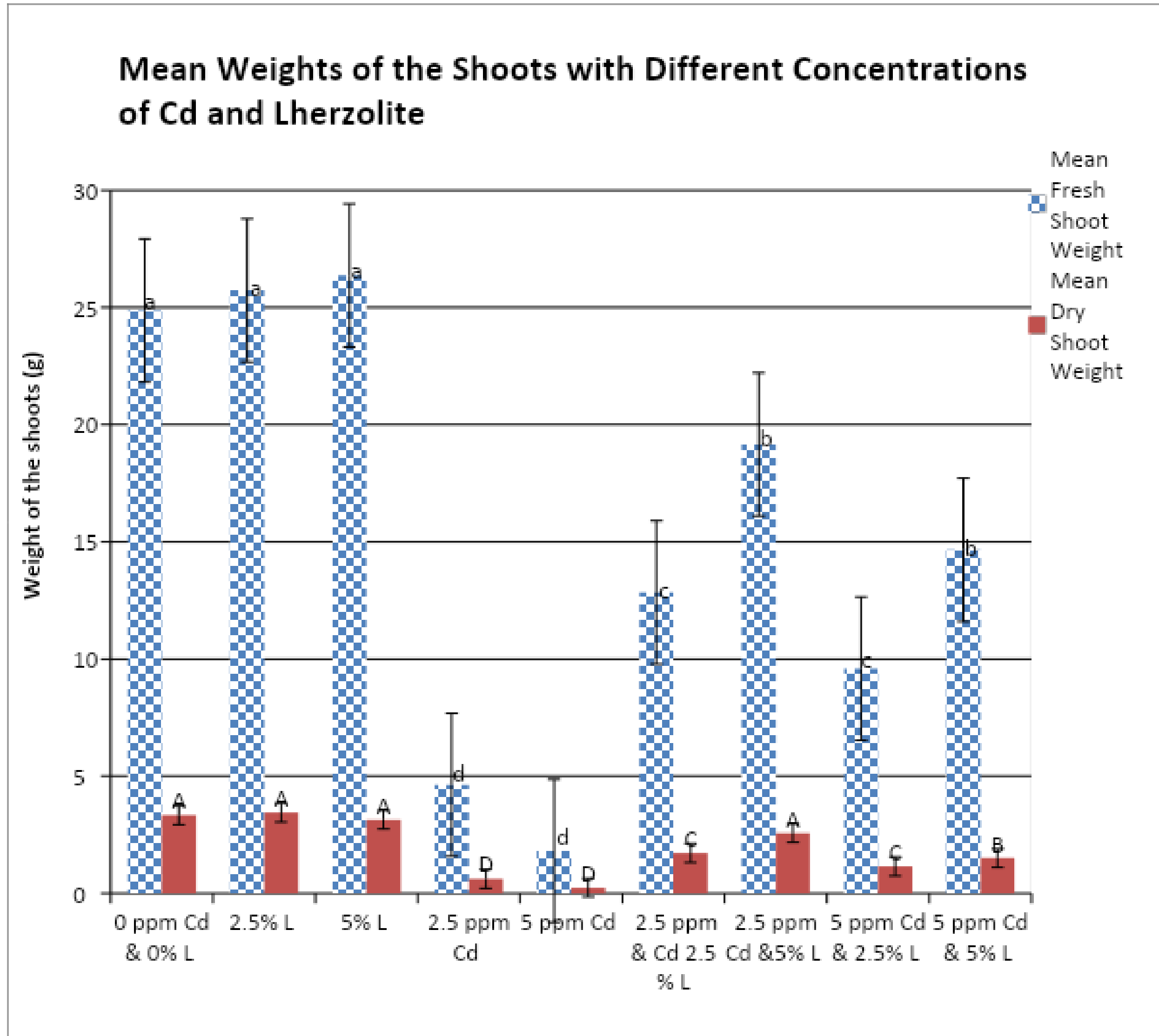


Figure 7: Fresh and Dry Weights of Shoots and Roots

According to the results, plants treated with lherzolite alone do not result a high growth compared to the plants grown in the lherzolite free soil. However, lherzolite does increase the growth of shoot and roots grown in soils treated with cadmium and lherzolite compared to that of the plants grown in pure cadmium contaminated soil samples. Fresh and dry weights of shoots and roots indicate that increasing lherzolite with constant amount of Cd increases overall weight of the plant.

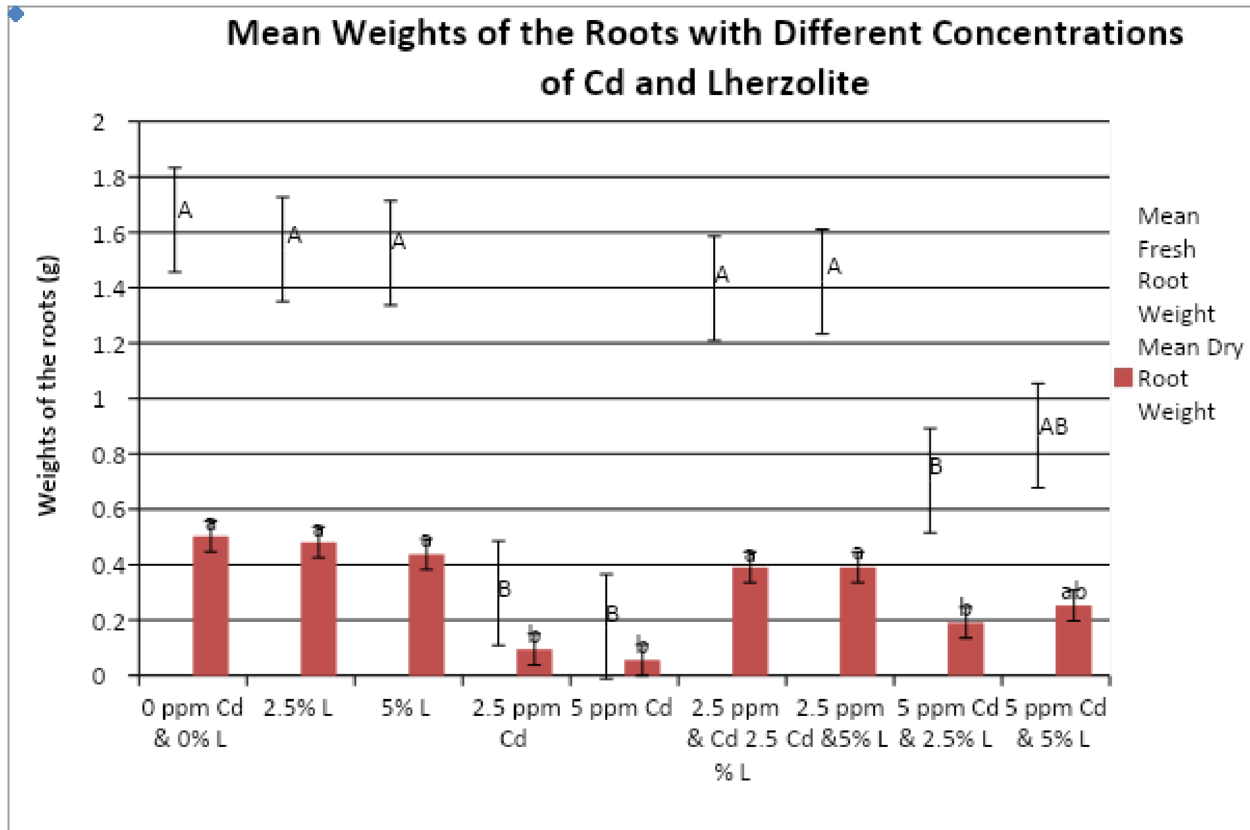


Figure 8: Dry and fresh weights of roots with different treatments

3.2. Cadmium Concentration in Shoot Tissues

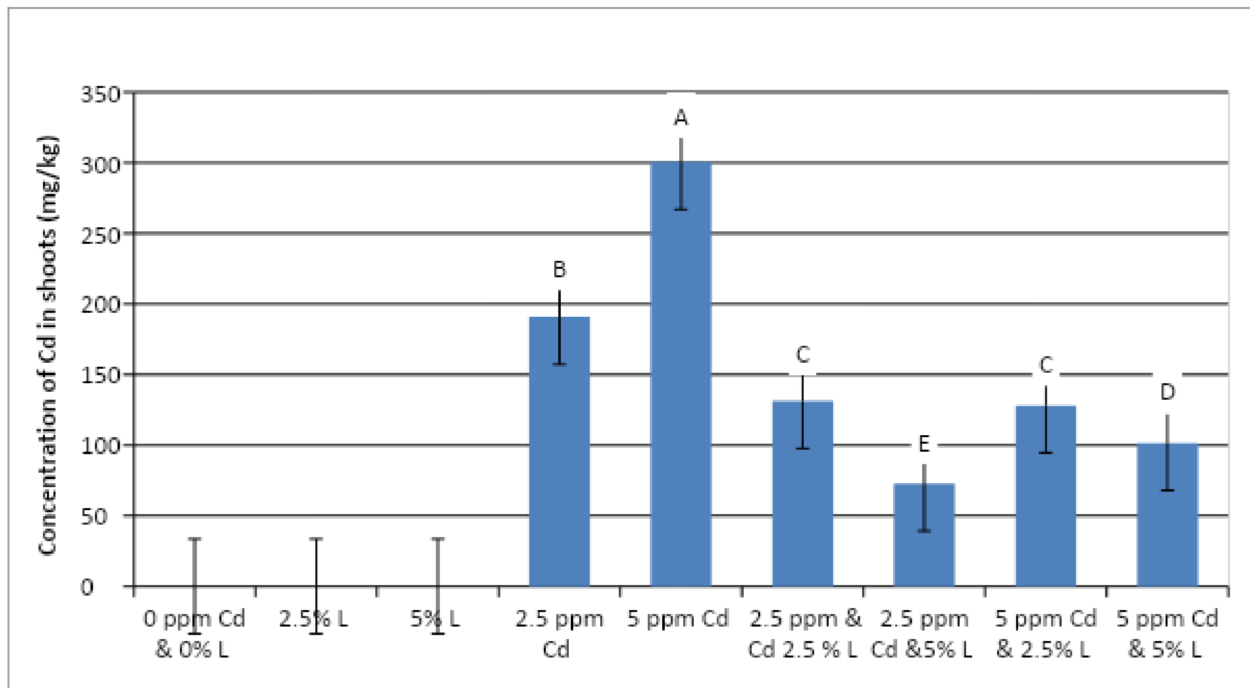


Figure 9: Cadmium concentration in shoot tissues

According to Figure 9, results reveal that tissue cadmium concentration in plant shoot is less in samples treated with lherzolite. It can be clearly seen that increasing amount of lherzilite with constant amount of soil cadmium decreases the amount of cadmium accumulated in plant shoots, indicating that lherzolite does show a positive effect by increasing the overll plant dry weights as a result of decreasing cadmium concentration in plant shoot tissues.

3.3. Results on Microbial Parameters

3.3.1. Effects of Cadmium and Lherzolite on Microbial Biomass Carbon

Microbial biomass carbon of microbes varied from 900 to 3700 (μg Cmic/ per gram moist soil). Unlike plant fresh and dry weights, microbial biomass carbon did not show a trend as a response to cadmium or lherzolite, which was unexpected.

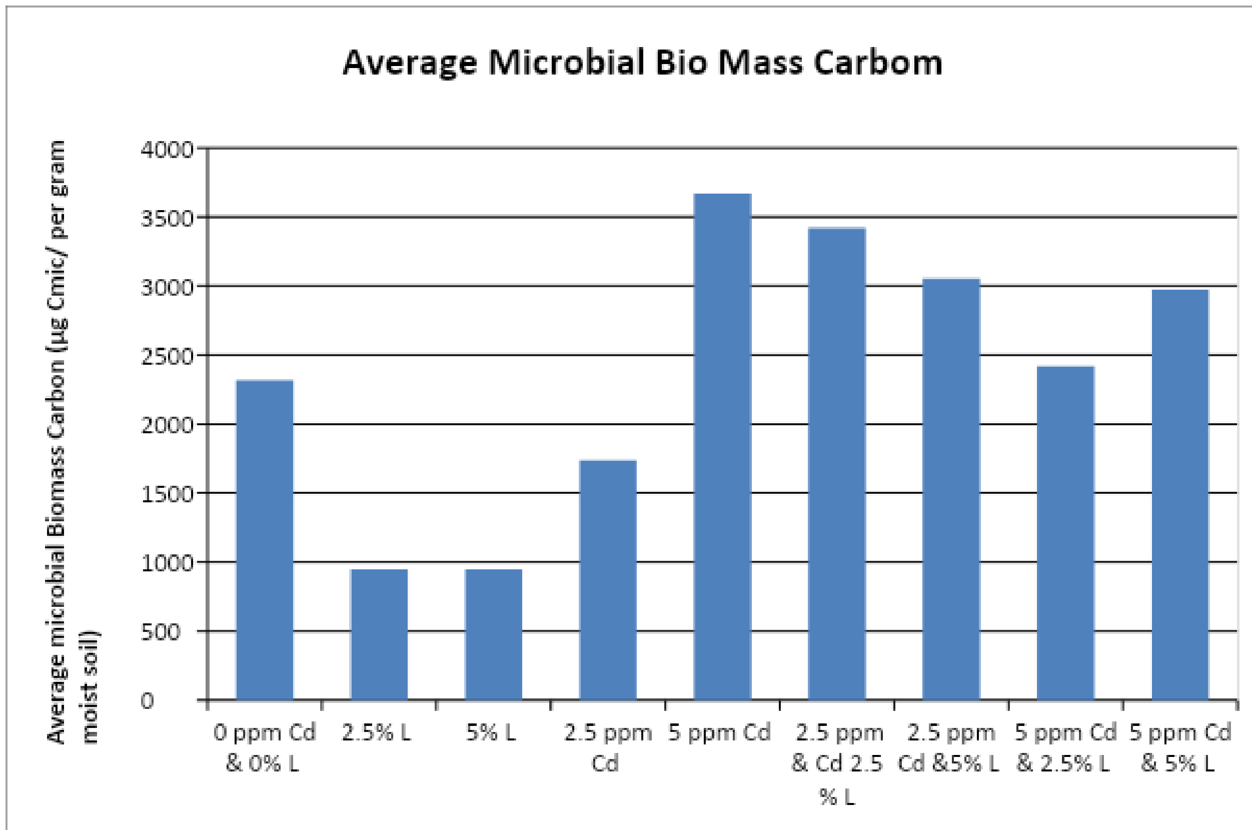


Figure 10: Average microbial biomass carbon for each treatment

3.3.2. Effects of cadmium and lherzolite on microbial population

Overall bacterial population and fungal population of the experimental soil samples fall in the range of 10^5 to 10^7 and in the range of 10^5 to 10^6 for almost all the samples. Data and detailed analysis are not provided here in detail.

3.3.3. Effects of cadmium and lherzolite on microbial basal respiration

Preliminary data on microbial basal respiration gives an average of 0.176mg CO_2/gram soil /day for soil samples that were not treated with Cd or lherzolite. However, hands onexperiments on microbial basal respiration for the respective soil samples were not done because of time, and instrumental limitations.

4. DISCUSSION

This thesis is an interdisciplinary research that combines two different fields of environment: environmental chemistry and toxicology and environmental microbiology. It was designed to explore the effect of lherzolite, a chemical mined in Japan, in artificially cadmium contaminated soil samples on plant growth and soil microbial population, microbial biomass carbon and microbial basal respiration.

4.1. Overall effects of Cadmium on Plant Growth

Fresh and dry weights of plant organs are commonly used parametersto asses and describe the toxicity effect of heavy metals (Kibria et al., 2010). According to the figures 7 and 8, based on plant fresh and dry weights, it can be seen that increasing cadmium display a significant decrease in plant growth, in terms of fresh and dry weights of both shoots and roots. Several others studies also have proven that increasing Cd concentration in soil will increase

bioavailability of Cd and thereby increase the uptake of Cd within plant tissues resulting in an overall decrease in plant yield (Kibria et al., 2010). Decrease in fresh and dry weights may also be related to the inhibition of photosynthesis in plant leaves (Kibria et al., 2010).

Most edible crops do not distinguish between essential nutrients and non-desirable toxic heavy metals (Kibria et al., 2010). It is scientifically proven that various plant species are able to absorb Cd by roots and translocate this heavy metal to the shoots (Kibria et al., 2010). Hence, intake of Cd by plants during plant growth and development is the crucial point of entry of heavy metals into the food chain. Figure 9illustrates that increasing soil cadmium concentration significantly increases cadmium accumulation in plant tissues. For example, plants grown in 5ppm Cd contaminated soil has significantly high amount of cadmium in plant shoot tissue compared to that of plants grown in 2.5ppm Cd contaminated soil.However, according to Figure 9, doubling the soil cadmium concentration in soil does not necessarily double the amount of cadmium accumulated in plant shoot tissues. Intake of cadmium through plant tissue results in decreased plant growth and development. For instance, shoot and root growth of plants grown in 5ppm cadmium contaminated soil have a significantly less fresh and dry weight of plants shoots and roots compared to that of the plants grown in 2.5ppm cadmium contaminated soil. This is in agreement with Kibria et al., (2010) who mentiones that Cd application at the highest amount, which was 9mg per kg soil, produced the lowest dry weight biomass of plant parts in all three soil samples experimented. This proves that increasing Cd concentration decreases plant fresh and dry weights.

Furthermore, during the growth period of the plants, it was observed that pots treated with cadmium alone did not provide a favorable soil environment for the seeds to germinate well and fast as compared to the rest of the seeds; hence, germination of the seeds in Cd contaminated

soil was delayed. Even between 2.5ppm Cd contaminated soil and 5ppm Cd contaminated soil, plants grown in 5 ppm Cd contaminated soil took a lot of time to start germinating. Also, not all of the seeds in those pots germinated except only two or three, and their overall growth was very poor. This observation is in agreement with Miles and Parker (1980) whose objective of the study was to explore whether Cd can affect plant germination, it is discovered that addition of Cd significantly reduced the process of germination for all the three species that were tested. This is because Cd is a toxic heavy metal that can suppress plant germination and adversely affect plant growth.

Interestingly, the results obtained in this research study were very similar to the results obtained by Kibriaet al., done in Indian Spinach – *Basellarubra L.* (2010). Kibria et al., (2010) claims that increasing Cd concentration from 1 to 9 mg Cd per kg soil significantly increases Cd concentrations in plant parts such leafs, stem, and roots. However, in this research, only the Cd concentration in shoots were measured; hence, it did not cover extensive experiments on each plant part such as shoot, root, and stem. However, according to Kibria et al.'s research, roots accumulated a high concentration of Cd, whereas shoots and stems accumulated almost similar amounts when they were grown in sandy loam soil. However, it was the opposite when they were grown in clay loam soil Kibria et al., (2010). Therefore, it can be concluded that the amount of accumulation of cadmium in different plant parts such as shoots, roots, and stem varies with plant species as well the soil types that are used to cultivate them.

4.2. Overall Effects Lherzolite on Plant Growth

As shows in Figures 7 and 8, increasing lherzolite concentration affects plant shoots and roots differently. Increasing lherzoite shows an increase in the fresh weight of shoots; in contrast,

increasing lherzolite decreases fresh and dry weights of the roots. However, it does not display a significant differencein any of the plant parts' weights. This suggests that lherzolite does show discrimination between *SpinaciaOleracea* L. shoots and roots weights. Therefore, lherolite alone should not be used as a fertilizer in agricultural fields. Though lherzoite does not displace a significant effect in plant shoot and root weights when applied alone, it shows an increase in plant fresh and dry weights when applied in soil contaminated with toxic cadmium.

4.3. Effect of Cadmium and Lherzolite on Shoot Weight

Increasing lherzoite concentration show positive results in terms of fresh and dry weights of shoots and roots.Increasing lherzolite concentration significantly increased shoot fresh and dry weight compared to that of the plants grown in cadmium contaminated soils. Regardless of whether the treatment was for 2.5 ppm Cd or 5ppm Cd, and whether they were all treated with 2.5% lherzolite or 5% lherzolite, they all displayed a significant increase in shoot fresh and dry weight compared to that of pure cadmium contaminated soil. This indicates that lherzolite does really work on decreasing the bioavailability of Cd and thereby increase plant shoot growth.

However, constant amount of lherzolite with different concentrations of cadmium displayed different behaviors in plant dry weight. For example, plants grown in 2.5ppm with 2.5% lherzolite and 5ppm cadmium with the same amount of lherzolitedo not show a significant difference in fresh and dry weights of plant shoots. However, plants grown in 2.5ppm Cd with 5% lherzolite and 5ppm cadmium with the same amount of lherzolite shows a significant difference in the dry weights of plant shoots but not in the fresh weight of plant shoots. Therefore, regardless of whether the treatment was 2.5ppm Cd or 5ppm Cd, addition of particular amount of lherzolite for both did not have a significant difference in terms of plant

fresh weights of the shoots. This indicates that certain amount of lherzolite can be effective for a particular range of cadmium concentration. Therefore, further research investigations are necessary to establish the minimal level of effective dose oflherzolite for a particular concentration of cadmium as cadmium concentration of soil contaminated with cadmium varies from place to place.

One of the objectives of the study was to explore whether lherzoite can be used as a fertilizer in agriculture. According to the results obtained for fresh and dry weight of plant shoot, there is a slight increase in shoots' fresh weight, but there is no considerable increase in roots' fresh weight with increasing lherzolite concentration. However, there is no significant effect in shoots' or roots' dry weights. Therefore, application of lherzolite alone has not increased plant growth more than the normal growth level of plants grown in the control treatments.

4.4. Effect of Cadmium and Lherzolite on Root Weight

According to Figure 8, application of lherzolite gives a negative relationship in terms of its effects on plant roots' fresh and dry weight, i.e., increasing lherzoite concentration slightly decreases fresh and dry root weight. However, there is no significant decrease in their weights. This suggests that application of lherzolite alone on *Spinaciaoleracea* L. beyond a certain level might decrease root weight considerably and hamper root development. Hence, further research needs to be done to investigate whether lherzoite can be applied on agricultural lands.

In fact, effect of lherzolite on roots is very different from that of shoots. All plants grown in lherzolite treated samples vs. samples treated with 2.5ppm cadmium and different amounts of lherzoilte do not show a significant difference in both fresh and dry weights of roots. Moreover, plants grown in the same amount of cadmium treated soil with different amounts of lherzolite do

not show a significant difference either. For example, 5ppm Cd treated with 2.5% lherzolite vs. 5ppm Cd treated with 5% lherzolitedo not show a significant difference. Hence, more work needs to be done to investigate the optimal level of lherzolite that can be applied to remediate a particular amount of cadmium in soil.

4.5. Effects of Lherzolite in Tissue Cadmium Concentration

Regardless of whether lherzolite increased plant shoot and root dry weight, it displayed a significant effect on shoot tissue cadmium concentration. Increasing lherzolite concentration in cadmium contaminated soil decreased the amount of cadmium accumulated in *SpinaciaOleracea* L. plants. Pure cadmium contaminated soil samples when compared to that of soil samples treated with lherzlite showed significantly smaller value for plant shoot and root fresh and dry weights as well as high amount of cadmium concentration in shoot tissues. For example, plants grown in 5ppm Cd contaminated soil has significantly high amount of cadmium in shoot tissue whiledry and fresh weights of shoots and roots were significantly less compared to theplants grown in 2.5 ppm cadmium contaminated soil samples.

Same amount of lherzolite has shown significant reduction in accumulation of shoots' cadmium concentration in different levels of cadmium contaminated soils compared to that of the samples that were not treated with herzolite. For instance, 2.5 ppm vs 5ppm cadmium contaminated soil samples both treated with 2.5% lherzolite does not show a significant difference between those two in terms of shoot tissue cadmium concentration, but it shows a significant reduction when compared with any of the cadmium contaminated soil samples that were not treated with lherzolite.

Increasing lherzolite concentration with constant amount of cadmium shows a significant reduction in shoot tissue cadmium concentration. For example, plants grown in soil with 5% lherzolite and 5 ppm cadmium had less cadmium accumulated in plant shoot tissues compared to that plants grown in in soil with 2.5% lherzolite and 5 ppm cadmium soil. However, in case of 5ppm cadmium contaminated soil, the reduction in tissue cadmium concentration is significantly lower. Tissue Cd concentration could have been further decreased if more lherzolite were applied. This suggests that lherzolite increases shoot and root growth by decreasing the uptake of cadmium in shoot tissues. However, it is difficult to predict the exact amount of lherzoliteneeded to remediate 2.5ppm cadmium because neither shoot tissue cadmium concentration nor shoot dry weight show significant difference between 2.5ppm cadmium contaminated soil treated with both 2.5% lherzolite vs. 5% lherzolite. Hence, further research needs to be done to investigate the optimal effective level of lherzolite that should be added to remediate a certain amount of cadmium in cadmium contaminated soils.

Application of lherzolitedecreases the amount of cadmium accumulated in plant shoot tissues. Therefore, application of lherzolitein cadmium contaminated soil increases fresh and dry weights in most of the plants compared to the plants grown in pure cadmium contaminated soil.

4.6. Effects of Cadmium and Lherzolite on Microbial Parameters

4.6.1. Effect of Lherzolite on Microbial Biomass Carbon

Results indicate thatlherzolite is a chemical that can remediate cadmium and decreases the harmful effects that Cd exerts on plants. However, it is also important to investigate whether lherzoliteaffects microbial population and their activities as soil microbes are one of the important components of soil ecology.

According to Figure 10, results reveal that the microbial biomass carbon of all the soil samples varies between the rangeof 900 to 3650 µg carbon per gram of moist soil. These results indicate a higher range of microbial biomass carbon compared to the study done by Rasid M., 2014, on soil microbial biomass carbon in different forest plantations.

There are some limitations in the way this experiment was conducted. The experiments for microbial biomass carbonwere not replicated because of time constraints. Moreover, since the sample size was large, carrying out experiments in series was effective, but it was difficult to maintain the time for the follow up experiments. Hence, an effective conclusion cannot be drawn from the results obtained.

4.6.2. Effect of Lherzolite on Microbial Population

Overall bacterial and fungal population of the experimental soil samples varied from 10^5 to 10^7 and 10^5 to 10^6colony forming units (CFU) per gram of moist soil, respectively. Similar to $C_{mic,}$ results on microbial population also did not show a trend. It was not possible to observe a decrease in C_{mic} or even in microbial population in cadmium contaminated soil samples as expected because some microbes shows inhibitions even from three weeks of incubation (Abdousalam, 2010).However, the reason why it was difficult to see a change might be because according to Abdousalam, highly significant effects of toxic heavy metals on soil microbes could be estimated only after six weeks of incubation period (2010).

Effect of cadmium contamination on microbial population also can vary based on their types: whether it is oligotrohic, organotrophic or copiotrophic. For example, Cd displayed a greater effect on organotrophic bacteria than oligotrophic bacteria in a research conducted by Wyszkowska and Wyszkowski (2002). Effect of heavy metals can also vary based on when the

experiments were taken place. For instance, “In response to Cd contamination, the number of copiotrophic bacteria in Mg untreated soil increased considerably on the first date, but did not show any significant changes on the second date of analyses (Wyszkowska and Wyszkowski., 2002).

Hence, a clear conclusion cannot be made about the effect of lherzolite in cadmium contaminated soil on soil microbial population without extensive research on this topic, and without overcoming the limitations this research underwent.

4.6.3. Effect of Lherzolite on Microbial Basal Respiration

Usually, biological activity of a soil sample is evaluated by measuring CO_2evaluation of soil microbes through which a combine measurement of CO_2 evolved per unit of biomass can be calculated (Abdousalam, 2010), if microbial population was considered. However, this experiment was not performed during this study because of instrumental and time limitations.

The decomposition of plant and animal residues as well as other organic materials in soil and the maintenance of soil structure are all highly dependent on proper functioning of soil microbial ecosystem (Abdousalam, 2010). Hence, it is very important to determine and predict the adverse effects of soil pollutants and toxic heavy metals on soil microbes. There are papers about toxic metals and how they affect soil microbes, but there are no comparable reports in which the effects of heavy metals on soil microbes have been compared, singly or in combination (Abdousalam, 2010). Therefore, it is difficult to estimate the toxicity of Cd with respect to other heavy metals on soil microbes.

5. CONCLUSION

Cadmium significantly decreases plant growth and development. Fairly large amount of Cd can accumulate in plant tissues even without showing stress (Moreno et al., 2002). High soil cadmium concentration accumulates high amount of Cd in tissues and decreases the overall plant weight.

Lherzolitesignificantly increases plant fresh and dry weight by decreasing the amount of cadmium absorbed and accumulated in shoot tissues. However, lherzolite per se cannot be used as a good fertilizer as there was no significant difference in plant fresh or dry wegihts between the plants treated with pure lherzolite and the control.

There are no papers that have been published on the effect of application oflherzolite on microbial population, growth, metabolic activities, and its functions. Hence, further research on lherzolite has to be expanded and more work needs to be done before actually introducing this chemical into the world of agriculture as a good source of Cd remediating agent.

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