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Influence of Biosolids upon the uptake of Mn and Cd by Radish (Raphanus sativus L.)

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INFLUENCE OF BIOSOLIDS UPON UPTAKE OF MANGANESE AND CADMIUM BY RADISH (*Raphanus sativus L.*)

By

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ABSTRACT

The influence of biosolids upon the uptake of Mn and Cd by radish (*Raphanus sativus L.*) was investigated through the characterization of biosolids, sequential extraction of the biosolids, and the determination of the metal content in the root, shoot and leaves of radish (*Raphanus sativus L.*).The biosolid samples from Nacogdoches Wastewater Sludge (NWWS), Lufkin Wastewater Sludge (LWWS), Soil Therapy Compost (STC) had pHs between 5.33 – 6.74. The elemental compositions of the biosolid samples were determined using inductively coupled plasma optical emission spectroscopy (ICP-OES) and SEM/EDX. Major elements (K, Mg, Mn, P) needed for plant growth were found in the biosolid samples while toxic elements Cd, Cr, As, Pb were determined below the USEPA maximum ceiling limit. The functional groups in the biosolids were determined using Fourier Transform Infrared (FTIR) spectroscopy. The FTIR spectra showed peaks at 3386, 2921, 1640, 1375, 1000, 695, 563 cm^{-1} attributed to -OH, C-H, C=O, C-N, C-F, C-Cl, and C-Br groups. A scanning electron microscope was used to determine the particle size of the biosolid. The biosolids have particle diameter in the range ~ 25 – 120 µm. X-ray diffraction analysis showed the existence of vermiculite, alunogen, and quartz in the biosolids. By using a modified Tessier sequential extraction protocol Mn and Cd were found bioavailable in biosolid samples. The accumulation of Mn and Cd concentrations in radish were in the order $[\text{Mn}]_{\text{leaves}} > [\text{Mn}]_{\text{shoot}} > [\text{Mn}]_{\text{root}}$, and $[\text{Cd}]_{\text{root}} > [\text{Cd}]_{\text{shoot}} > [\text{Cd}]_{\text{leaf}}$, respectively.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Biosolids are generated from wastewater treatment processes.[1] They are rich in essential nutrients and organic matter which makes it useful to boost crop production in agriculture. Although biosolids contain essential nutrients, the presence of toxic metals can limit their use. The risk of biosolids contaminating the soil and transferring heavy metals into the food chain may also result in potential health disorders in humans. The determination of total metal concentrations and bioavailable metals in biosolids is important because it provides information on the kind of metals present in biosolids and the metals available for uptake by plants. In this Chapter, an overview of the generation of biosolids from wastewater treatment processes, the importance of biosolids, effects of biosolids on human health and selected metals of special interest (Mn and Cd) are provided.

1.1 WASTEWATER TREATMENT PROCESSES

The goal of wastewater treatment process involves the removal and decomposition of contaminants from wastewater generated from household, homes, institutions, factories and different industries. Wastewater can include but are not limited to liquid waste from showers, toilets, bathrooms, kitchens in residential homes or liquid wastes from manufacturing industries.[2] Wastewater treatment, also referred to as sewage treatment involves the conversion of "used water" from domestic, industrial, agricultural and commercial activities into an outflowing water which goes into a natural body of water and therefore returning into the hydrological cycle with little impact on the environment.[2]

1.1.1 Importance of wastewater treatment

Wastewater treatment is essential because it helps to get rid of contaminants and pollutants, thus safeguarding both the ecology and public health. [3,4] If untreated wastewater is disposed into the water bodies, it can harm the aquatic environment. Untreated wastewater in bodies of water cause reduced oxygen levels and increased levels of organic matter which can result in the death of aquatic life. In addition, the bodies of water develop an awful odor and can lead to the spread of waterborne diseases.[3]

The stages used in wastewater treatment are outlined in Figure 1.1.[5] In general, wastewater treatment occurs in the following stages; namely, preliminary, primary, secondary, and tertiary treatment stages.

1.1.1.1 Preliminary treatment

Influent (incoming wastewater) from homes, factories, offices and industries flow into the wastewater treatment plants from sewers. The incoming wastewater goes through upright screen bars that are 3 inches apart.[6] The upright screen bars get rid of solid trash such as sticks, newspaper, cans, cups and any other similar material, to protect the sewage pumps. After passing through the screening bars, the influent wastewater is lifted by the sewage pump to the wastewater treatment plant’s surface level.[7]

1.1.1.2 Primary treatment

From the preliminary treatment stage, the wastewater moves into a sedimentation tank or primary settling tank for 60 – 120 minutes.[8] The movement of water is reduced to allow large solids particles to settle below the tank and the lighter particles float on top of the tank. At the end of the primary treatment particles such as small plastics and grease are scooped from the surface of the liquid.[7]

The large solid particles at the bottom of the tank referred to as primary sludge is passed through cyclone degritters. A cyclone degritter utilizes centrifugal force to segregate heavy solids such as sand, grit, and gravel from the wastewater. After the primary sludge has been degritted, it is passed into the plant’s sludge facility for more processing. The wastewater that has been treated partially in the sedimentation tank moves to the secondary treatment.[8]

1.1.1.3 Secondary treatment

In the activation sludge process also called secondary treatment, air and "seed" sludge from the wastewater treatment processes are mixed with the wastewater to decompose the sludge further. Large aeration tanks are pumped with air, that combines with the wastewater and sludge. Combining the wastewater, sludge and air stimulates the activity of aerobic microorganism present in sewage. The micro-organisms are used to decompose a large amount of the remaining organic matter, which generates heavier sediments. Wastewater moves through the bubbling tanks for 180 to 360 minutes.[9]

After aeration, the wastewater moves to a final settling tank as seen in Figure 1.1. Most of the secondary sludge goes back to the aeration tank as "seed" to enhance the activated sludge process. The recirculating sludge is made of millions of tiny organisms that aid in ensuring the right amount of air and bacteria in the tank and help to get rid of other pollutants. The secondary sludge left over in the settling tank is removed and combined with the primary sludge for additional processing. Wastewater moves through the settling tank in 120 to 180 minutes before it moves to the disinfection tank.[10]

1.1.1.4 Tertiary treatment

Tertiary treatment (Figure 1.1)[5] includes the following processes: disinfection, sludge thickening, digestion, and sludge dewatering.

1.1.1.4.1 Disinfection

Disinfection is important in the wastewater treatment process because even after passing through primary and secondary treatment, pathogens are still present in the treated wastewater. To get rid of dangerous organisms and disinfect the treated wastewater, the wastewater spends about 20 minutes in a chlorine tank by combining with sodium hypochlorite.[11] After disinfection, the effluent can be dispensed in surrounding water bodies. Equation 1.1 shows the chemical reaction of the disinfection process in the wastewater treatment plant.

$$Cl_{2(g)} + H_2O_{(l)} \longrightarrow HOCl_{(aq)} + HCl_{(aq)} \qquad (1.1)$$

Disinfection is a very important process for wastewater treatment because, the water bodies in which the effluent is released are used by people for fishing and other recreational purposes, treating the effluent help prevent any health risks associated with using such water. [12]

1.1.1.4.2 Sludge thickening

Sludge thickening is the process by which sludge generated during the primary and secondary wastewater treatment processes (which contains about 99% of water) is thickened or concentrated for additional processing. The sludge is collected in a tank where it separates out into a layer of solid and liquid for almost a day. The water obtained from this process is transferred back to the aeration tank for further treatment.[6] Dry or emulsion polymers such as dry or emulsion polyacrylamides are usually used to thicken thin sludge.

1.1.1.4.3 Digestion

After the sludge thickening process, the sludge undergoes additional treatment to make it less harmful to the environment. The sludge is collected inside an oxygen-free digester. Next, the digester is raised to a temperature of about 35 ^{0}C for about 21 - 28 days. This promotes the performance of anaerobes, which decompose the organic matter in the sludge. Unlike the aerobic bacteria in the aeration tanks, anaerobic bacteria perform well in an environment without oxygen. During the digestion process, almost all the organic matter in the thickened sludge is transformed into water, carbon dioxide and methane gas.[7] After digestion, what is left is a black sludge (digested sludge) which is very thick with little odor. The black sludge is thereafter transferred to a dewatering facility from a sludge storage tank.[12]

1.1.1.4.4 Sludge dewatering

Dewatering entails the removal of about 90% liquid content from sludge. In dewatering facilities, the black sludge (digested sludge) is passed through centrifuges that operate like the spin cycle of a washing machine. The centrifugal force from the centrifuges, removes most of the water content from the sludge, generating what is referred to as biosolids. The water removed from the centrifugation returns to the head of the plant for more processing. Addition of an organic polymer (polyacrylamide, $(C_3H_5NO)_n$) enhances the thickness of the "cake", which generates a sample which is firmer and easy to manage. Biosolid cake consists about 27% of the solid matter and 73% moisture.[13]

1.2 BIOSOLIDS AND THEIR USES

Biosolids often referred to as treated wastewater sludge are dark organic matter rich in beneficial nutrients.[14] They are the main by-products of the wastewater treatment process. The United States Environmental Protection Agency (USEPA Federal Regulations Code title 40, part 503) describes, the standards to be met by sewage sludge and biosolids products.[15] Biosolids are required to comply with the USEPA contaminants and pathogen requisite for land use and disposal.[15]

Biosolids have several uses. They are often used as soil conditioners or fertilizers, to improve soil nutrient and boost crop production.[16] In addition, biosolids can help redeem top soils of strip mines by supplying essential nutrients and organic matter. They also promote forestland and serve as a source of compost for gardening and subsistence farming.[16]

Since the USEPA regulation for land application of biosolids in 1994, the amount of metals in biosolids has reduced because of improved wastewater treatment facilities. Biosolids generated from across the USA consist of a low amount of metals.[17] Biosolids may consist of both macronutrients and micronutrients. Macronutrients, such as N, K and P, are needed in large amounts by plants. Micronutrients, such as Mn, Cu, Fe are needed by plants in trace amounts. It is noted that biosolids may contain heavy toxic metals including Cd, Cu, Pb, Hg, and As.[1,18]

1.2.1 Classes of Biosolids

The USEPA groups biosolids into Class A, Class A EQ, (Exceptional Quality) or Class B. In Class A biosolids, disease-causing micro-organisms must be lowered to a non-detectable limit and the material must also adhere to the strict regulations specified by the USEPA, in terms of the total metal content, odor and measure of attraction of disease vectors to biosolids, also referred to as vector attraction reduction (VAR).[19] Vectors might include houseflies, anopheles mosquitoes, rats and birds. For Class A biosolids to meet USEPA rules for land use, it must undergo processes such as oxygen-free digestion, composting, lime stabilization and thermal hydrolysis. After undergoing these processes, Class A biosolids can be applied to land as fertilizers and can also be used for gardening.[20]

Class A EQ (exceptional quality) biosolids, not only meet but also surpass, the regulations for Class A metal content reduction and vector attraction reduction. [19]

Although class B biosolids have undergone treatment, they still have a high number of disease-causing organisms. Class B biosolids can only be used on land, after the USEPA has given a permit with restrictions in terms of crop cultivation and access to the public. Class B biosolids have almost the same nutrient and organic material as Class A biosolids. Class B biosolids are not frequently used as compared to Class A and Class A EQ biosolids because of the increased number of disease-causing organisms, foul odor and the inability to achieve a great vector attraction reduction standard. [20]

1.3 UTILIZATION OF BIOSOLIDS IN SOIL RESTORATION PROJECTS

Biosolids provide important nutrients that are lacking in chemical fertilizers.[21] The major aim of using biosolids by farmers is to limit the use of chemical fertilizers on land. The use of biosolids on lands and farms helps to promote, restore and encourage healthy soil by incorporating essential nutrients including P, K, Mn, Mg, and Ca into the soil. This makes the soil rich in nutrients and boosts crop yield. Also, biosolids contain a high amount of organic matter, which makes them function as soil conditioners to increase the activity of bacteria, aerate compacted soils and improve soil thickness in sandy soils.[16] Soil texture enhancement helps to promote good root growth and the transport of nutrients to the plants.[22]

Soil erosion can be limited by using biosolids.[23] Biosolids contain organic material which helps to hold soil particles firmly together. This helps to improve the texture and the ability of soil to retain water, which enhances the root growth.[21] Biosolids can also be used as good topsoil for recreational purposes. Biosolids can supply adequate nutrient necessary for use in sports fields, gardens and golf courses. Composted biosolids which usually release organic matter, nitrogen and phosphorus very slowly are often used for this purpose.[21]

Heavy machinery used in quarries, construction sites and strip mines can strip the top layer of the soil away and cause soil compaction.[24] This results in the exposure of the subsoil, causing the soil nutrients to be readily washed off by flood or heavy downpour. The soil becomes deprived of the organic matter and necessary nutrient which inhibits the growth of plant on such land. Biosolids can restore the soil nutrient and fertility in such lands. Land reclamation with biosolids can provide a balanced fast and slow release of nutrients and organic matter content. Fast release of nutrients allows for the increased establishment of seedling while slow release allows for a fast growth of a plant which helps to establish a permanent community of plants. The role of the organic matter is to restore the moisture content of the soil, soil porosity and prevent soil compaction.[25]

Furthermore, biosolids can also be used to boost forest production, as soil fertilizer to improve timber production, growth of hybrid poplars and Christmas trees.[26,27] In addition, biosolids can reduce the generation cycle of lumber and pulpwood, especially in very rich soils by promoting vegetative growth. Wildlife in turn benefits from the abundant vegetation.[21] Biosolids have also been used for making brick and construction material, in glass manufacture (through vitrification), and as a biofuel substitute. [28]

1.4 EFFECTS OF USE OF BIOSOLIDS UPON THE ENVIRONMENT

While biosolids are important, there are potential hazards to the environment. Biosolids may contain inorganic heavy metals pollutants, organic pollutants (such as pharmaceuticals and polychlorinated biphenyls), bacteria, virus and other disease-causing organisms, [29,30] and radioactive pollutants.[31] The continual land use of biosolids changes the chemistry of the soil. Depending on the amount and bioavailability of pollutants or contaminants present in the soil, biosolids can be harmful. Crops cultivated on biosolids can uptake metals. The ingestion of such crops can lead to severe health conditions.[32,33,34] In addition, heavy metals can be leached into the water table through land use.[35]

1.5 SELECTED INORGANIC POLLUTANTS

This section describes the possible health hazards that can arise from the ingestion of selected heavy metals found in biosolids.

1.5.1 Arsenic

Arsenic is regarded as a non-toxic pollutant in its organic form, but its inorganic compounds can be of high toxicity. Inorganic arsenic in drinking water can cause skin cancer through ingestion. Studies have shown that arsenic can cause urinary bladder cancers.[36] There are researches that have speciated As in the soil.[37,38] Arsenic is present in domestic sources, its organic forms but can be converted into an inorganic form in biosolids. Examples of organic arsenic compounds include arsenobetaine $(CH_3)_3AsCH_2CO_2$), dimethylarsinic acid $(CH_3)_2AsO_2H$), arsanilic acid ($NH_2C_6H_4AsO_3H_2$), methylarsonic acid ($CH_3AsO_3H_2$).[39]

Major public health concern in the USA, Taiwan, Mexico and Bangladesh has resulted from the exposure of arsenic through groundwater. Reports have shown that over 100 million people are chronically exposed to arsenic through drinking water contaminated with high level of arsenic.[40] A high number of people are exposed to arsenic in Bangladesh. The maximum allowed level of arsenic in drinking water is 50 ppb but in Bangladesh, the level of arsenic is between 150 – 200 ppb in tube well water.[40]

Over 80 million people in Bangladesh are exposed to arsenic. In 1993 arsenic pollution in groundwater was first detected in Bangladesh. The greatest risk of epidemic associated with arsenic poisoning was detected in Bangladesh.[41]

1.5.2 Cadmium

The major effect of cadmium, either through digestion or inhalation from aerosols is proteinuria, a condition that affects and may damage the kidneys.[42,43] Compared to other metals, plants can readily take up Cd. Dietary cadmium might be a major exposure pathway from sewage sludge or biosolids. Deficiencies in calcium, iron, and zinc, which are dietary factors can affect the toxicity of cadmium.[44] Organometallic forms of cadmium include dimethylcadmium (CH_3CdCH_3), cadmium acetate $Cd(CH_3COO)_2$, cadmium stearate $Cd(C_{36}H_{72}O_4)$. Other compounds of cadmium include cadmium chloride ($CdCl_2$), cadmium hydroxide $Cd(OH)_2$, cadmium sulfide (CdS), cadmium oxide (CdO), cadmium sulfate ($CdSO_4$), cadmium nitrate $Cd(NO_3)_2$, and cadmium carbonate ($CdCO_3$).[45]

1.5.3 Copper

Copper is deemed an inorganic pollutant in biosolids by USEPA because of its effect on plants.[20] Exposure to high concentration of Cu at > 0.1 ppm affects the structure and root growth of plants. Copper is known to reduce seed germination and lower the availability of iron. In humans, the toxicity of copper is mostly reported in conjunction with dialysis. Thus, Cu is likely not to pose health risks from biosolids.[36]

Copper occurs in various forms such as chalcopyrite ($CuFeS_2$), chalcocite (Cu_2S), digenite (Cu_9S_5), bornite (Cu_5FeS_4), covellite (CuS), tetrahedite-tennantite ($(Cu,Fe)_{12}(As,Sb)_4S_{13}$), enargite ($Cu_3AsS_4$), azurite $Cu_3(CO_3)_2(OH)_2$, malachite $Cu_2CO_3(OH)_2$, cuprite (Cu_2O), and tenorite (CuO).[46]

1.5.4 Lead

Exposure to low concentrations of Pb might not be harmful but it can accumulate over time in the body system. Ingestion of Pb can affect the blood, gastrointestinal tract, nervous systems, kidney, and cardiovascular blood forming systems.[47,48] Lead inhibits red blood cell enzyme systems, and in high concentration can cause anemia.[49,50,51] Lead can occur in forms such as galena (PbS), anglesite ($PbSO_4$), cerussite ($PbCO_3$), linarite ($PbCuSO_4(OH)_2$), pyromorphite ($Pb_5(PO_4)_3Cl$), mimetite $Pb_5(AsO_4)_3Cl$, vanadinite ($Pb_5(VO_4)_3Cl$ and wulfenite ($PbMoO_4$).[42] Examples of organically bonded lead include lead acetate ($C_4H_6O_4.Pb$) and lead subacetate ($C_4H_{10}O_8Pb_3$).[52]

1.5.5 Mercury

Mercury exists in different forms. The known organic mercury compounds forms include methylmercury $(CH_3Hg)^+$, dimethylmercury (CH_3HgCH_3), ethylmercury $(CH_3CH_2Hg)^+$ while inorganic forms are mercury (II) chloride ($HgCl_2$), and mercury (I) chloride (Hg_2Cl_2). The form in which mercury occurs, explains the exposure route as well as the effects. Ingestion is the greatest exposure route for inorganic mercury.[53] The exposure route for metallic mercury is through aerosol and it can cause harm to the respiratory, gastrointestinal systems, and the skin.

Methylmercury is the major source of epidemics that have occurred from mercury poisoning. Methylmercury $(CH_3Hg)^+$ is formed from industrial pollution of water with elemental and inorganic mercury. One well known case of mercury poisoning was documented from Minamata Bay and Shiranui sea in Japan. The mercury poisoning occurred through consumption of fish and shellfish contaminated with methylmercury discharged in wastewater from a chemical plant (Chisso Co. Ltd.). Studies have detected methylmercury in biosolids.[5]

1.5.6 Selenium

Selenium is less toxic compared to arsenic, cadmium and lead when ingested. The gastrointestinal tract and lungs usually absorb selenium readily.[38] Selenium occurs in inorganic forms such as selenide (Se^{2-}) and selenate (SeO_4^{2-}).[55] Selenium can also be bonded to amino acids found in living systems. Seleno-amino compounds are selenomethionine ($C_5H_{11}NO_2Se$), selenocysteine ($C_3H_7NO_2Se$) and methylselonocysteine ($C_4H_9NO_2Se$)[56], and selenium can also exist in an organic form such as dimethyl selenide (CH_3SeCH_3).[57]

1.5.7 Zinc

The earth's crust contains about 0.0075% of zinc.[58] Zn occurs in soils to concentration of 5 - 770 ppm with an average of 64 ppm. About 30 ppb of zinc is present in seawater while the atmosphere contains about 0.1 - 4μg/m^3.[59] Although zinc is an essential nutrient needed for good health, excess amounts can be toxic. Solutions of free ion (< 20 ppm) are very toxic to plants. [59] Zinc can damage the nerve receptors in the nose leading to a condition known as anosmia. Inhalation of zinc fumes can also lead to zinc chills.[60]

Besides the selected metals briefly reviewed, other microelements such as Fe, B, Si, Mo or macroelements such as N, P, K, and Ca are required for plant growth, and for good health for humans and animals. The selected elements were chosen as they are toxic and are not needed by plants.

1.6 LITERATURE REVIEW

Although biosolids contain essential nutrients (such as N and P) and organic matter needed to boost crop production and performance, they also contain heavy metals and pollutants which can pose serious health risks.[14]

This research is focused on determining the influence of biosolids upon the uptake of metals by radish (*Raphanus sativus L.*). Biosolid samples were collected from Nacogdoches Wastewater Treatment Plant (NWWTP), Lufkin Wastewater Treatment Plant (LWWTP), and the Angelina-Neches Compost Facility in Jacksonville (NCWF). This section reviews the bioavailability of metals and other pollutants found in biosolids-amended soils.

1.6.1 Investigations of Plant Uptake of Metals in Literature

In 1988 the United States banned the disposal of sewage sludge into the ocean. Thus, the bulk of the sewage sludge are deposited on land. Numerous investigations have been carried out to determine how biosolids affect the ecology, plants, and humans. These investigations have focused on the high amounts of pharmaceutical and personal care products, and other toxic contaminants present in biosolids.[61] Some sample research studies carried out on biosolids by USEPA[61,62,63,64] are herein reviewed. On analysis the biosolid specimens obtained by the USEPA were found to contain organic contaminants such as triclocarban, triclosan, ciprofloxacin, ofloxacin, and several tetracycline antibiotics.[61,62] These studies provided information for future risks associated with the use of biosolids for agricultural purposes. These risks include the boost of antibiotic resistance in the surrounding, unfavorable effects on soil and plants, susceptibility of consumers to antibiotics, and the presence of pharmaceutical remains in crops cultivated from biosolids-amended land.[61] Other investigations have been carried out over the years to determine the effects of biosolids on plant uptake.[63,64]

Cotching and Coad [65] investigated the metal uptake of plants by vegetables (silverbeet, potato) and wheat after the application of biosolids. During the investigation, the amount of metals in the edible parts of silverbeet, potato and wheat cultivated in a glasshouse after adding lime amended biosolids and digester sludge into the sludge to the soil were measured. There was no significant uptake of As, Hg and Se. The amount of As was determined below the maximum permitted limit (1 mg/kg in all crops). The maximum limit of Pb (0.1 mg/kg in vegetables)[65] was exceeded in the silverbeet planted with lime amended biosolids. No significant amount of Cd was present in the different application rates of the lime amended biosolids and digester sludge in the potato or wheat grain. Silverbeet was found to have high amounts of Al, Fe, Zn, Cu, Ni, Cd and Co compared to the potato and wheat grain. The Cd concentration in silverbeet was also found above the maximum permitted limit (0.1 mg/kg) in all the treatments including the control.[65]

Ghulam et al.[66] studied the plant uptake and effects of aging biosolids with soils of different pH on subsequent concentrations of Cu and Zn in pore water.[66] Examination of the application of biosolids to the soil after a short time showed lowering of the solubility and the phytotoxicity ability of biosolids-borne Zn and Cu. In the study,[66] aging biosolids at 0, 60 and 120 days were applied with at four contrasting soil pH values (acidic (pH 4), neutral (pH 7), and alkaline (pH 8.4). The amount of Zn, Cu, and dissolved organic carbon were examined in spinach over two months growing season utilizing rhizon pore water samplers. Rhizon pore water samplers are used to extract low amount of pore water from soil in a non-destructive way. It was observed that increase in the aging period in the acidic and neutral soils brought about a decrease in the amount of Cu and Zn with an increase in the solution pH. The alkaline soil showed little effect with an increase in its aging period. In both the alkaline and neutral soil, the amount of soluble Zn and Cu were positively related, and the amount of dissolved organic carbon was negatively related with the soil pH. However, the amount of dissolved organic carbon positively correlated with the pH in the acidic soil. It was also noticed that the yield of harvested spinach for the neutral and alkaline soil was very low and increased with increasing rates of biosolids in the acidic, alkaline and neutral soils. The concentration of tissue Zn and Cu were found very high in the shoots of the radish plants cultivated in the acidic soil. In addition, in all the amended soils, the amount of tissue Cu was found to be low in the radish plants cultivated after two months rather than no aging. From this study, it was concluded that aging biosolids were likely to reduce the solubility and phytotoxicity of biosolids-borne Zn and Cu especially in acidic and neutral soil.[66]

Chenzi et al.[29] studied the uptake of pharmaceutical and personal care products in soybeans grown in biosolids. Pharmaceutical and personal care products are present in biosolids and discharge from wastewater treatment plants.[29] This study found that the use of such biosolids can result in the transfer of pharmaceutical and personal care products into the terrestrial and aquatic habitats leading to its build up in plants. Chenzi et al.'s study[29] carried out at a greenhouse, and investigated the uptake of three pharmaceuticals (carbamazepine, diphenhydramine, and fluoxetine) and two personal care products (triclosan and triclocarban) by soybeans. Soybean was cultivated 60 to 110 days, and analysis was carried out on the plant's tissues and soil. In the root tissues, the carbamazepine, triclosan and triclocarban were present in high concentrations. The pharmaceuticals were also transported to the upper parts of the plant in beans while presence and transportation of diphenhydramine and fluoxetine was limited.[29] Judy et al.[67] investigated the outcome of mixing soil with biosolids obtained from a wastewater treatment plant consisting of metal-based engineered nanomaterials to boost production using *Mendicago truncatula* (barrel-clover) and its symbiotic association with *Sinorhizobium meliloti* (gram-negative bacterium). The study involved soils treated with biosolids produced with Ag, ZnO, TiO_2 engineering nanomaterial (ENM biosolids), $AgNO_3$, $ZnSO_4$ and TiO_2 (dissolved or bulk metal biosolids) and an influent without any metal (control). The soils were mixed with biosolids to replicate 20 years of metal loading of Zn, Ti and Ag in the dissolved/bulk or ENM treatments. It was discovered that the amount of tissue Zn in the plants cultivated with ENM treatment was higher than those cultivated with bulk or dissolved treatment. The result of this study shows the difference in bioavailability and toxicity between ENM and bulk/dissolved metals at amounts applicable to regulatory limits.[67]

Brown et al.[30] studied the relative uptake of cadmium by garden vegetables and fruits grown on long-term biosolids amended soil. The pollution of soil with cadmium is the most hazardous form of soil pollution. The major hazard caused by cadmium contamination is through the consumption of vegetables cultivated on a cadmium contaminated soil. The study was done with different vegetables (cabbage (*Brassica oleracea*), carrot (*Daucus carota*), potato (*Solanum tuberosum*), navy bean (*Phaseolus vulgaris*), tomato (*Solanum lycopersicum*), maize (*Zea Mays*)) selected from different classes or families cultivated on a long-term sludge. In addition, a reference plot at low and high pH levels was used to figure out the cadmium uptake patterns in relation to a reference crop, lettuce, used as the indicator crop. This was carried out to investigate the potential of a relative uptake index. This relative uptake index can be used to determine the risk associated with transferring food cultivated with a cadmium-polluted soil to the food chain.[30]

Bon-Jun et al.[68] studied the availability and plant uptake of biosolids-borne metals using corn plants. In this study, corn plants were cultivated on a sand medium with and without biosolids treatment, both rich in similar nutrients excluding microelements like Zn, Cu, Mn and Ni. After germination, metal analysis on the corn plants showed that the root contained higher concentration of metals compared to the shoot. The corn plants cultivated on the sand medium amended with biosolids had more prominent measure of metals than those cultivated without biosolids. In the tissue of corn plants cultivated in biosolid treatment, the concentration of cadmium in the shoot and root, and nickel in the root were high. Aside from the varying amount of cadmium and nickel in the shoot and root in the two-soil media, the concentration of metals in the plant tissue diminished with the length of growth in the plants.[68]

Marta and Raul[69] carried out research to determine the heavy metal content in lettuce plants cultivated in biosolid compost.[69] Varying amounts of compost biosolids (0 - 100%) w/w, was used to cultivate lettuce plant in greenhouse conditions. Both the dry and fresh biomass of the plant were determined, including the leaf area and metal uptake of the plants. It was observed that the control treatment had lesser dry and fresh matter production of the plants. The biomass of the lettuce plants increased at 20% and 40% (w/w) of compost biosolids. In all treatments, the concentration of Cd and Pb were found below detection limit in the leaves. As compost composition decreases, the amount of Zn in the leaves increases. The use of the biosolid-amended soil caused the amount of Cu and Ni to increase in the lettuce plant. From the research[69] it was concluded composted biosolids can be utilized as soil amendment on a short-term basis for the cultivation lettuce without any harmful effects on its chemical make-up. The results obtained showed that by varying amounts of the composted biosolids, the amount of metals taken up by the plants are present in less toxic concentrations.[69]

Tapia et al.[70] investigated the movement of metals in biosolid compost and pruning waste using shrubs *Atriplex halimus* (Mediterranean saltbush) and *Rosmarinus officinalis* (rosemary). The shrubs were transplanted in the biosolid compost and pruning waste and sprayed with citric acid and nutrients for 60 days. It was discovered that the citric acid raised the amount of soluble Fe and Mn present in the nutrient substrate solution determined by suction probes. The concentrations of Cu and Zn differed little while Cd and Pb were found below detectable levels. From the research,[70] the concentration of Cu and Mn was raised by citric acid in the leaves of *Rosmarinus officinalis* (rosemary) while only the concentration of Mn increased in *Atriplex halimus* (Mediterranean saltbush). The research results showed increase in the solubility of Fe and Mn on addition of citric acid. It was concluded that citric acid enhances the nutrition of plants by increasing the uptake of essential nutrients.[70]

Santibanez et al.[71] examined the effect of metal uptake in *Lolium perene* (ryegrass) under greenhouse pot experiments to explain or determine how the use of biosolids affect metal uptake. In this research, biosolids were photostabilized with copper mine tailings at 0, 6 and 12% (w/w). After 6 months of cultivating *Lolium perene*, the total metal content in the roots and shoots of the plants was analyzed. Results from the research showed that biosolids increased the dry biomass of *Lolium perene*, and the amount of nitrogen and chlorophyll in the shoot. The biosolids also increased the amount of Cu and Zn in the plant tissues. There was no sign of phytotoxicity with the increased amount of Cu and Zn in the plant tissues, and the amount of metals was below the normal range for plants (0.05 ppm and 0.10 ppm for Cu and Zn respectively)[71]. In addition, it was observed that biosolids can lower the Mo uptake and shoot accumulation in plants. The plants took up metals in the order Cu > Zn > Mo > Cd. From the experiment, it was concluded that the metals are generally incorporated into the roots and only a minimal amount is transported to the shoots.[71]

Sridhar et al.[72] examined the consequences of biosolid-amended soil on the metal and nutrient uptake in five different vegetable plants; collard, radish, lettuce, tomato and pepper. After harvesting the vegetable plants, elemental concentration analysis was carried out on the soil, shoot, root and fruit samples. The chemical concentrations present in the soils and all the plant parts increased with increasing amount of biosolids. The observed increase in Cu and Zn concentrations in the shoot of the plant was in the order: collard < radish < lettuce < tomato < pepper. The amount of Cu and Zn accumulated largely in the tomato fruit compared to others. The shoot concentration factor of Zn was larger in the pepper plant than others. This implies a greater increase in the uptake of Zn. From this result, the increase in shoot relative uptake index for Cu and Zn was given as collard < radish < lettuce < tomato < pepper. The shoot dry weight and the spectral reflectance of the radish plants in the near-infrared region (800 – 1300 nm) lessened with increasing amount of biosolid compared to other plants.[72] The purpose of the spectral reflectance was to monitor stress-sensitive plant species and their physiology, which indirectly affects the chemical concentrations in soils and plants.

Residual effects of biosolids and farm manure were investigated by Hamidpour et al.[73] in a calcareous soil using the wheat. After three years of applying biosolids and farm manure to a calcareous soil in 0 (control), 25, 50 and 100 Mg ha^{-1}, the chemical speciation and availability of Cu, Ni, Pb and Zn were investigated using wheat plants. It was observed that the amount of Pb and Ni in the wheat grains cultivated in the biosolid and farm manure treatment were significantly smaller than those grown in the control. On the other hand, the amounts of Zn and Cu in the wheat grains cultivated in the biosolid and farm manure-amended soils was higher compared to those cultivated in the control soil. It was also observed that the amount of diethylenetriaminepentaacetic acid (DTPA)-extractable Zn, Cu, Pb and Ni present in the biosolid-amended soils were more than in the control soil. Results obtained from series of extraction showed almost all of Cu, Zn and Ni were in residual fraction while Pb was majorly bound with Fe – Mn oxides. It was concluded that very little of the residual fraction of heavy metals is bioavailable in conditions usually found in calcareous soils. Based on the speciation of heavy metals upon the use of the soil amendments after three years, no difference was observed in the value of the mobility factor.[73]

From the different studies, it can be concluded that there is no complete removal of contaminants from biosolids during wastewater treatment. Biosolids still contain organic or inorganic contaminants even after treatment. Also, the continual use of biosolids to amend soil might lead to the incorporation of these contaminants into the soil and uptake of such contaminants by plants.

1.7 OBJECTIVES OF RESEARCH

The objectives of this study are to:

1. Determine the total metal content; micro-, and macroelements (including toxic metals) present in biosolids.
2. Speciate and determine the bioavailable metals in the biosolids via Tessier sequential extraction procedures.
3. Examine the effect of uptake of Mn (an essential metal) and Cd (a toxic metal) to radish (*Raphanus sativus L.*).
4. Examine how changes in pH affects radish (*Raphanus sativus L.*) uptake of metals from biosolids.

This data will be useful in providing information for assessing the risks, health and environmental impact of using biosolids to amend soils. In this study, pot experiments (experiments carried out with pot-grown plants) were performed.

1.8 SIGNIFICANCE OF RESEARCH

The deposition and land use of biosolids in the environment poses threats to the environment.[74,75,76] The investigation of the influence of biosolids upon the uptake of metals by plants will provide data important for:

(i) environmental risk analysis, and

(ii) assessing the environmental impact of using biosolids-soil amendments in crop production.

This study focuses on the uptake of Mn and Cd by radish (*Raphanus sativus L.*). Radish (*Raphanus sativus L.*) plants were cultivated on biosolids collected from Nacogdoches Wastewater Treatment Plant (NWWTP), Lufkin Wastewater Treatment Plant (LWWTP) and Angelina-Neches Compost Facility.

The influence of biosolids on uptake of Mn and Cd by radish (*Raphanus sativus L.*) was achieved through:

- Determination of the total concentrations of macro- (N, P, Mg, K, S) and microelements (Fe, Mn, B, Zn, Cu, Mo, Ni) including toxic metals (Pb, As, Hg, Cd) from the three biosolids.
- Analysis of physio-chemical parameters such as pH, conductivity of biosolids.
- The use of Tessier sequential extraction procedures to provide information on the bioavailability of metals in the biosolids.
- Examination of how pH changes affect Mn and Cd uptake by radish (*Raphanus sativus L.*). For this study pH values 6.74 (acidic) and 7.30 (alkaline) were considered.

1.8.1 Justification for use of Mn and Cd in this study

1.8.1.1 Manganese

The earth's crust consists about 0.1 % of manganese. In the soil, manganese is found to be about 7 - 9000 ppm, with an average value of 440 ppm.[77] Manganese exists majorly as pyrolusite (MnO_2), braunite ($Mn^{2+}Mn^{3+}{}_6[O_8|SiO_4]$), psilomelane ($Ba(Mn^{2+})(Mn^{4+})_8O_{16}(OH)_4$), and rhodochrosite ($MnCO_3$).[78]

Manganese is an essential element for human health, needed for development, metabolism, growth and antioxidant system.[79] Although Mn is an essential element, chronic exposure or ingestion can lead to manganism, a neurodegenerative disorder; a condition that causes dopaminergic neuronal death with symptoms associated with Parkinson's disease.[79]

Biosolids are known to have high affinity for metals.[80,81,82,83] Since Mn is an essential microelement needed for plant growth, this study will determine whether Mn is accumulated in the biosolids (depriving the radish (*Raphanus sativus L.*) of the essential nutrient) or taken up by the radish (*Raphanus sativus L.*).

1.8.1.2 Cadmium

The earth crust contains about 0.1 ppm of cadmium. Cadmium is chemically similar to Zn (a trace element needed by animals, plants and human). Significant amount of cadmium is not found in ores. Greenockite (CdS) is the major mineral of cadmium of importance and is closely related to sphalerite (ZnS).[84] It is also notable that Cd can complex with other organic compounds in soil.[85,86] Cadmium has been implicated in atmospheric trace amounts (0.27 – 15.5 ng/m^3).[87,88]

The major effect of cadmium, either through digestion or inhalation from aerosols is proteinuria, a condition that affects the kidney.[36] Chronic exposure to cadmium can lead to renal failure, obstructive lung disease, or cancer.[89] It can also lead to bone defects at minimal concentrations.[90] Cadmium is a toxic metal known to be readily bioavailable.[91,92,93] Cadmium can be taken up by plants if the soil is contaminated. This research investigates the amount of cadmium taken up by the radish (*Raphanus sativus L.*), whether above recommended USEPA Ceiling Concentration Limit. In addition, this study evaluated if land application of biosolids should be encouraged by determining the uptake of metals, Cd and Mn by radish (*Raphanus sativus L.*). This investigation also gives insight into the recommended ratios for amending soils with biosolids.

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FIGURES

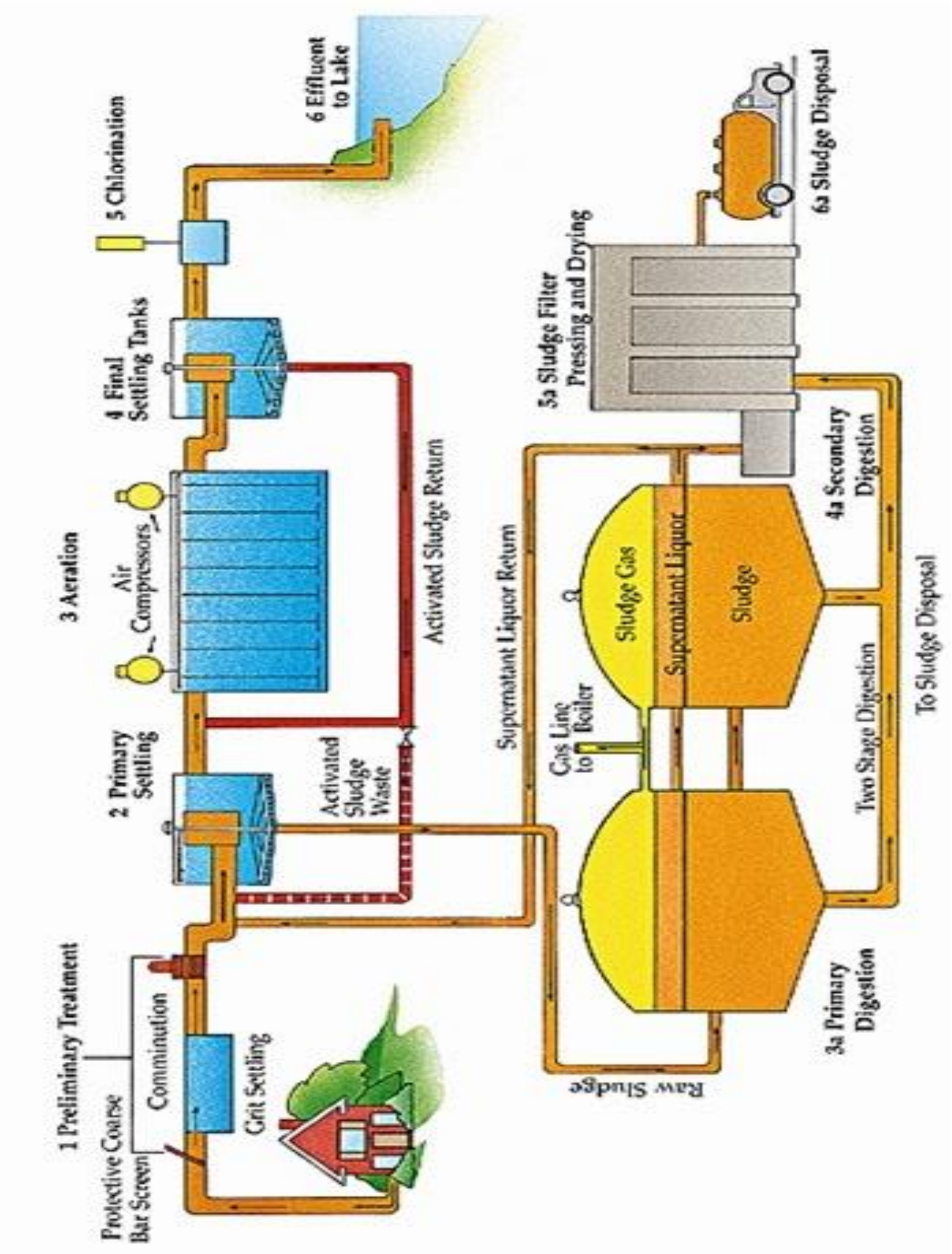

Figure 1.1: Schematic diagram of a wastewater treatment process.[5]

CHAPTER 2

SPECTROSCOPIC CHARACTERIZATION AND THERMOGRAVIMETRIC ANALYSIS OF BIOSOLIDS

2.1 ABSTRACT

Physical-chemical (pH, conductivity), spectroscopic (Fourier-Transform Infrared spectroscopy (FT-IR), scanning electron microscopy (SEM), inductively coupled plasma-optical emission spectroscopy (ICP-OES), X-ray diffraction (XRD)) characterization and thermogravimetric analysis was carried out on biosolids collected from three wastewater treatment plants; Nacogdoches Wastewater Treatment Plant (NWWTP), Lufkin Wastewater Treatment Plant (LWWTP) and the Angelina-Neches Compost Facility (NCF), to collect quantitative data, and generate information on the quality of the biosolids. The pH of the biosolid samples was determined between 5.33 - 6.74. The elemental concentrations of biosolid samples were measured using inductively coupled plasma optical emission spectroscopy (ICP-OES). In general, the concentration of macroelements was higher in Nacogdoches wastewater sludge. The biosolid samples were found similar in metal concentrations. Macroelements concentrations in the biosolid samples followed the order; Al: (NWWS (15591 ± 1692 ppm) > STC (12424 ± 824 ppm) > LWWS (12271 ± 1985 ppm)); Ca: (LWWS (21552 ± 3186 ppm) > NWWS (18738 ±

1644 ppm) > STC (11760 ± 1358 ppm)); Fe: (NWWS (32890 ± 2695 ppm) > LWWS (15163 ± 2212 ppm) > STC (13352 ± 2731 ppm)); K: (NWWS (6126 ± 229 ppm) > LWWS (3328 ± 548 ppm) > STC (2207 ± 82 ppm)); Mg: (NWWS (7293 ± 647 ppm) > LWWS (4116 ± 561 ppm) > STC (1362 ± 98 ppm)); P: (NWWS (26102 ± 1522 ppm) > LWWS (20855 ± 2594 ppm) > STC (8623 ± 426 ppm)) and S: (LWWS (12116 ± 1784 ppm) > NWWS (8365 ± 331 ppm) > STC (2838 ± 89 ppm)). Heavy metals (As: (STC-5.48 ± 0.62 ppm, LWWS-20 ± 3 ppm, NWWS-12 ± 1 ppm); Cd: (STC-0.2 ± 0.1 ppm, LWWS-0.20 ± 0.03 ppm, NWWS-Below detection); Cr: (STC-4.3 ± 0.2 ppm, LWWS-0.9 ± 1.7 ppm, NWWS-17 ± 2 ppm); Cu: (STC-338 ± 14 ppm, LWWS-531 ± 78 ppm, NWWS-386 ± 35 ppm); Pb: (STC-17 ± 1 ppm, LWWS-29 ± 3 ppm, NWWS-27 ± 27 ppm); Hg: (STC, LWWS, NWWS-Below detection) and Mo: (STC-4 ± 1 ppm, LWWS-8 ± 1 ppm, NWWS-7.3 ± 0.7 ppm)) were found below the USEPA Ceiling limit. The FT-IR spectra showed peaks attributed to -OH, C-H, C=O, C-N, C-F, C-Cl and C-Br functional groups. SEM micrograph showed biosolids particle diameter sizes were in the range 25 – 120 µm. The XRD pattern showed that the soil therapy compost contains compounds such as vermiculite, alunogen and quartz at 2θ and d-spacing values (6.070°, 14.55), (20.58°, 4.55) and (26.40°, 3.36), respectively.

2.2 INTRODUCTION

In the previous chapter, an overview of the generation of biosolids from wastewater treatment processes, the importance of biosolids and its effects on human health was discussed. This chapter outlines several spectroscopic techniques that were used to characterize biosolids collected from three wastewater treatment plants (LWWTP and NWWTP) and compost treatment facility (the Angelina-Neches Compost Facility, NCF).

2.2.1 Analytical Spectroscopic Techniques used for Characterization of Biosolids

The spectroscopic characterization of biosolids is an important analysis to assess the impact of the land application of biosolids to plants and human. Studies done for characterization of biosolids, have utilized analytical techniques including nuclear magnetic resonance spectroscopy (NMR), X-ray Absorption Near Edge Structure Spectroscopy (XANES), Transmission Electron Microscopy (TEM), Fourier-Transform Infrared spectroscopy (FTIR), Raman spectroscopy, ion chromatography (IC) and Scanning Electron Microscopy (SEM).

2.2.1.1 Nuclear magnetic resonance spectroscopy (NMR)

Solid-state ^{13}C NMR spectroscopy is an analytical method used for determining the chemical composition of complex organic matter.[1] Ronald et al.[2] used a solid-state carbon-13 nuclear magnetic resonance spectroscopy to characterize biosolids' organic matter. Characterization of sewage sludge using solid-state ^{13}C NMR spectroscopy was first carried out in 1984 by Piotrowski et al.[3] Several other studies have used solid-state ^{13}C NMR spectroscopy to characterize sewage sludge.[4,5,6] In one study[2] six biosolid samples from five wastewater treatment plants in Australia were characterized using solid-state ^{13}C NMR spectroscopy. Solid-state ^{13}C NMR requires a pretreatment which involves the use of hydrofluoric acid to remove the organic mineral in biosolids.[7,8] Two NMR techniques were applied in this study; the standard cross polarization (CP) technique and the Bloch decay (BD) technique. The Bloch decay is a less sensitive decay compared to cross polarization because of the longer recycle delay it requires which results in a lower signal to noise ratio. In addition to the NMR techniques used, a spin counting technique was utilized in this study.[2] The Bloch decay spectrum obtained for each biosolid sample, before and after hydrofluoric acid treatment showed the biosolid samples contain notably more alkyl carbon. The difference between the CP and BD spectra was ascribed to the presence of alkyl carbon with high molecular mobility which affects the efficiency of cross-polarization. From the BD and CP NMR spectra, the distribution of signal intensity were between four chemical shift regions: 190 – 165 ppm (ascribed to carbonyl carbon in carboxylic acids, esters and amides), 165 – 110 ppm (aryl carbon), including (O-aryl) 165 – 145 ppm and 145 – 110 ppm (C- and H-substituted aryl carbon), 110 – 45 ppm (O-substituted alkyl carbon in carbohydrates, including methoxyl carbon and N-substituted alkyl carbon in protein), and 45 – 0 ppm (alkyl carbon).[2] The spectral results obtained showed that the organic matter of biosolids is different in chemistry to the soil organic matter and the land application of biosolids might have some implications.

Mao et al.[9] characterized biosolids-derived organic matter using various solid-state nuclear magnetic resonance spectroscopy techniques including ^{13}C, ^{1}H, and ^{15}N. The NMR spectra obtained indicated five distinct peaks, namely, 174.5 ppm (attributed to COO/CON groups), 100 ppm (anomeric O-CH-O), 73 ppm (OCH), 55 – 65 ppm (OCH_3 or NCH) and 24 – 19 ppm (carbon-bonded CH_3 groups). The peaks attributed to OCH and O-CH-O groups are indicative of large polysaccharide fractions in the biosolids.[9]

2.2.1.2 X-ray absorption near edge structure spectroscopy (XANES)

XANES is a local bonding-sensitive and element specific spectroscopic technique that analyses spectra obtained in X-ray absorption spectroscopy experiments. It also determines the partial density of the empty states of a molecule.

Amy et al.[10] characterized phosphorus species in biosolids using XANES (X-ray absorption near edge structure) spectroscopy. The aim of the study was to determine the phosphorus species in biosolids and other manures to provide explanations on how land application of biosolids or manures can lead to a long-term potential loss of phosphorus species in biosolids.[10] Some studies have associated loss of phosphorus species to surface and ground water in agricultural runoff and leachate to soils extremely fertilized with biosolids or manures.[11,12,13] The XANES spectroscopy was used to identify the dominant phosphorus species in the biosolids that will control phosphorus solubility. Based on the

XANES spectra, phytic acid was a main component of lime stabilized biosolids. Phytic acid ($C_6H_{18}O_{24}P_6$) accounted for 8 – 15% of total P in the lime stabilized biosolids. The Fe-treated or digested biosolids, however, had phytic acid as its minor component. The study concluded that the presence of phytate in the biosolids might be as a result of biosolids being generated from residential or industrial wastewater treatment.[10]

Hettiarachchi et al.[14] investigated metal binding mechanisms in biosolids using µ-XANES (micro XANES) and µ-XRF (micro X-ray fluorescence). Both µ-XANES and µ-XRF was used to identify Fe and Mn phases and their association with two biosolid samples (lime composted and Nu-Earth). The µ-XANES and µ-XRF were used to also determine the elements distribution and speciation in the biosolid samples. Results obtained for the biosolid samples before and after treatment (elimination of organic carbon) with elemental mapping of the XRF images showed spatial correlations, which suggested strong correlations between Fe and Cd, Pb, Cr, or Zn ($r^2 = 0.65 – 0.92$) before and after elimination of most of the organic carbon in the biosolids.[14] Strong correlation was observed for Fe and Cu in the biosolid samples before the removal of organic carbon. The weak correlation between Fe and Cu after the organic carbon removal was due to the Cu associated with the organic carbon coatings that may have been present in the Fe compounds.[14] With exceptions to Fe and Cr, the spatial correlations of other metals with Mn improved after removal of organic carbon suggesting that the treatment (to remove organic carbon) changed more than that. In addition, the Fe µ-XANES spectra of the biosolid samples showed that every point of the biosolids had different mixtures of (Fe^{2+}

and Fe^{3+}) species and no two points were alike.[14] From the results obtained from this study, it was concluded that, the variation in Fe species in the biosolid samples suggests the heterogeneity and complexity of biosolids.[14]

2.2.1.3 Inductively coupled plasma-optical emission spectroscopy (ICP-OES)

ICP-OES is an analytical technique used to determine the elemental composition of samples. Biosolids from seven wastewater treatment plants in Swaziland were analyzed for a range of physical-chemical properties such as organic matter, nutrients, cation exchange capacity, pH and trace elements.[15] The results showed that the organic matter and nutrient contents of biosolid samples were found in high concentrations.[15] The organic matter of the biosolid samples was in the range 20 – 60%. The high cation exchange capacity (71 – 615 meq/100 gm) recorded for the biosolid samples is expected to increase the cation exchange capacity of soils especially sandy and loamy soils that have poor cation binding. The pH of the biosolid samples was in the range 5.90 – 7.00. The heavy metal concentrations for Cr (317 – 1396 mg/kg), Pb (12 – 96 mg/kg), Zn (478 – 2311 mg/kg) and Ni (0 – 327 mg/kg) in the biosolid samples analyzed with ICP-OES were below the regulatory limits in Swaziland and other countries such as USA, South Africa, China and the European Union.[15] From the results obtained from the study, it was concluded that all the biosolid samples showed high concentrations of organic matter, nutrients and trace elements necessary for plant growth despite undergoing different wastewater treatment.[15]

Dede at al.[16] analyzed the metal content of biosolids used for soil amendment in a kiwi fruit farm with ICP-OES. The analysis of metal concentrations, Cu (19 ppm), Zn (1435 ppm), Cr (243 ppm), Ni (79 ppm), Pb (34 ppm) and Cd (3 ppm) were found below the USEPA limit.[16]

2.2.1.4 Transmission electron microscopy (TEM)

Transmission electron microscopy is a microscopy technique that is used to generate an image by passing beam of electrons through a specimen. TEM is an important tool for analysis of nanoscience in biological and materials fields.

Yang et al.[17] investigated metal and nanoparticle occurrence in biosolid-amended soils using transmission electron microscopy (TEM) coupled with energy dispersive X-ray spectroscopy (EDX) analysis. The major objective of the study was to show the possibility of nanomaterials used in the society entering the wastewater treatment system and be deposited in biosolids. Application of the biosolids to agricultural field can result in the accumulation of nanomaterial into the soil over time. Transmission electron microscopy (TEM) coupled with energy dispersive X-ray spectroscopy (EDX) was used to characterize and determine the chemical composition of nanoparticles in biosolid amended soils.[17] Biosolid amended soil samples were collected from two biosolid land application sites in Texas. From the results obtained, Ti-containing particles were identified in the biosolid amended soils. The Ti-containing particles had a diameter of 50 nm. The EDX spectrum showed an atomic ratio of 5.5 for O to Ti. The EDX spectrum also showed the presence of elements such as Al, Fe, Ca and K in the biosolid amended soil samples.[17]

Bojeong et al.[18] analyzed biosolids product from the USEPA TNSSS (Targeted National Sewage Sludge Survey) to determine nano-, and larger TiO_2 present in the biosolid samples. Titanium dioxide (TiO_2) is the most widely used engineered nanoparticle. Land application of biosolids is a major entry route for TiO_2. In this study, transmission electron microscopy and scanning electron microscopy was used to determine the nature of TiO_2 nanoparticles in the biosolid samples. Results obtained from the study showed that TiO_2 particle size was between 40 nm - 300 nm. In addition, the TiO_2 nanoparticle was crystalline in structure with a faceted shape.[18]

2.2.1.5 Fourier transform-infrared spectroscopy (FT-IR) studies of biosolids

Fourier-transform infrared spectroscopy (FT-IR) is an analytical technique that provides an infrared spectrum for the emission and absorption of any state of matter (solid, liquid and gas).

Zhou et al.[19] characterized dissolved organic matter derived from biosolids and composted biosolids using FT-IR spectroscopy. Dissolved organic matter is a major factor that affects the availability of heavy metals in biosolid amended soils. The hydrophilic and hydrophobic acid fractions of the dissolved organic matter in the biosolid and composted biosolid were 78% and 73% of total dissolved organic matter respectively.[19] Similar IR spectra obtained for both the biosolid and the compost biosolid suggested that both samples had similar functional groups.[19] The hydrophilic acid fraction for both samples showed a broad band near 3400 cm^{-1} (-OH stretching of the carboxyl groups or phenolic groups). Both samples also had an absorption near 1630 cm^{-1} (C=O or C=C stretching vibration). An absorption peak at 1462 cm^{-1} was observed for both samples (O-H deformation vibration). The combination of the absorptions at 3400 cm^{-1}, 1630 cm^{-1} and 1462 cm^{-1} suggest the present of carboxyl group and polyhydroxyl phenol in both samples. The IR spectra established that the hydrophilic acid contained larger amount of polyhydroxyl phenols and carboxyl group compared to hydrophilic base because of a stronger absorption of C-O stretching vibration and a smaller number of H-bonded C-C in hydrophilic acid.

Comparison between the IR spectra showed that more carboxyl and polyhydroxyl phenols were present in the hydrophilic acid, more C-N group in hydrophilic base (1125 and 1200 cm^{-1} peaks from C-N of amino acids, amino sugars, amines and pyridine) and more C-H and C=O in the hydrophilic neutral (a sharp peak at 1010 – 1085 cm^{-1} for C-O from carbohydrates and polysaccharides and a band at 836 cm^{-1} and 722 cm^{-1} for C-H of pyridine).[20] On the other hand, the IR spectra of hydrophobic acid and base was different from that of the hydrophilic acid and base. In the hydrophobic acid of both biosolid and compost biosolid, a strong band at 1636 cm^{-1} (C=C stretching from aromatic ring) and (C=O of carboxylate) with bands at 843 cm^{-1} and 715 cm^{-1} (C-H deformation of aromatic ring), bands at 3400 – 3500 cm^{-1} (O-H stretching of hydroxyl) and 1462 cm^{-1} (O-H deformation). The IR spectra of the hydrophobic acid for both samples showed that larger amounts of aromatic acids or phenols are present. The hydrophobic base had fewer peaks in both biosolids and biosolid compost dissolved organic matter. A broad band around 3500 cm^{-1} (O-H stretching of hydroxyl) and band at 1642 cm^{-1} (C=O stretching of carboxylate) were observed. The study concluded that since the hydrophilic fraction of the biosolid was greater than that of the composted biosolid, land application of biosolid might have a greater possibility in lowering the trace metal adsorption capacity of soils compared to the composted biosolid.[19]

Ghezzi et al.[21] characterized environmental nano- and macrocolloid particles extracted from biosolids using Fourier-transform infrared spectroscopy. The following peaks were observed in the IR spectra: 3700 – 3000 cm^{-1} (attributed to the presence of O-H stretching vibrations), 950 – 650 cm^{-1} (O-H bending vibrations), 900 – 1200 cm^{-1} (Si-O stretching), a broad peak at 1404 – 1425 cm^{-1} (carboxyl group), 1634 cm^{-1} (might be a combination of 3 features (C=O stretching of amide functional groups, aromatic C=C stretching and asymmetric COO^{-} stretching)) and a shoulder peak at 1720 cm^{-1} (C=O stretch of COOH groups).[21]

2.2.1.6 Raman spectroscopy

Tatiane et al.[22] used Raman spectroscopy to characterize biosolids-derived hydrochar (sewchar). Sewchar are hydrothermally converted biosolids. From the Raman spectra, the biosolids and sewchar showed D (1320 – 1350 cm^{-1}), and G (1540 – 1590 cm^{-1}) bands. These results indicate the presence of aromatic hydrocarbon and graphitic carbon structures in the biosolids.[23,24.25]

2.2.1.7 Thermogravimetric analysis (TGA)

William et al.[26] performed kinetic analysis on dried biosolid sample using a thermographic analyzer 550. The heating temperature range was from 373.15 K to 1273.15 K at 4 heating rates (5, 10, 15, 20 K/min).[26] From the TGA and DTGA data, five major reactions were predicted to occur with peaks near 473 K, 547 K, 596 K, 738 K and 840 K. The peaks were attributed to reactions of low stability organic compounds, hemicellulose,[27,28] cellulose, lignin – plastics and inorganic compounds respectively.[29]

Elsa et al.[30] carried out thermogravimetric analysis on biosolids to better understand the thermal decomposition of the biosolids. The nitrogen atmosphere range at which the thermal analysis was carried out was 25 °C to 1000 °C with a heating rate of 10 °C/min. From the TGA result, two major peaks were observed with maximum mass loss rates around 330 °C and 420 °C. The first peak was attributed to water and carbon dioxide release while the second peak was indicative of carbonization involving C-H stretching, methane and ammonia release.[30]

Onchoke et al.[31] carried out thermogravimetric analysis on biosolid samples. In the study, the biosolids were decomposed at 10 °C/min and 20 °C/min from 34 °C to 1000 °C. Five decomposition stages in the range 34 – 175 °C, 175 – 216 °C, 326 – 385 °C, 388 – 521 °C and 522 – 800 °C were observed. The range at 35 – 100 °C and 85 –

120 °C were attributed to moisture loss. The stage at 210 – 310 °C was attributed to the breakdown of small volatile compounds with a weak hydroxyl bond. The final stages at 388 – 521 °C and 522 – 805 °C were associated with inorganic species SiO_2, CaO, MgO, Fe_2O_3 and Al_2O_3 in biosolid samples.[31]

2.2.1.8 Ion Chromatography (IC)

Lomonte et al.[32] used ion chromatography to determine the concentration of soluble anions (F^-, Cl^-, NO_2^-, Br^-, NO_3^-, $H_2PO^-_4$, SO_4^{2-}) in biosolid water extracts. From the study, it was observed that Br^- was found below detection limit. The concentrations of anions were present in the biosolids water extract in the order SO_4^{2-} (1119 ± 21 mg/kg) > NO_3^- (456 ± 5 mg/kg) > PO_4^{3-} (181 ± 13 mg/kg) > Cl^- (111 ± 2 mg/kg) > NO_2^- (1.9 ± 0.3 mg/kg) > F^- (1.4 ± 0.4 mg/kg).[32]

Using ion chromatography, Onchoke et al.[31] determined the anion concentrations of biosolid samples. The observed order of concentration was PO_4^{3-} (22.60 ± 1.55 mg/L) > F^- (1.55 ± 0.09 mg/L) ≈ Cl^- (1.52 ± 0.02 mg/L) > SO_4^{2-} (1.32 ± 0.07 mg/L) > NO_3^- (1.31 ± 0.03 mg/L) > Br^- (1.22 ± 0.01 mg/L) > NO_2^- (0.32 ± 0.01 mg/L. All anion concentrations in the biosolid samples were found below the USEPA guideline limit with the exception to PO_4^{3-} .[31]

In the present research, inductively coupled plasma optical emission spectroscopy (ICP-OES) was employed to quantitate elements (Ag, Al, As, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Mo, Ni, P, Pb, S, Se, Zn, V, Na) in the biosolid samples from

NWWTP, LWWTP, and NCF (Angelina-Neches Compost Facility). The morphology, particle size and crystalline nature of the biosolid samples were determined using scanning electron microscopy and X-ray diffraction (XRD). Information on the loss of mass (or decomposition) of biosolid samples was also provided using thermogravimetric analysis. Although some analysis has been reported for NWWTP and LWWTP,[31] this study was done to provide supporting data that serves as a basis for comparison with other reported studies. On the other hand, no reports have been presented on the metal concentration, morphology and crystalline nature of biosolids obtained from Angelina-Neches Compost Facility (NCF).

2.3 MATERIALS AND METHODS

2.3.1 Sampling site

Biosolids samples were collected from three field sites: namely, Nacogdoches Wastewater Treatment Plant (NWWTP), Lufkin Wastewater Treatment Plant (LWWTP) and Angelina-Neches Compost Facility (NCF). Figures 2.1 and 2.2 show the sites NWWTP, LWWTP and NCF, respectively. The Nacogdoches Wastewater Treatment Plant (NWWTP) is located at 2977 Rayburn road, latitude 31°33’31.2444’’N, longitude 94°38’52.1808’’W, Nacogdoches, Texas. It has a treatment capacity of 12.88 MGD.[31] Lufkin Wastewater Treatment Plant (LWWTP) is located at 300 E. Shepherd, latitude 31°17’13.8804’’N, longitude 94°44’56.2416’’W, Lufkin, Texas. It has a treatment capacity of 11.3 MGD. The Angelina-Neches Compost Facility (NCF) is located at 1805, Highway 79 W, 31°54’1.552’’N, longitude 95°24’16.451’’W, Jacksonville, Texas.

2.3.2 Collection of biosolids

The biosolid samples were collected in plastic containers, brought into the laboratory, air dried, and passed through a 2.36 mm diameter U.S.A. standard testing sieve (from A.S.T.M., Milwaukee, Wisconsin).

2.3.3 Reagents

In all cases, nano-pure water (18.2 MΩ), HNO_3 (Flinn Scientific Inc.), H_2O_2 (Sigma Aldrich), and KBr (Sigma Aldrich Chemical Co.) was used. All reagents used were of high analytical purity.

2.3.4 Instrumentation used for study

The instrumentation used in this study include Fourier-transform infrared spectroscopy (diffuse reflectance infrared-Fourier transform spectroscopy, Perkin Elmer, 100 spectrometer) with a DTGS detector, inductively coupled plasma-optical emission spectroscopy (Agilent ICAP 7400 ICP-OES, dual view, Thermoscientific), scanning electron microscopy coupled with an energy dispersive X-ray analyzer (JEOL-JSM 6100 SEM equipped with Horiba energy-dispersive X-ray (EDAX)), X-ray diffraction (Bruker AXS D8 Advance diffractometer equipped with an X-ray tube (Cu Kα radiation: λ = 1.54060 Å, 40 kV and 40 mA) using a Ni filter and a LynxEye detector). A thermogravimetric analyzer (Perkin Elmer, TGA thermogravimetric simultaneous thermal analyzer) was used.

2.3.4.1 Inductively coupled plasma-optical emission spectroscopy (ICP-OES)

ICP-OES is an analytical technique used to determine the elemental composition of samples. Figure 2.3 shows a block diagram of ICP-OES. Analytes are converted to aerosol by conducting them with a peristaltic pump through a nebulizer into a spray chamber.[33] The aerosol generated is led into an argon plasma. The end of a quartz touch in ICP-OES is used to generate plasma through a cooled induction coil where high frequency alternate current flows. Because of the current flow, an alternate magnetic field is generated which makes electrons to accelerate in a circular path. The argon atom and ionized electrons collide to form a stable plasma. The plasma is very hot, ranging from 6000 – 7000 K.[34] Atomization and ionizations of the analyte occurs in the torch desolvation. The electrons become highly excited due to the thermal energy taken up. As the electrons drop to the lowest energy level, energy is liberated as photons. Each element has different emission spectrum, which is measured by a spectrometer. The emission intensity on the wavelength is measured and the calibration is calculated into concentration.[33]

2.3.4.2 Fourier-transform infrared spectroscopy (FT-IR)

Fourier-transform infrared spectroscopy[35] is an analytical technique that provides an infrared spectrum for the emission and absorption of any state of matter (solid, liquid and gas). Figure 2.4 shows a schematic diagram of Fourier-transform infrared spectroscopy. FT-IR operates by exposing the analyte to different wavelengths. It collects high-spectral resolution data over a wide spectral range. The wavelengths absorbed by the analytes is measured by FT-IR, the computer processes the data and produces a meaningful absorbance spectrum.[35]

The biosolids were analyzed using diffuse reflectance infrared-Fourier spectroscopy (DRIFTS) with Perkin Elmer Spectrum 100 spectrometer in the spectral region, 230 - 4000 cm^{-1} with a resolution of 4 cm^{-1}.

2.3.4.3 Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) is used to determine the morphology, structure and elemental composition of samples. Figure 2.5 shows the schematic diagram of a scanning electron microsope. SEM operates by focusing beams of electron over a sample to generate an image. In SEM, electrons are first generated with an electron gun.[36] The electron beam generated from the electron gun are confined. A condenser lens reduces the electron beam as they pass through it. The diameter of the beam of electrons can be adjusted with an aperture. After passing through the aperture, the objective lens focuses the electron beam on the sample. The electron beam moves to the next chamber, sample chamber. In the sample chamber, the sample is held under high vacuum to get rid of hindrances from undesirable particles. The sample to be analyzed needs to be conductive to avoid charging in order generate an image of greater quality. The detector is the last part of the SEM. The detector observes or identifies different signals produced when the electron beam scanning the sample strikes it.[36]

The procedure for SEM analysis is adopted from Onchoke et a.l[31] report. The morphology and elemental composition of the biosolid samples were determined using a JEOL-JSM-6100 scanning microscope equipped with Horiba energy-dispersive X-ray spectroscopy (EDAX). The electron microscope was operated at an accelerated voltage of 20 KV and filament current of 200A. An Automatic Platinum Sputter Coater System (Quorum Q150RS was used to coat biosolid samples to lower electron-charging.

2.3.4.4 Powder X-ray diffraction (XRD) spectroscopy

The powder X-ray diffraction (XRD) spectroscopy determines the crystalline phases, morphology and elemental composition of a sample by using, neutron, X-ray or electron diffraction on powder.[37] Figure 2.6 shows the schematic diagram of an X-ray diffractrometer. X-ray diffractometers consist of three major components[37] a sample holder, an X-ray tube and an X-ray detector. Electrons are generated by heating a filament in the X-ray tube. Electrons are directed towards the sample material. Voltage is applied to the sample material. When the electrons acquire enough energy to remove electrons from the inner shell of the sample material, X-ray spectra are generated. The detector measures the intensity of the reflected X-ray. The X-ray signal is recorded and transferred into a count rate by the detector.[37]

The procedure for XRD analysis is adopted from Onchoke et al. report.[31] A Bruker AXS D8 Advance diffractometer equipped with an X-ray tube (Cu Kα radiation: λ = 1.54060 Å, 40 kV and 40 mA) was used with a Ni filter and one-dimensional LynxEye detector.

2.3.4.5 Thermogravimetric analysis (TGA)

Thermogravimetric analysis is a thermal analysis that measures the amount of a sample over time at varying temperature. Figure 2.7 shows a schematic diagram of a thermogravimetric analysis block. In TGA, the increase or decrease of the mass of a sample is determined upon heating the sample.[38] Thermogravimetric analysis is usually performed using a thermogravimetric analyzer.

The procedure for thermogravimetric analysis is adopted from Onchoke et al report.[31] About 20.0000 mg of biosolid samples was used for analysis with a Perkin Elmer TGA thermogravimetric simultaneous thermal analyzer (STA 6000) at 10 °C/min, 20 °C/min and 30°C/min heating rates in a nitrogen atmosphere ranging from 30 °C - 1000 °C.

2.3.5 Soil digestion

The USEPA method 3050B[39] was used for digestion of the biosolids samples and perlite (Nacogdoches wastewater treatment sludge (NWWS), Lufkin wastewater sludge (LWWS), Soil therapy compost (STC) and hydroponic sample (perlite) to determine their total metal content. The digestion procedure used in this study was adopted from Onchoke et al.[31] Approximately 0.5000 g of the samples (STC, PER, NWWS, LWWS and CRM (Certified Reference Material) were weighed in quadruplicates (STC, PER, CRM) and triplicates (NWWS, LWWS) into digi-tubes. Digestion was done using 1:1 nitric acid (70% v/v ACS reagent, Flinn Scientific Inc, Batavia, IL, USA) and hydrogen peroxide (35% w/w, Sigma Aldrich, St. Louis, MO). A digestion block (SCP Science, www.scpscience.com, Graham, NY) was used to reflux the biosolid samples. After digestion, the biosolid samples were filtered using 0.45 µm filter. Standard solutions and dilutions of the filtered biosolid samples were prepared in 18.2 MΩ nanopure water.

An Agilent ICAP 7400 inductively coupled plasma-optical emission spectrometry (ICP-OES, dual view, Thermoscientific) was used to analyze the metal content in the biosolid samples.

2.3.6 Physical-chemical Characterization of Biosolids

The physical-chemical characterization (pH, electrical conductivity) of biosolids was determined using USEPA methods (9050D, 9050A).[40,41]

About 20.0000 g of the samples (Soil therapy compost (STC), Nacogdoches wastewater sludge (NWWS), Lufkin wastewater sludge (LWWS) and Perlite (PER)) was mixed with 40 mL of 18.2 MΩ nanopure water. The USEPA method 9054D was used to determine the pH of the biosolids samples. The soil-water slurry was suspended for about 1 hour. A pH 211 microprocessor pH meter (from HANNA instruments) was used to determine the pH of the samples.

Electrical conductivity determines the amount of soluble ions in the soil. The USEPA method 9054A was used to determine the electrical conductivity of the biosolid samples. Biosolid samples were weighed and a soil-water slurry was made with 50 mL of 18.2 MΩ nanopure water. To dissolve the soluble salt in the biosolids, the soil-water suspension was shaken at 15 rpm for 60 minutes. The conductivity meter was calibrated using KCl as the reference and the conductivity of the soil-water suspension was measured using the conductivity meter.[41]

2.3.7 Quality Control and Quality Assurance

Method validation was carried out by comparing the ICP-OES results obtained for the concentrations of elements in biosolid samples with the certified values for certified reference material (CRM, EnviroMAT SS-2) from SCP Science, Clark Graham, Canada. The results from 80-120% percent agreement with the certified reference material were regarded as useful analysis. Table 2.1 shows the percent agreement between the measured values and the certified reference material.

The limit of detection was determined using the formula 3 x standard deviation / mean (n = 4). The limit of detection (ppm) for each element analyzed are given as follows: Ag/0.001868, Al/0.002812, As/0.008147, B/0.025747, Ba/0.000469, Ca/0.502776, Cd/0.000407, Co/0.000473, Cr/0.001257, Cu/0.004264, Fe/0.002599, Hg/0.002549, K/0.235606, Mg/0.008697, Mn/0.0002, Mo/0.000408, Ni/0.001508, P/0.006847, Pb/0.00666, S/0.00829, Se/0.014936, Zn/0.000322, V/0.001627, Cd/0.000391, Na/0.843562.

2.4 RESULTS

2.4.1 Physical-chemical Characterization of Biosolids

Table 2.2 shows the pH of the biosolid samples and perlite. The pH of the biosolid samples are given as: STC (6.74 ± 0.03), NWWS (5.33 ± 0.01), LWWS (5.78 ± 0.01). Plants are known to thrive well in the soil pH range of 5.50 - 7.50.[42] Since all biosolid samples and perlite, have pH values in this range, they are all suitable as soil media for plant growth.

2.4.2 Analysis of Metal Concentration in Biosolids with ICP-OES

The macro- and microelements present in the biosolids were determined using ICP-OES following protocol in 2.3.5 with USEPA 3050B. Table 2.3 shows the elemental composition of the biosolid samples and their corresponding USEPA maximum concentration limit (mg/L).

Macro elements

Figure 2.8a and 2.8b shows the concentration of macroelements present in the biosolids samples, STC, NWWS, LWWS and perlite (PER). From the figure, essential nutrients such as P and K necessary for plant growth are present in the biosolid samples. Except for Fe, LWWS and NWWS had relatively similar metal concentrations (LWWS (15163 ± 2212 ppm), NWWS (32890 ± 2695 ppm)). Perlite contained relatively low amount of the macroelements (Al (1176 ± 322 ppm), Ca (3632 ± 1035 ppm), Fe (1338 ± 248 ppm), K (9337 ± 1281 ppm), Mg (654 ± 236 ppm), P (4649 ± 790 ppm), S (2097 ± 227 ppm), Na (195 ± 1658 ppm)). The order of concentrations of macroelements in the biosolids and perlite is NWWS (Al (15591 ± 1692 ppm), Fe (32890 ± 2695 ppm), Mg (7293 ± 647 ppm), P (26102 ± 1522 ppm)) > LWWS (Al (12217 ± 1985 ppm), Fe (15163 ± 2212 ppm), Mg (4116 ± 561 ppm), P (20855 ± 2594)) > STC (Al (12424 ± 824 ppm), Fe (13352 ± 2731 ppm), Mg (1362 ± 98 ppm), P (8623 ± 426 ppm)) > PER (Al (1176 ± 322 ppm), Fe (1338 ± 248 ppm), Mg (645 ± 236 ppm), P (4649 ± 790 ppm)) , for Ca: LWWS (21552 ± 3186 ppm) > NWWS (18738 ± 1644 ppm) > STC (11760 ± 1358 ppm) > PER (3632 ±1035 ppm), and K: PER (9337 ± 1281 ppm) > NWWS (6126 ± 229 ppm) > LWWS (3328 ± 548 ppm) > STC (2207 ± 82 ppm).

Microelements

Figure 2.9a and 2.9b shows the concentration of microelements in biosolids samples, (STC, NWWS, LWWS) and hydroponic material PER (perlite). From figure 2.9, toxic elements (As, Cd, Cr, Hg, Co, Pb) were detected in concentrations < 30 ppm.

The microelements Ba: (LWWS – 319 ± 44 ppm, NWWS – 563 ± 45 ppm, STC – 308 ± 14 ppm), Cu (LWWS – 531 ± 78 ppm, NWWS – 386 ± 35 ppm, STC – 338 ± 14 ppm), Mn (LWWS – 1262 ± 192 ppm, NWWS – 1136 ± 102 ppm, STC – 794 ± 39 ppm) and Zn (LWWS – 883 ± 127 ppm, NWWS – 810 ± 71 ppm, STC – 409 ± 14 ppm) were present in higher concentrations compared to other microelements. Microelements were in the order Ba: NWWS (563 ± 45 ppm) > LWWS (319 ± 44 ppm) > STC (308 ± 14 ppm) > PER (31 ± 7.2 ppm), Cu, Mn, Zn: LWWS (Cu (531 ± 78 ppm), Mn (1262 ± 192 ppm), Zn (883 ± 127 ppm)) > NWWS (Cu (386 ± 35 ppm), Mn (1136 ± 102 ppm), Zn (810 ± 71 ppm)) > STC (Cu (338 ± 14 ppm), Mn (794 ± 39 ppm), Zn (409 ± 14 ppm)) > PER (Cu (2 ± 0.6 ppm), Mn (27 ± 6 ppm), Zn (10 ± 2 ppm)).

Microelements (As, Cd, Cr, Cu, Pb, Hg, Mo, Ni and Zn) in biosolid samples were found below the USEPA maximum concentration limits (75, 85, 3000, 4300, 840, 57, 75, 420 and 7500 ppm respectively).

2.4.3 Spectroscopic Analysis of Biosolids

2.4.3.1 FT-IR spectroscopy of biosolids

Figure 2.10 shows an FT-IR spectrum of Soil Therapy Compost (STC) in the spectral region 240 – 4000 cm^{-1}. The broad band in the range 3600 - 3200 cm^{-1} indicates the presence of an O-H group. Bands at 3000 - 2900 cm^{-1} are ascribed to sp^3 C-H stretching. The peak at 1640 cm^{-1} might indicate a carbonyl group. The peaks between 1250 - 1000 cm^{-1} indicate a C-N group. The peaks at 1375 cm^{-1}, 695 cm^{-1}, 563 cm^{-1} might be due to the presence of C-F, C-Cl and C-Br respectively.[43]

2.4.3.2 Elemental composition of biosolids with SEM/EDX

Figures 2.11a and 2.11b show the SEM micrographs obtained for both soil therapy compost (STC) and Nacogdoches wastewater treatment sludge (NWWS) respectively. The particle diameter of the biosolids was 25 – 50 µm. Biosolids are known to have particle diameters in the range 20 µm to 500 µm.[31,44]

Figures 2.12a, 2.12b and 2.12c show the elemental composition of elements in STC, NWWS and LWWS acquired with EDX. Tables 2.4, 2.5 and 2.6 show the percentage compositions of elements corresponding to the STC, NWWS and LWWS micrographs. The result for the elemental composition confirms the presence of macroelements (Al, Ca, Fe, Na, K, P, S) which were initially detected using ICP-OES. Toxic metals (Cd, Hg, As, Pb) were not detected in the biosolids with SEM-EDX because EDX does not have low detection limits. Other elements (C, O, Si) were detected with EDX. The order of concentration of elements in STC is: O (56.0 %wt/wt) > Si (24.3 %wt/wt) > Al (7.4 %wt/wt) > Ca (3.4 %wt/wt) > P (2.9 %wt/wt) > Fe (2.4 %wt/wt) > S (2 %wt/wt) > K (1.2 %wt/wt) > Mg (0.6 %wt/wt). The order of concentration of elements in NWWS is: O (47.6 %wt/wt) > C (38.5 %wt/wt) > Si (6.0 %wt/wt) > P (2.3 %wt/wt) > Fe (2.0 %wt/wt) > Al (1.4 %wt/wt) > Mg (1.3 %wt/wt) > S (0.9 %wt/wt). The order of concentration of elements in LWWS is: O (41.2 %wt/wt) > C (40.6 %wt/wt) > Si (6.8 %wt/wt) > P (3.0 %wt/wt) > Al (2.4 %wt/wt) > S (1.4 %wt/wt) ≈ Ca (1.4 %wt/wt) > Fe (1.3 %wt/wt) > Mg (0.9 %wt/wt) > Na (0.6 %wt/wt) > K (0.3 %wt/wt).

In addition, results obtained from EDX confirmed the presence of metals previously detected with ICP-OES.

2.4.3.3 X-ray powder diffraction patterns (XRD)

Figure 2.13 shows the X-ray powder diffraction pattern of Soil Therapy Compost (STC). Figure 2,14 shows the XRD pattern from Nacogdoches and Lufkin wastewater sludge. From literature study,[31] the 2θ and d-spacing values, (6.070°, 14.55), (20.58°, 4.55) and (26.40°, 3.36) can be attributed to the presence of vermiculite ($CaSO_4.2H_2O$), alunogen ($Al_2(SO_4)17H_2O$), quartz (SiO_2), respectively.

2.4.4 Thermogravimetric analysis

Figure 2.15 shows the phase changes that occur upon decomposition of the biosolid sample. The biosolid sample was heated at 10 °C/min, 20 °C/min and 30 °C/min from 30 °C to 1000 °C. From the TGA curve, decomposition stages were observed over the ranges 33 – 110 °C, 110 – 220 °C, 220 – 400 °C, 400 – 800 °C. The initial stage at 33 – 110 °C might be attributed to the loss of moisture content.[45] The second stage at 220 – 400 °C might indicate the breakdown of small volatile compounds with weak hydroxyl bonds.[50] The final stage at 400 – 800 °C might be due to inorganic compounds (SiO_2, Al_2O_3, MgO, Fe_2O_3, CaO).[47]

2.5 DISCUSSION

It is important to characterize biosolids before they are applied to agricultural fields. Characterization of biosolids helps to estimate the possible nutrients or factors that will contribute to plant growth and yield.[15] This further provides information on the suitable application rates of biosolids to agricultural fields and means of investigating the possible pollutants in the biosolids.[15] In the study, the biosolid samples were analyzed for physical-chemical properties such as pH and electrical conductivity. The pH of the biosolid samples varied between 5.33 – 6.74. pH can control the uptake of metals by plants especially those present in labile form.[15] Ronald et al.[1], and Joseph et al.[15], have reported the pH of the biosolids analyzed to be between 5.00 – 7.00. Plants are known to thrive well at pH 5.50 – 7.00.[42,48] As it is known that more plant nutrients are available at pH of around 6.[48] Since the pH values of biosolid samples are within this range, the biosolids are suitable for land application.

Analysis of biosolids with ICP-OES provided information on the metal content (macro- and microelements) present in the biosolid samples. Metal analysis also provided information on possible toxic pollutants in the biosolid samples. Notable essential macroelements (P and K) needed for plant growth were present in the biosolid samples. Microelements (Mn, Zn, Fe, Cu, Mo, Ni) essential for plant growth were also found present in the biosolids. Heavy metals As (STC-5.48 ± 0.62 ppm, LWWS-20 ± 2.79 ppm, NWWS-12 ± 0.64 ppm), Cd (STC-0.19 ± 0.07 ppm, LWWS-0.20 ± 0.03 ppm, NWWS-Below detection), Cr (STC-4.3 ± 0.18 ppm, LWWS-0.93 ± 1.67 ppm, NWWS-17 ± 1.71

ppm) and Pb (STC-17 ± 0.57 ppm, LWWS-29 ± 3.44 ppm, NWWS-27 ± 0.59 ppm) in the biosolid samples were determined below the USEPA ceiling limit (Table 2.3). This is in agreement with other studies which have found the metal concentrations in the biosolids to be lower compared to the USEPA regulatory limits.[15,49,50] The results obtained from metal analysis of the biosolids in this study suggests a reasonable recommendation for the land application of the biosolid samples. However, with the continued use, the risk of accumulation of metals in soils may be envisioned.

The FT-IR spectra obtained for the biosolids showed absorption peaks indicative of functional groups (-OH, C-H, C=O and C-N).[43] Zhou et al.[19] and Onchoke et al.[31] studies showed similar peaks indicative of these functional groups. In addition, peaks observed on the IR-spectra are indicative of the presence of groups such as C-F, C-Cl and C-Br.[43] The presence of organic compounds such as -COOH, -OH in the biosolids can result in complexation of heavy metals in the biosolids. The complexation of heavy metals can lead to the solubility and mobility of the heavy metals in the biosolids and thus result in the uptake of such metals by plants.[51,52]

The SEM/EDX was used in this study to determine the morphology, structure and metal concentration of the biosolid samples. The SEM micrograph obtained showed a particle diameter between 25 µm – 120 µm.[31,44] This is in correlation with what has been observed in other studies.[44,53] The elemental composition determined with EDX further confirms the presence of elements such as Al, Ca, K, P, Cu. Zn, Cd which were detected using ICP-OES.

The XRD provided information on the crystalline phases of the biosolid. The presence of vermiculite ($CaSO_4 \cdot 2H_2O$), alunogen ($Al_2(SO_4) \cdot 17H_2O$) and quartz (SiO_2) with d-spacing 14.55, 4.55 and 3.36, respectively, in the biosolid sample is also evident in other studies.[54,55] Ling et al.[54] showed a peak with similar 2ϴ and d spacing for quartz at (26.641°, 3.346).[54] Also, the peak observed for vermiculite and alunogen had similar 2ϴ and d spacing with the study done from Onchoke's lab (6.217°, 14.21) and (20.76°, 4.28) respectively.[31]

From the TGA curves, it can be deduced that small volatile organic compounds with weak hydroxyl bonds[46] and inorganic species (SiO_2, Al_2O_3, MgO, Fe_2O_3, CaO)[47] are present in the biosolid samples. The presence of the nanoparticles in the biosolids can affect the release of essential nutrient for plant growth from the organic matter fraction and also disrupt the plant-microbe relationship that enhance soil fertility.[56]

2.6 CONCLUSIONS

Spectroscopic methods (ICP-OES, FT-IR, SEM, EDX, XRD, TGA) were used to determine the metal content in the biosolids samples and provide information on the functional groups, morphology and crystalline nature of biosolids. From the metal content analysis, heavy metals concentrations (As, Cd, Cr, Pb, Hg) were found below the USEPA regulatory limit. The FT-IR data reports the presence of groups (-OH, C=O, C-H) that can complex with heavy metals in the biosolids and enhance the uptake of such metals by plants. From the SEM micrograph, it was concluded that the particle diameter is between 25 – 120 µm in the nanocrystalline regime. The EDX data supports the presence of elements previously detected in the biosolids using ICP-OES. The XRD pattern shows the presence of compounds (vermiculite, alunogen, quartz) in the biosolids. The presence of compounds containing elements (Ca, Al, S) shows the presence of elements that have been found in the biosolids with ICP-OES and SEM/EDX analysis.

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TABLES AND FIGURES

Table 2.1: ICP-OES Analysis of Certified Reference Material (CRM), (SCP Science, SS-2, EnvironMAT) the standard deviations, (mean ± concentration) and the approximate percent agreement. (Bd = Below detection)

Element	Number of reference samples (n)	CRM SS-2 (Reference) (Mean), ppm	Measured (Mean ± SD), ppm	Approximate percent agreement
Al	4	13265 ± 1151	15102 ± 275	87.8
As	4	75 ± 10	94 ± 4	80.2
Ba	4	215 ± 13	244 ± 5	87.9
Ca	4	112861 ± 4872	121381 ± 2658	92.9
Cd (228.80)	4	2	4.0 ± 0.1	50.3
Co	4	12 ± 1	16.0 ± 0.2	76.6
Cr	4	34 ± 4	40 ± 0.8	86.1
Cu	4	191 ± 9	213 ± 6	89.8
Fe	4	21046 ± 1449	22524 ± 174	93.4
K	4	3418 ± 352	5774 ± 142	59.2
Mg	4	11065 ± 606	12208 ± 166	90.6
Mn	4	457 ± 24	521 ± 8	87.6
Mo	4	0.09 ± 0.01	2.0 ± 0.2	58.8
Ni	4	54 ± 4	69 ± 1	78.7
P	4	734 – 770	1022 ± 36	73.5
Pb	4	126 ± 10	132 ± 1	95.8
S	4	2193 ± n/a	5628 ± 51	38.9
Zn	4	467 ± 23	577 ± 5	80.9
V	4	34 ± 3	41 ± 1	82.8
Cd (226.50)	4	2	4.00 ± 0.03	54.3
Na	4	456 – 660	1078 ± 49	51.8

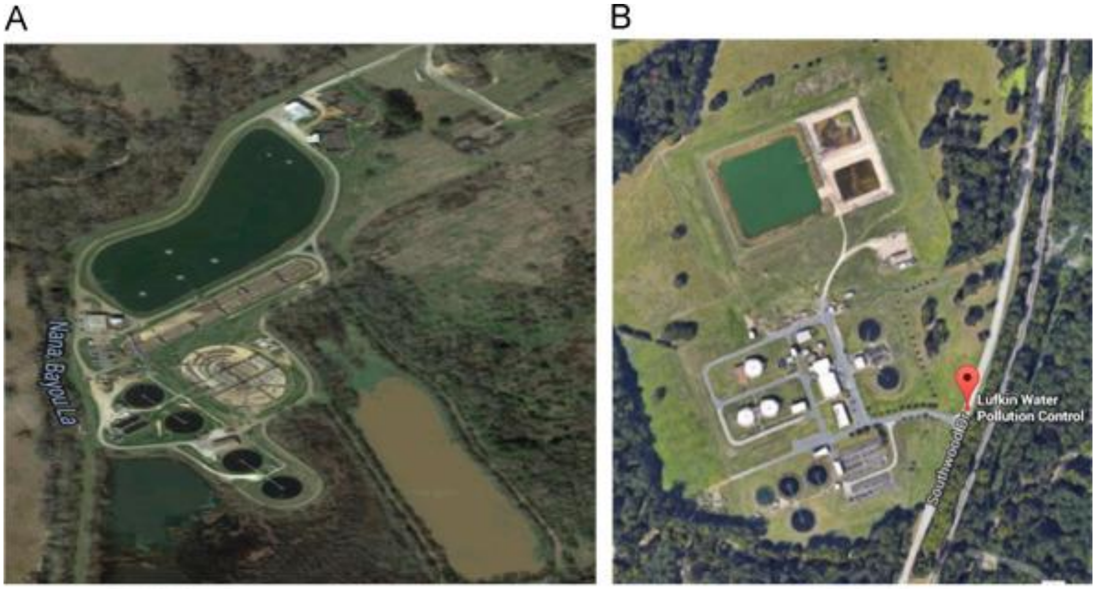
Table 2.2: pH of biosolid samples and perlite

SAMPLE	**pH**
STC	6.74 ± 0.03
NWWS	5.33 ± 0.01
LWWS	5.78 ± 0.01
PER	5.77 ± 0.03

STC = Soil Therapy Compost (from Angelina-Neches Compost Facility), NWWS = Nacogdoches wastewater sludge, LWWS = Lufkin wastewater sludge, PER = Perlite.

Table 2.3: Mean ± standard deviation of metals in the biosolid samples analyzed with ICP-OES and their corresponding USEPA limit. (n = 4 for STC (Soil Therapy Compost), n = 3 for NWWS (Nacogdoches wastewater sludge), n = 3 for LWWS (Lufkin wastewater sludge), n = 4 for PER (Perlite), Bd = Below detection)

Element	Samples				
	STC (ppm) (n = 4)	PER (ppm) (n = 4)	LWWS (ppm) (n = 3)	NWWS (ppm) (n = 3)	USEPA Ceiling Limit (ppm)
Ag	1.1 ± 0.3	Bd	Bd	0.6 ± 0.4	-
Al	12424 ± 825	1176 ± 322	12217 ± 1985	15591 ± 1692	-
As	6 ± 1	3 ± 1	20 ± 3	12 ± 1	75
B	Bd	Bd	8 ± 0	107 ± 21	-
Ba	308 ± 14	31 ± 7	319 ± 44	563 ± 45	-
Ca	11760 ± 1358	3632 ± 1035	21552 ± 3186	18738 ± 1644	-
Cd	0.2 ± 0.1	0.05 ± 0.03	0.20 ± 0.03	Bd	85
Co	4.3 ± 0.2	0.9 ± 0.4	11 ± 2	17 ± 2	3000
Cr	14.4 ± 0.4	5 ± 2	25 ± 4	26 ± 4	-
Cu	338 ± 14	2 ± 1	531 ± 78	386 ± 35	4300
Fe	13352 ± 2731	1338 ± 248	15163 ± 2212	32890 ± 2695	-
K	2207 ± 82	9337 ± 1282	3328 ± 548	6126 ± 229	-
Mg	1362 ± 98	654 ± 236	4116 ± 561	7293 ± 647	-
Mn	794 ± 39	27 ± 6	1263 ± 192	1136 ± 102	-
Mo	4 ± 1	Bd	8 ± 1	7 ± 1	75
Ni	17.5 ± 0.4	9.1 ± 1.3	33.5 ± 1.2	44 ± 4	420
P	8623 ± 426	4649 ± 790	20855 ± 2594	26102 ± 1522	-
Pb	17 ± 1	Bd	29 ± 3	27 ± 1	840
S	2838 ± 89	2097 ± 227	12116 ± 1784	8365 ± 331	-
Zn	409 ± 14	10 ± 2	883 ± 127	810 ± 71	7500
V	12 ± 2	5 ± 1	8 ± 1	33 ± 3	-
Na	668 ± 92	2630 ± 0	524 ± 696	Bd	-



NWWTP **LWWTP**

Figure 2.1: Aerial view of Nacogdoches Wastewater Treatment Plant (NWWTP) and Lufkin Wastewater Treatment Plants (LWWTP). Figure adopted from Ref 57.

Figure 2.2: Aerial view of Angelina-Neches Compost Facility.

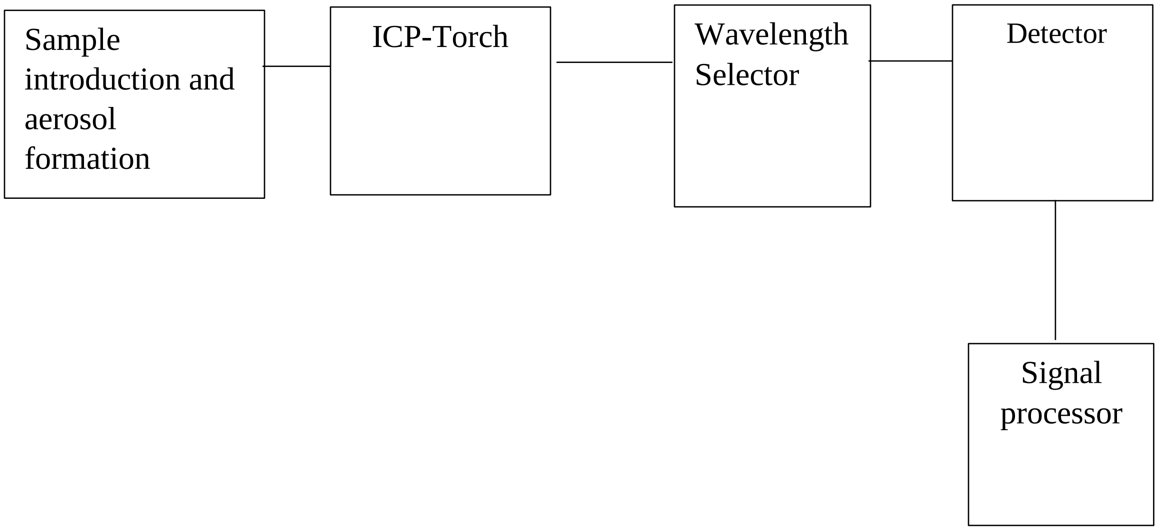


Figure 2.3: Block diagram of inductively coupled plasma-optical emission spectroscopy.[58]

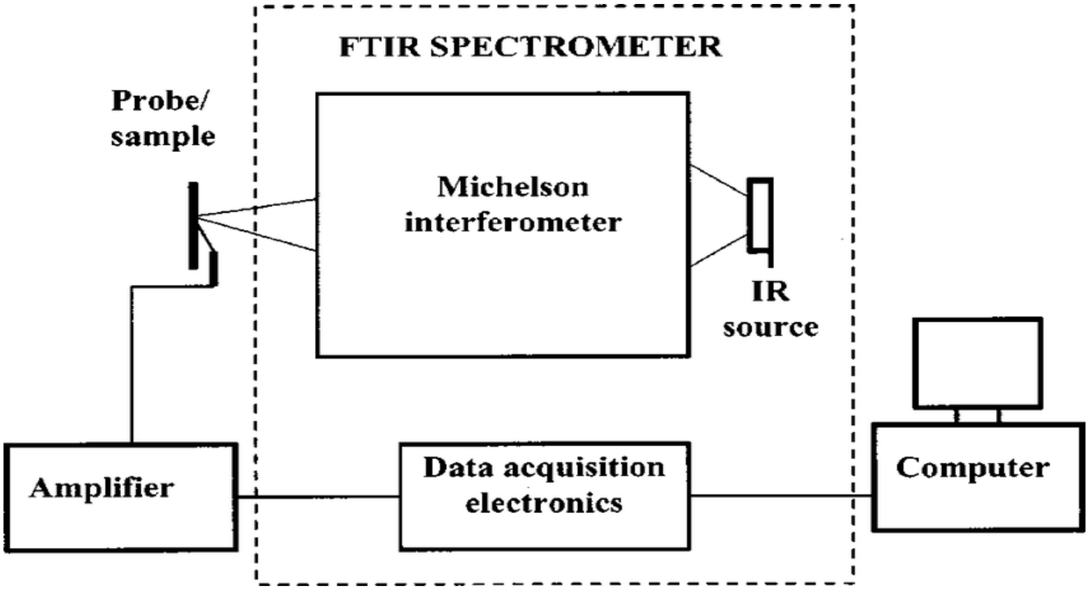


Figure 2.4: Schematic diagram of Fourier-transform infrared spectroscopy (FTIR)[59]

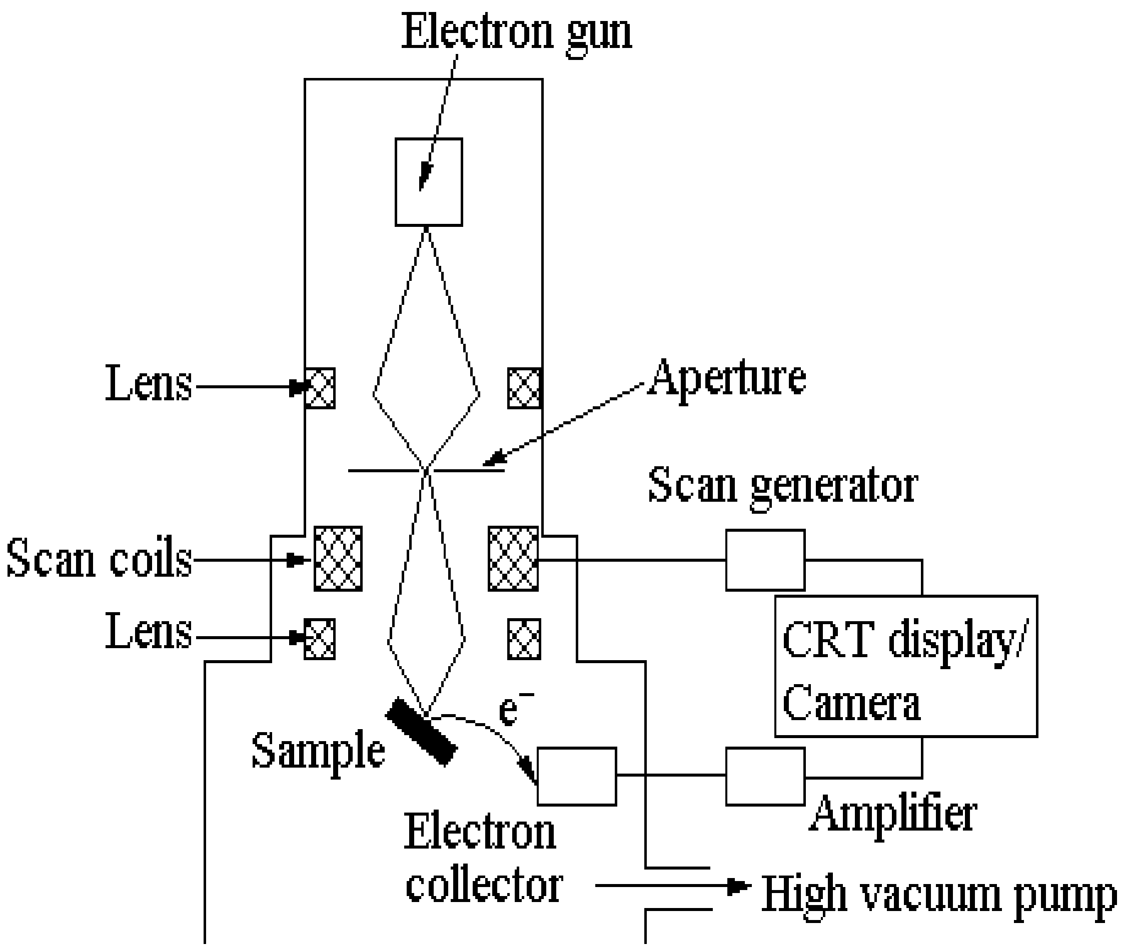


Figure 2.5: Schematic diagram of scanning electron microscope (SEM).[60]

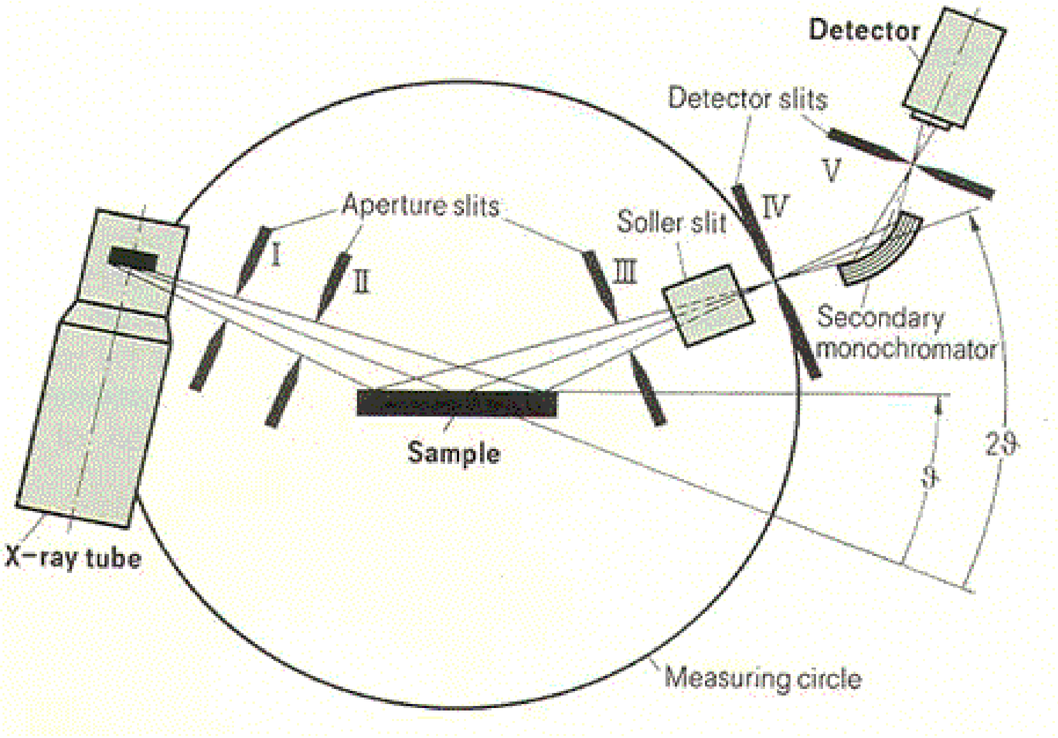


Figure 2.6: Schematic diagram of powder X-ray diffractometer.[61]

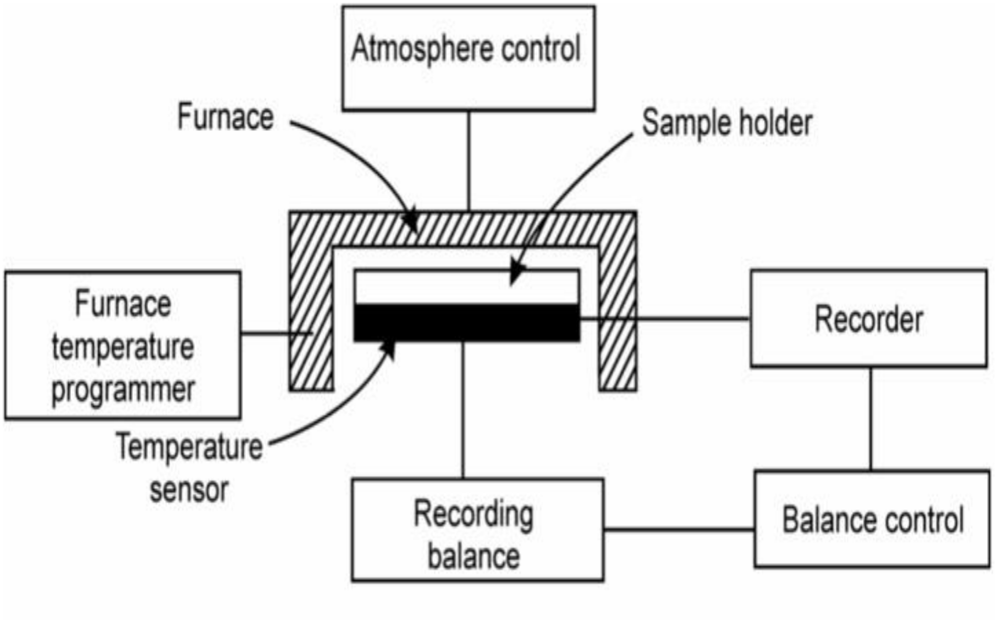


Figure 2.7: Schematic diagram of a thermogravimetric analysis block.[62]

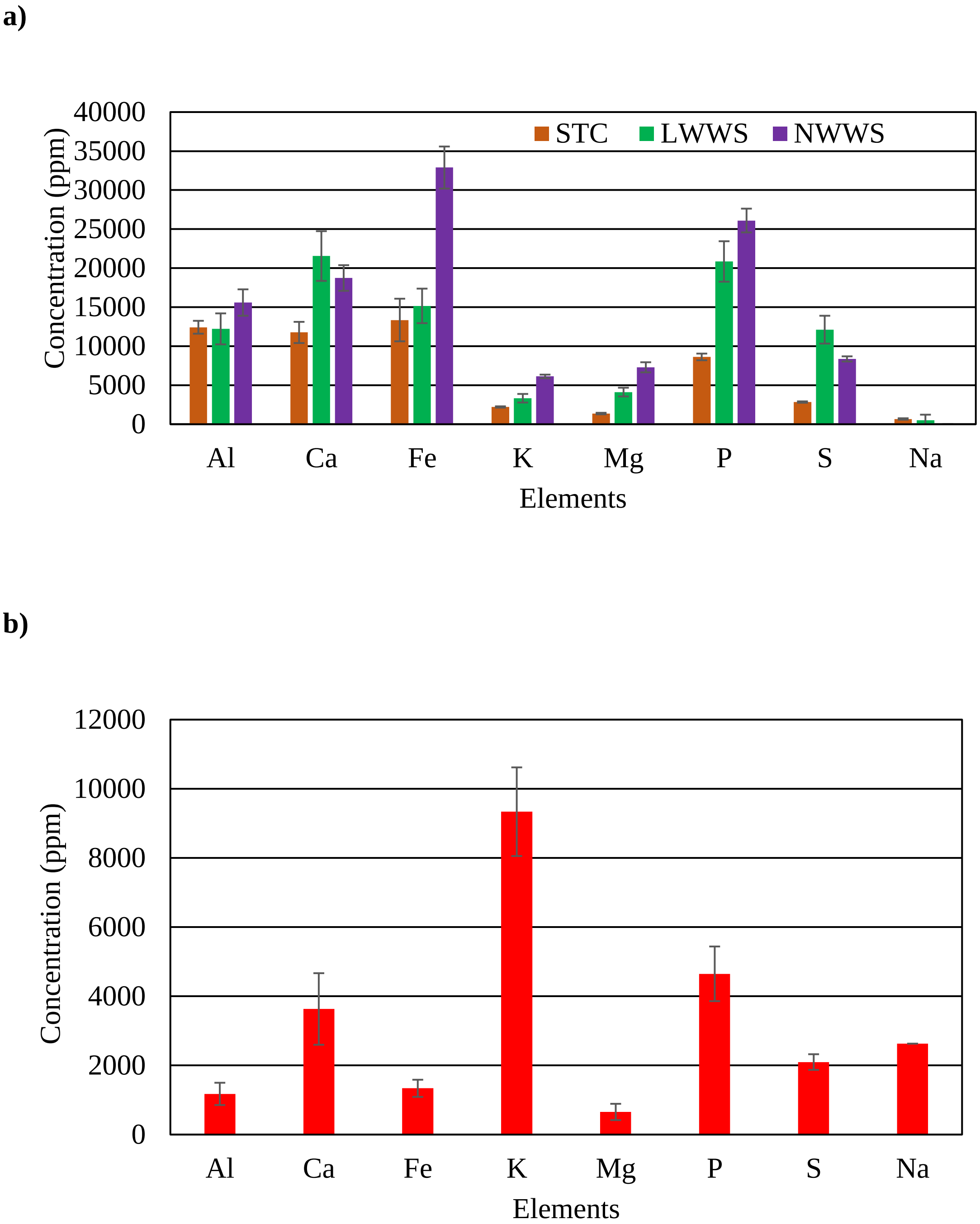
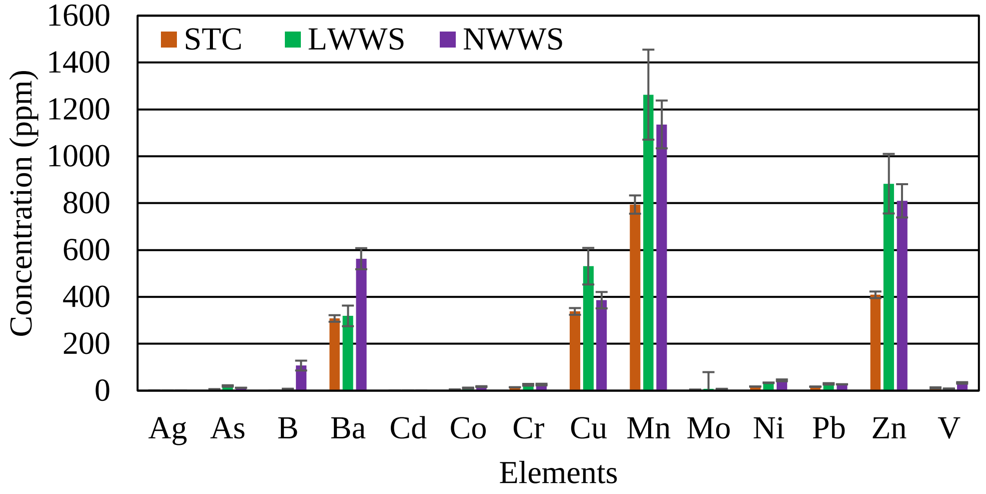


Figure 2.8: Concentrations (ppm) of macroelements in STC (n = 4), LWWS, NWWS (a) (n =3) and perlite (b) (n = 4). Error bars depict standard deviations.

a)



b)

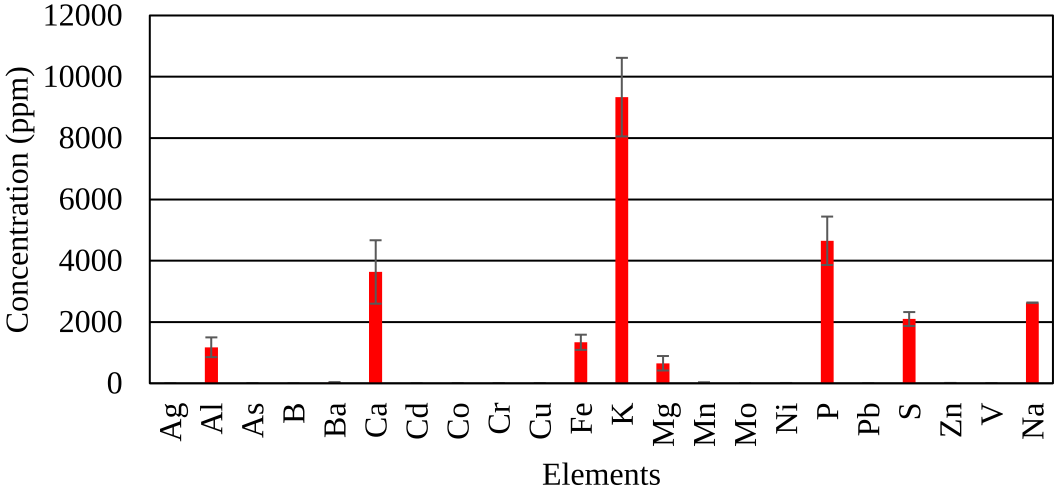


Figure 2.9: Concentrations (ppm) of microelements in STC (n = 4), LWWS, NWWS (a) (n = 3) and perlite (b) (n = 4). Error bars depict sstandard deviations.

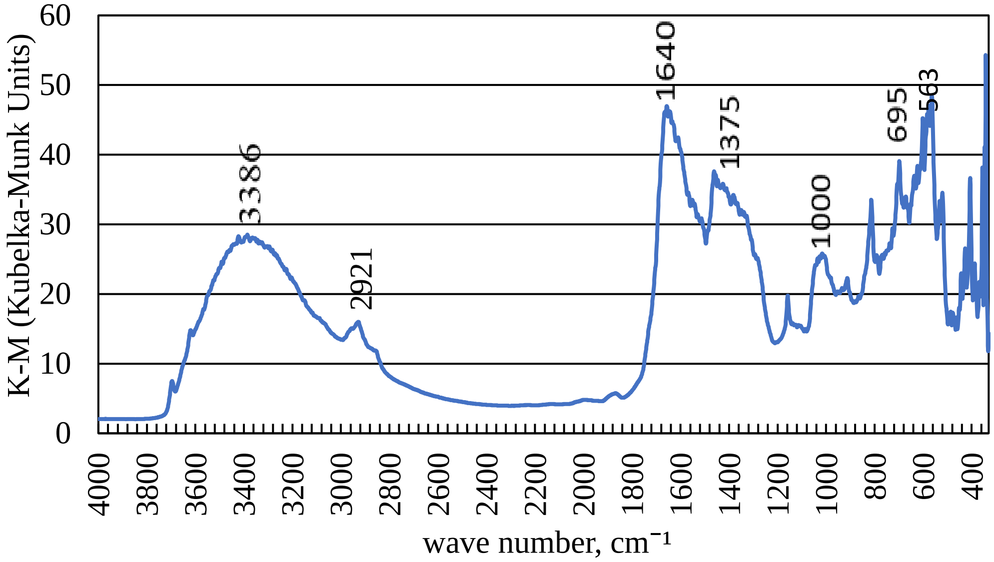
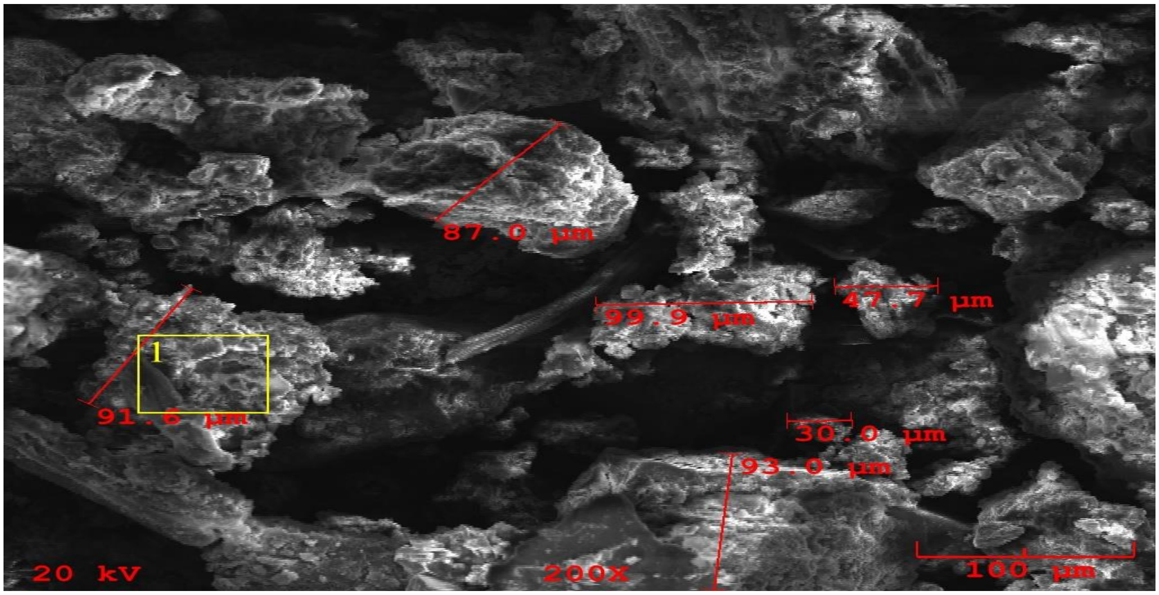
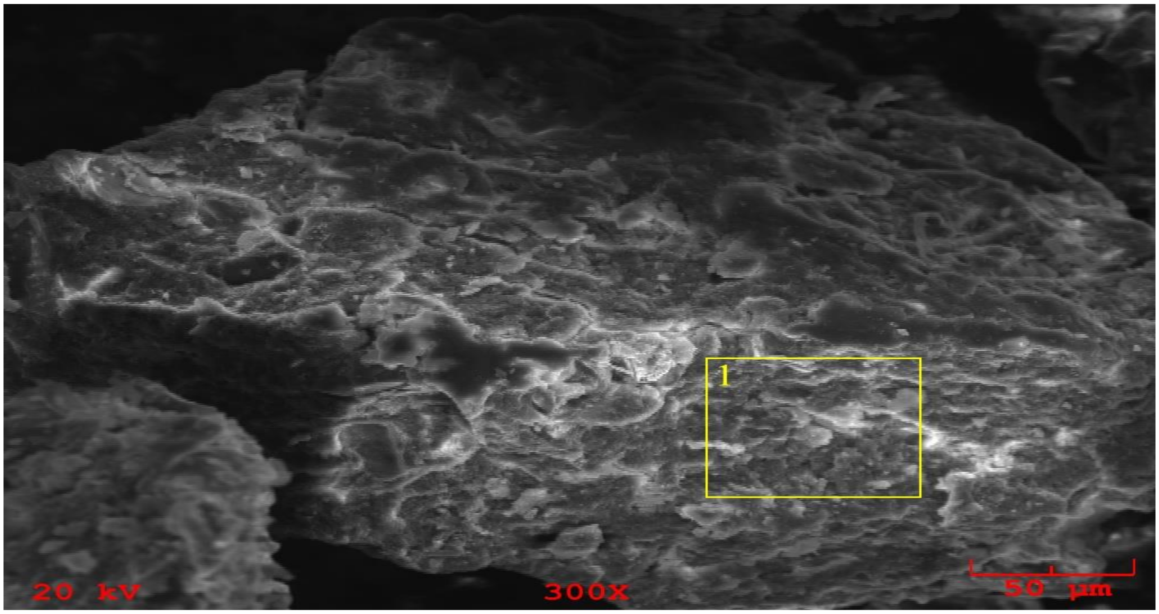


Figure 2.10: FT-IR spectrum for soil therapy compost (STC) acquired at 4 cm^{-1} resolution.

a)


87.0 µm
99.9 µm
47.7 µm
1
91.6 µm
30.0 µm
93.0 µm
20 kV
200X
100 µm

b)


1
20 kV
300X
50 µm

c)

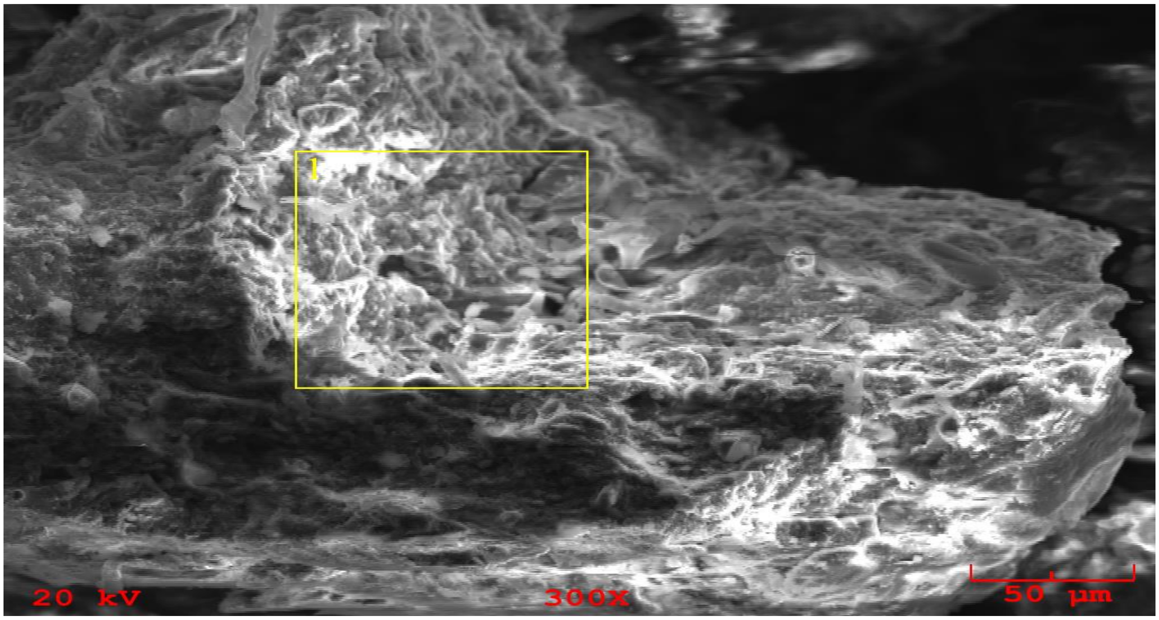
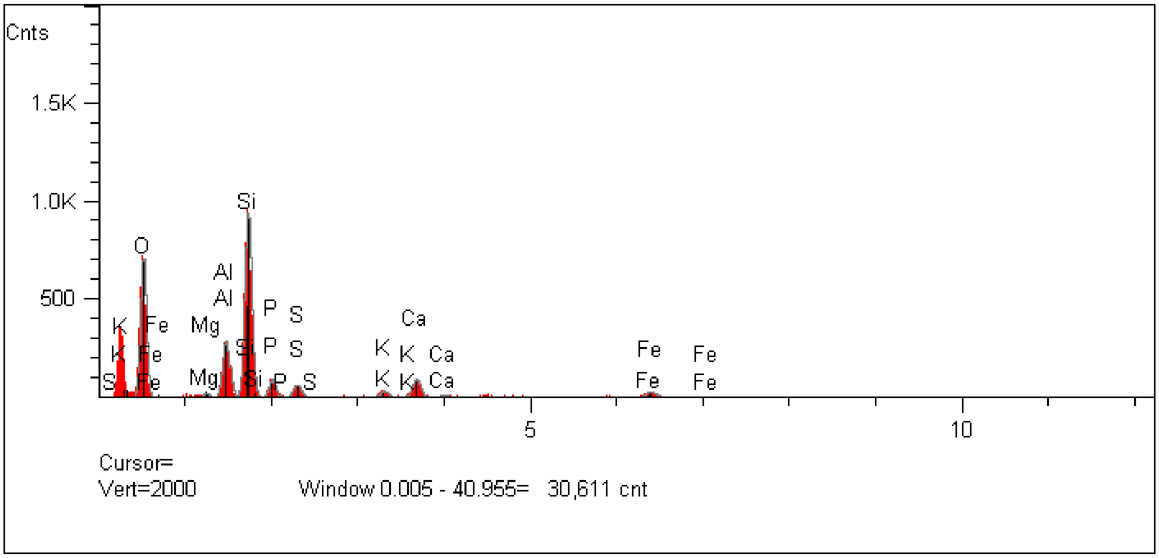


Figure 2.11: SEM micrograph for STC (a), NWWS (b), and LWWS (c) at a X300 magnification, an accelerated voltage of 20 KV, and filament current of 200 A. The red boxes in the SEM micrographs indicates the elemental composition taken at the region.

a)


Cnts
1.5K
1.0K
500
Si
O
Al
Al
K
Fe
Mg
P
S
K
Fe
Si
P
S
S
Fe
Mg
Si
P
S
K
K
Ca
K
Ca
K
Ca
Fe
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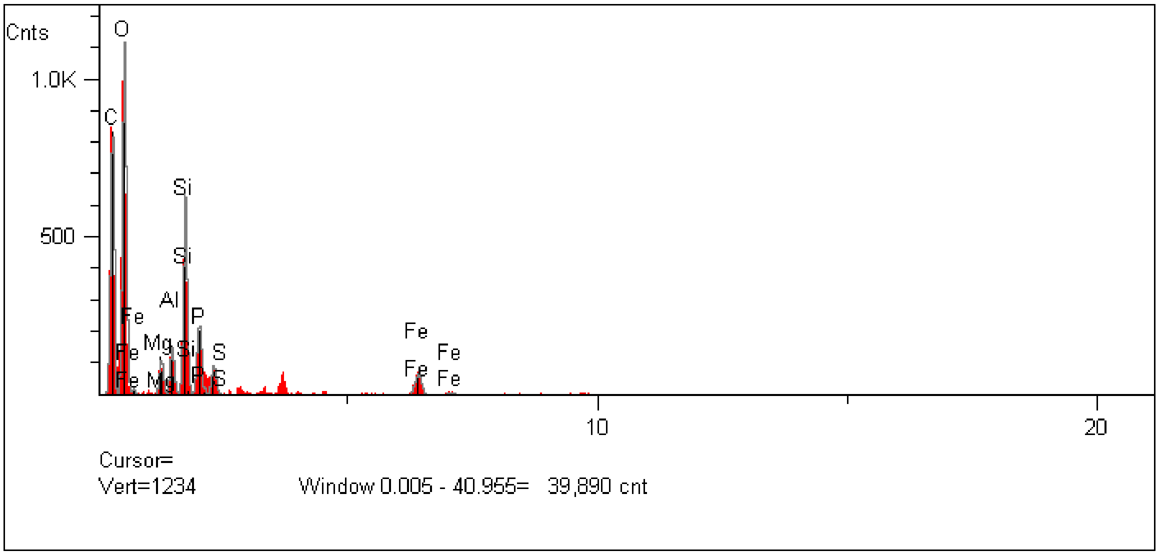
Fe
Fe
5
10
Cursor=
Vert=2000
Window 0.005 - 40.955=
30,611 cnt

b)


Cnts
O
1.0K
C
500
Si
Si
Al
Fe
P
Mg
Si
S
Fe
Fe
Mg
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S
Fe
Fe
Fe
Fe
10
20
Cursor=
Vert=1234
Window 0.005 - 40.955=
39,890 cnt

c)

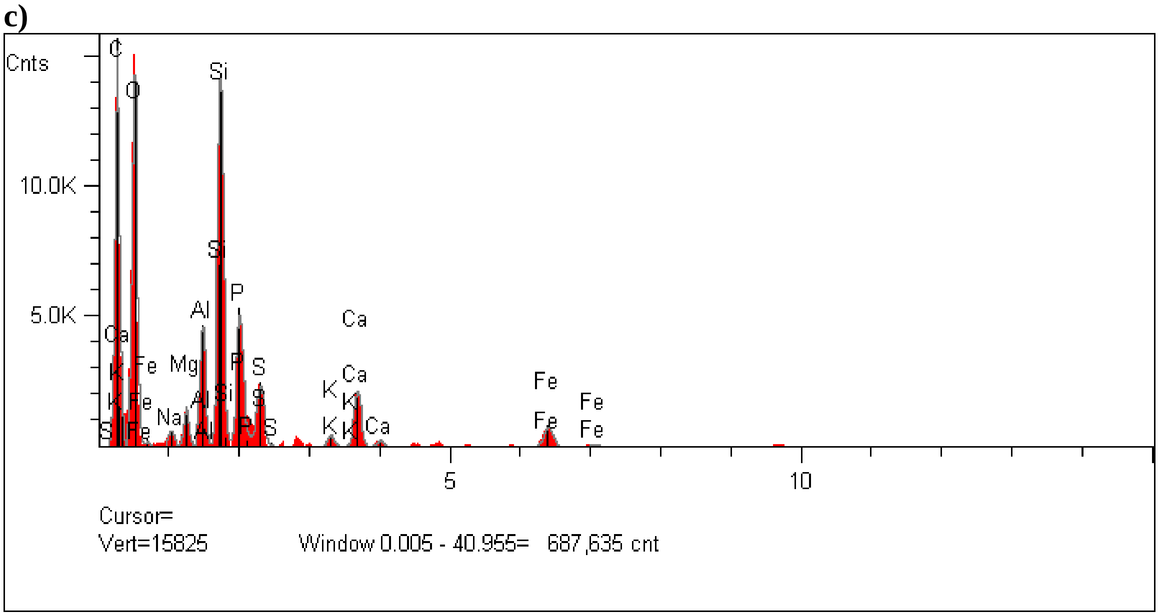

Figure 2.12: Elemental composition of Soil therapy compost (STC) (a), Nacogdoches Wastewater Sludge (NWWS) (b), and Lufkin wastewater sludge (LWWS) (c) at a magnification of X300, an accelerated voltage of 20 KV and filament current of 200 A.

Table 2.4: Percentage composition (% wt/wt) of elements in Soil therapy compost corresponding to Figure 2.12a.

Elt.	Line	Intensity (c/s)	Error 2-sig	Atomic %	Conc	Units	
O	Kα	110	2.7	70	56.0	wt.%	
Mg	Kα	3.5	0.48	0.5	0.6	wt.%	
Al	Kα	50	1.8	5.5	7.4	wt.%	
Si	Kα	168	3.4	17	24.3	wt.%	
P	Kα	16	1.0	1.9	2.9	wt.%	
S	Kα	12	0.88	1.2	1.9	wt.%	
K	Kα	7.2	0.69	0.6	1.2	wt.%	
Ca	Kα	21	1.2	1.7	3.4	wt.%	
Fe	Kα	8.0	0.73	0.9	2.4	wt.%	
				100.0	100.0	wt.%	Total

Table 2.5: Percentage composition (% wt/wt) of elements in Nacogdoches wastewater sludge (NWWS) corresponding to Figure 2.12b.

Elt.	Line	Intensity (c/s)	Error 2-sig	Atomic %	Conc	Units	
C	Kα	135	3.0	48	38.5	wt.%	
O	Kα	171	3.4	45	47.6	wt.%	
Mg	Kα	19	1.1	0.8	1.3	wt.%	
Al	Kα	25	1.3	0.8	1.4	wt.%	
Si	Kα	121	2.8	3.2	6.0	wt.%	
P	Kα	44	1.7	1.1	2.3	wt.%	
S	Kα	18	1.1	0.4	0.9	wt.%	
Fe	Kα	18	1.1	0.5	2.0	wt.%	
				100.0	100.0	wt.%	Total

Table 2.6: Percentage composition (% wt/wt) of elements in Lufkin wastewater sludge (LWWS) corresponding to Figure 2.12c.

Elt.	**Line**	**Intensity (c/s)**	**Error 2-sig**	**Atomic %**	**Conc**	**Units**	
C	Kα	2,262	12.	52	41	wt.%	
O	Kα	2,299	12	39	41	wt.%	
Na	Kα	110	2.7	0.4	0.6	wt.%	
Mg	Kα	256	4.1	0.6	0.9	wt.%	
Al	Kα	794	7.3	1.4	2.4	wt.%	
Si	Kα	2,497	13	3.7	6.8	wt.%	
P	Kα	995	8.1	1.5	3.0	wt.%	
S	Kα	509	5.8	0.7	1.4	wt.%	
K	Kα	104	2.6	0.1	0.3	wt.%	
Ca	Kα	458	5.5	0.5	1.4	wt.%	
Fe	Kα	213	3.8	0.4	1.3	wt.%	
				100.0	100.0	wt.%	Total

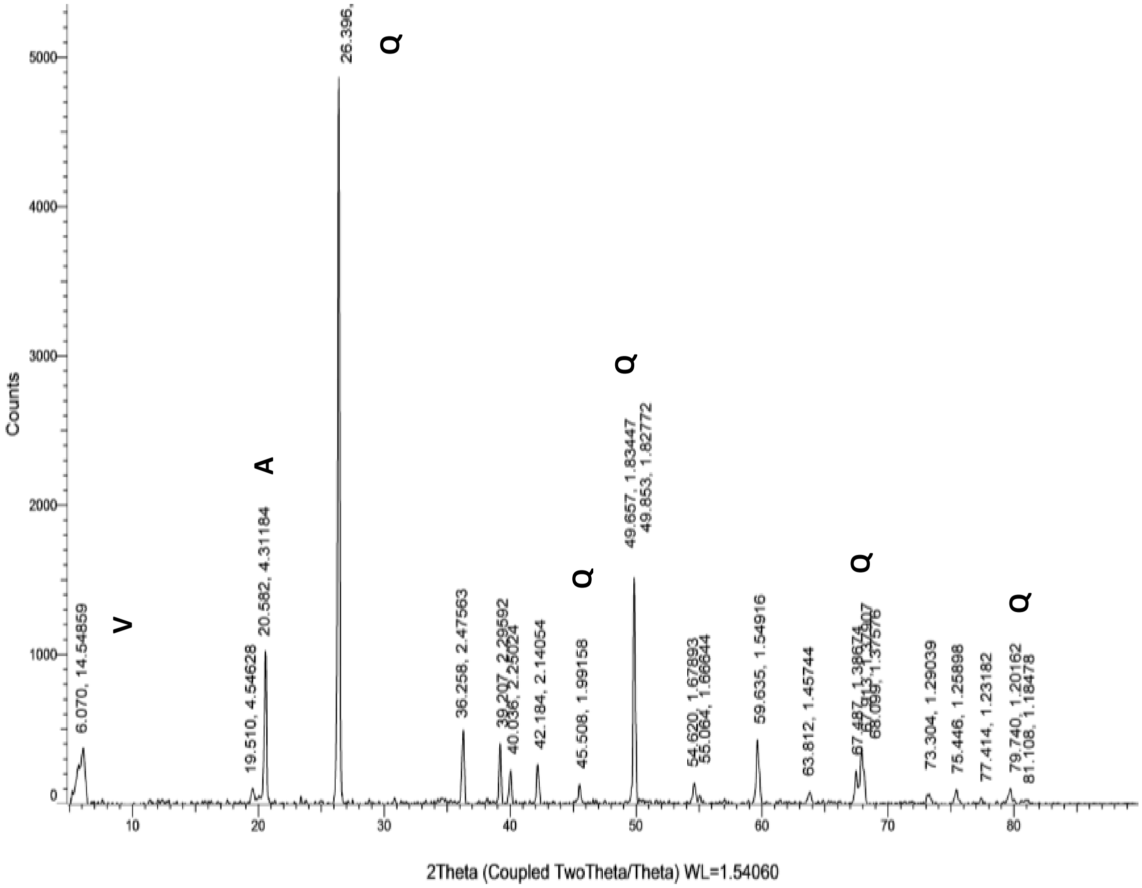

Figure 2.13: XRD Pattern for Soil Therapy Compost (STC) (V = Vermiculite, A = Alunogen, Q = Quartz).

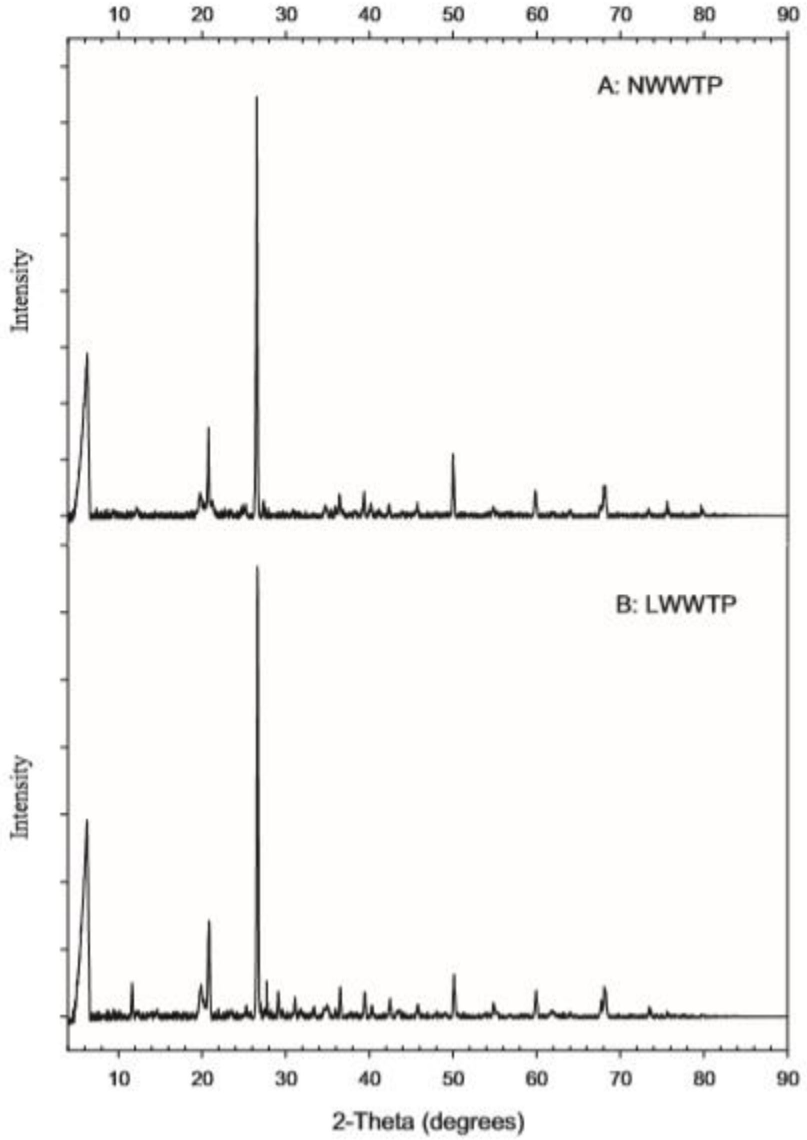

Figure 2.14: XRD Pattern for Nacogdoches wastewater sludge and Lufkin wastewater sludge.[35]

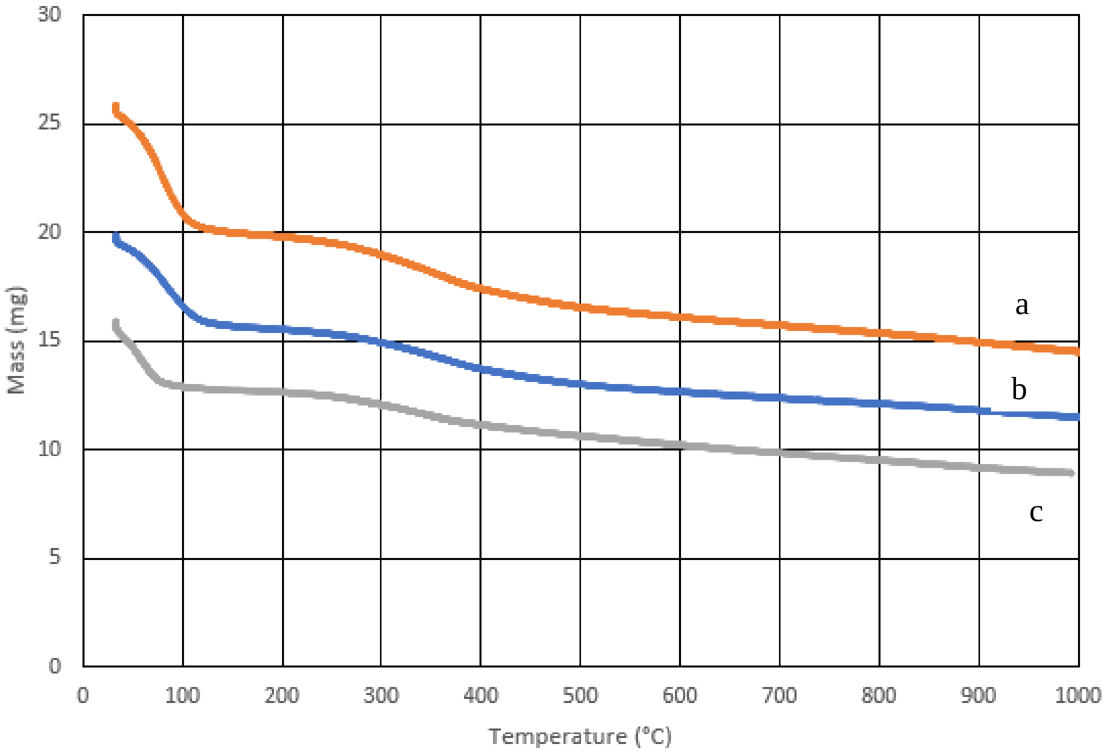

Figure 2.15: Thermogravimetric curve of Soil Therapy Compost (STC) (a = 30 deg/min, b = 20 deg/min, c = 10 deg/min).

CHAPTER 3

SPECIATION AND BIOAVAILABITY OF METALS IN THREE BIOSOLIDS FROM DIFFERENT WASTEWATER TREATMENT PLANTS

3.1 ABSTRACT

Sequential extraction of biosolids is an essential process because it helps to determine the metals that are available for plant uptake. In this study, a modified Tessier protocol was used to speciate metals in the Soil Therapy Compost (STC), Nacogdoches Wastewater Sludge (NWWS), and Lufkin Wastewater Sludge (LWWS). The sequential extraction process entails five extraction steps or fractions; the exchangeable fraction, the adsorbed fraction, the organically bonded fraction, the carbonate fraction and the sulfide or residual fraction. The first two fractions provide information on the bioavailable metals. From results obtained, macroelements (Ca (STC-2168 ± 20 ppm, NWWS-1682 ± 9 ppm, LWWS-7342 ± 11 ppm), Fe (STC-25.69 ± 0.34 ppm, NWWS-16.9 ± 0.1 ppm, LWWS-7.5 ± 0.1 ppm), K (STC-105140 ± 588 ppm, NWWS-117960 ± 205 ppm, LWWS-109980 ± 110 ppm), Mg (STC-417 ± 4 ppm, NWWS-1973 ± 12 ppm, LWWS-2722 ± 3 ppm), P (STC-259 ± 2 ppm, NWWS-3332 ± 16 ppm, LWWS-2424 ± 5 ppm), S(STC-2690 ± 10 ppm, NWWS- 2979 ± 10 ppm, LWWS-9848 ± 15 ppm) and Na (STC-643 ± 3 ppm, NWWS-2513 ± 5 ppm, LWWS-11750 ± 4 ppm)) were found in the

bioavailable fraction in all biosolids samples. Microelements (As (STC-0.190 ± 0.004 ppm, NWWS-2.10 ± 0.01 ppm, LWWS-1.38 ± 0.01 ppm) B (STC-5.34 ± 0.02 ppm, NWWS-6.82 ± 0.02 ppm, LWWS-15.42 ± 0.04 ppm), Mn (STC-14.53 ± 0.10 ppm, NWWS-6.34 ± 0.48 ppm, LWWS-60.07 ± 0.70 ppm), Mo (STC-1.59 ± 0.01 ppm, NWWS-1.54 ± 0.01 ppm, LWWS- 3.80 ± 0.03 ppm), Zn (STC-3.11 ± 0.11 ppm, NWWS-0.59 ± 0.05 ppm, LWWS-1.35 ± 0.03 ppm), Cu(STC-9.67 ± 0.08 ppm, NWWS-28.82 ± 0.076 ppm, LWWS-27.69 ± 0.72 ppm) were found in the bioavailable fraction but in very low concentrations. The highest bioavailable element in the biosolid samples was potassium. Heavy metals (Cr, Pb, Co and V) were not detected in the mobile fraction.

In the previous chapter, spectroscopic characterization of biosolids was discussed. In this chapter, an overview of what speciation and bioavailability of metal entails and the different speciation methods that have been used was provided. In addition, the results obtained for the speciation and bioavailability of the biosolid samples in this study was also discussed.

3.2 INTRODUCTION

Biosolids are dark organic matter rich in beneficial nutrients.[1,2] Although biosolids contain essential nutrients, the presence of toxic metals can limit their use, the risk of biosolids contaminating the soil and transferring heavy metals into the food chain may result in potential health disorders in humans.[3] The determination of bioavailable metals in biosolids is important because it provides information on the metals bioavailable for uptake by plants.

Speciation is a chemical process that involves draining or leaching out of metals in biosolids and soils. The major function of speciation is to imitate the release of metals into solution under some environmental conditions.[4] Heavy metal speciation in composted sludge and biosolids helps to determine the metals available for plant uptake following the use of composted sludge. The methods involved in the speciation of heavy metals in composted sludge include chemical extractions, centrifugation, decantation, filtration, and analysis can be done using atomic absorption emission, inductively coupled plasma optical emission spectrometry or inductively coupled plasma mass spectrometry.[5] Different procedures used for the sequential extraction of metals in biosolids and soils are reviewed here below.

Tessier sequential extraction procedure has often been utilized in special studies. Tessier sequential procedure apportions metal distribution in the different stages with different reagents. The steps with the reagents used are exchangeable (1 M $MgCl_2$ / 1 M NaOAc), bound to carbonates (1 M NaOAc with CH_3COOH), bound to Fe-Mn oxides (0.3 M $Na_2S_2O_4$, 0.175 M Na-citrate, 0.025 M H-citrate / 0.04 M NH_2OH-HCl in acetic acid), bound to organic matter (0.02 M HNO_3, 30% H_2O_2) and residual (HF-$HClO_4$).[6]

Shrivastava and Dipak[7] investigated the bioavailability of Cu, Zn, Pb, Ni, Cr and Cd in biosolids and biosolids-amended soils by using a modified Tessier method. The sequential extraction was carried out to provide information on the metals associated with soils and their uptake by plants. In Tessier sequential extraction protocol, $MgCl_2$ was used to extract the exchangeable fractions. However, in this study, $Mg(NO_3)_2$ was used to replace this $MgCl_2$.[6] $Mg(NO_3)_2$ was used because the chloride ions from the $MgCl_2$ readily form a complex with metals,[8] hence the solubility of the metals in the biosolids to soils is increased.[9] The five sequential steps and reagents used in this study are:

1. The exchangeable phase (1 M $Mg(NO_3)_2$),
2. The oxidizable phase (bound to organic matter, $H_2O_2 + HNO_3 + CH_3COONH_4$),
3. The acid soluble fraction (bound to carbonates, Na_2EDTA),
4. The reducible fraction (bound to Fe/Mn oxides and hydroxides, $NH_2OH.HCl + CH_3COONH_4$),
5. The residual fraction (bound to silicates and detrital materials, $HCl\text{-}HNO_3/HF$).

From the speciation of the biosolids,[7] the concentrations of metals in various fractions were observed:

Copper: residual > acid soluble > oxidizable > reducible > exchangeable

Zinc: residual > reducible > acid soluble > oxidizable > exchangeable

Lead: residual > reducible > oxidizable > acid soluble > exchangeable

Nickel: residual > oxidizable > reducible > acid soluble> exchangeable

Chromium: residual > oxidizable > acid soluble > reducible> exchangeable

Cadmium: residual > acid soluble > reducible > oxidizable > exchangeable

The elements Cu and Zn are highly concentrated in the ratio 1:1 in the non-residual and residual fractions. The high concentration of Cu and Zn in the non-residual fraction shows they are likely bioavailable when used to amend soils. In the non-residual fraction, Cu (acid soluble fraction, 17.2%) and Zn (reducible fraction, 19.9%) had the highest concentration. In addition, elements Pb (72.8%), Ni (61.4%), Cr (79.1%), and Cd (65.7%) were found in greater abundance in the residual fraction suggesting they are less bioavailable.[7]

Sims and Kline[10] carried out sequential extraction and investigated the uptake of heavy metals by plants cultivated in soils treated with co-composted sewage sludge (CCSS). The sequential extraction method utilized reagents, KNO_3, H_2O, NaOH, Na_2-EDTA, and HNO_3, to fractionate Cd, Cr, Cat pH values in the range 5.3 –7.2. In this experiment, wheat (*Triticum aestivum* L.) and soybean (*Glycine max* L.) were cultivated in a greenhouse.[10]

The sequential extraction[10] showed all metals aside from Cd, differed in soils amended with CCSS. Higher rates were found in the NaOH and EDTA fractions and lower rate in the HNO_3 fraction in soils amended with CCSS in contrast with unamended soils. The bioavailable fractions (KNO3, H2O) for Cu, Ni, Pb, and Zn increased(<1 mg/kg) in soil amended with CCSS while there was no increase for Cd and Cr. Liming resulted in (< 5%) changes in the distribution of Cd, Cr, Ni, and Pb in the various fractions. In soils amended in CCSS, the decrease in NaOH fraction in Cu, the bioavailable fraction in Zn and the increase of Cu and Zn in the EDTA and HNO3 fractions were as a result of liming.[10]

Shakunthala et al.[1] performed speciation of heavy metals in biosolids obtained from Mysore Wastewater Treatment Plants in Karnataka, India to determine the properties of heavy metals in the biosolids. For this study, Tessier multi-step extraction procedure was used. This is a five step extraction procedure which involves the use of reagents; 1 M $MgCl_2$, CH_3COONa, $(NH_3OH)Cl + CH_3COOH$, $HNO_3 + H_2O_2$ for the exchangeable, the carbonate bound, Fe-Mn oxide, organic and sulfide metal fractions respectively. The residual metal fraction is calculated as difference between total metal content and sum of extracted metals. From results obtained, maximum amount of heavy metals was found in the residual fraction and only small amounts of Fe, Cd and Zn were found in the exchangeable and carbonate fractions (soluble fractions).[1]

Urasa and Macha[11] speciated heavy metals in soils, sediments and sludges using a modified Tessier sequential extraction procedure and a D.C plasma atomic emission spectrometry coupled with ion chromatography. The modified Tessier sequential extraction uses reagents KNO_3, distilled deionized water (exchangeable and absorbed phase), NaOH (organically bound phase), EDTA (carbonate phase) and HNO_3 (sulfide/residual phase). Metals extracted by KNO_3 and distilled deionized water are the mobile metals, readily bioavailable to plants.[11]

Onchoke et al.[12] utilized a five-step modified Tessier sequential extraction protocol[11] with use of reagents KNO_3, H_2O, NaOH, EDTA, and HNO_3 to speciate heavy metals in sewage sludge. From the study, elements Cu, Fe, Cr, Pb, Cd, Ni, Zn, and Mn were found bioavailable in the composted wastewater sludge. Compared to other metals, Cu had higher concentrations in the different metal fractions (exchangeable fraction (1.94%) adsorbed fraction (0.83%), organically bonded fraction (40.64%), carbonate fraction (26.89%) and sulfide/residual fraction (29.70%)).[12]

Morere et al.[13] Maiz et al.[14] He et al.[15] and Silveira et al.[16] used Tessier multi-step extraction procedure to determine the properties of heavy metals in the biosolids.

Morere et al.[13] utilized the five steps Tessier sequential extraction protocol (with reagents $MgCl_2$, NaOAc, $NH_2OH.HCl$, $H_2O_2 + HNO_3$, $HF + HClO_4$) and sorption isotherms to determine the bioavailability and distribution of metals (Cd, Cu, Ni, Pb and Zn) in four soils with different physicochemical properties. From the study, it was found that most of the metals were found in the more mobile fractions (exchangeable and carbonate fractions). This contrasts with what is observed in soils, in which metals are mostly associated with the residual fraction.[13]

Maiz et al.[14] utilized a two-step extraction method with four steps Tessier sequential extraction protocol to determine the availability of heavy metals in polluted soils. The two-step extraction method used involved; the mobile fraction and the mobilizable fraction. Aqueous solutions were used to extract both fractions. The mobile fraction was extracted with $CaCl_2$ solution while the mobilizable fraction was extracted with DTPA (diethylenetriamine pentaacetic acid) + $CaCl_2$ + TEA (triethanolamine). The four step Tessier extraction protocol with the reagents used involved; exchangeable

($MgCl_2$), bound to carbonates (NaOAc/HOAc), bound to Fe-Mn oxides ($NH_2OH.HCl$), bound to organic matter and sulfides ($HNO_3 + H_2O_2 + NH_4OAc$). From the study, it was concluded that there was possibility of Cd, Cu, Zn and Pb to be bioavailable in the soil on a short-medium term.[14]

He et al.[15] utilized four different sequential extraction procedures (Sposito, Community Bureau of Reference (BCR), Tessier and Silvera) to speciate soil metals. The Sposito protocol is a four-step procedure (exchangeable (KNO_3), organic matter (NaOH), Fe-Mn oxides (Na_2EDTA), and residual (HNO_3)).[17] The BCR method is also a four-step method (exchangeable, water, and acid-soluble (CH_3COOH), reducible (Fe and Mn oxides, NH_2-OH-HCl), oxidizable ($H_2O_2 + NH_4OAc$), residual (HNO_3-HCl)).[18] The Tessier procedure involves the following steps and reagents (exchangeable ($MgCl_2$), carbonates (NaOAc), Fe and Mn oxides (NH_2OH/HCl), organic matter ($HNO_3 + H_2O_2 + NH_4OAc$), residual (HNO_3-HCl)).[6] The Silveira procedure entails the following steps (soluble- exchangeable ($CaCl_2$), surface adsorbed (NaOAc), organic matter (NaOCl), Mn oxides (NH_2OH-HCl), poor crystalline Fe oxides (oxalic acid + oxalate), crystalline Fe oxides (HCl) and residual (HNO_3-HCl)).[16] From the results, Fe and Zn were mostly retained in the recalcitrant soil fractions while Cd was mostly found in the exchangeable fraction. Cu was highly retained in the organic matter fraction. It was concluded that there was variation in the extraction efficiency of the metals with the different sequential extraction procedure because of the different reagents and experimental conditions.

Community Bureau of Reference (BCR) procedure is another sequential extraction procedure.[19] This has been utilized by Kazi et al[20] and Jamali et al.[21]

Kazi et al.[20] utilized a four-step BCR sequential extraction method (exchangeable and acid-soluble (CH_3COOH), reducible (NH_2OH-HCl), oxidizable (H_2O_2 + CH_3COONH_4), residual (HCl/HNO_3) to determine the mobility of toxic metals (Cr, Pb, Ni and Cd) in untreated industrial wastewater sludge. From the study, Cd was found mostly in the easily mobilized form (acid exchangeable). The toxic metals (Cr, Pb, Ni, Cd) were all present in the oxidizable fraction.[20]

Jamali et al.[21] utilized the four-step BCR sequential extraction method to speciate Cd, Cr, Cu, Ni, Pb and Zn in untreated domestic wastewater sludge. From the study, all the metals except Cd were present in the oxidizable fraction. In addition, the concentration of Cd, Cr, Cu, Ni and Zn found in the acid-exchangeable fraction (mobile fraction) were 31.0%, 3.1%, 2.5%, 7.6%, 2.6% and 8.4%, respectively.[21]

Some other procedures used for speciation of metals are the Galan (Galan et al.)[22] and modified Geological Society of Canada (Benitez and Dubois)[23] procedures.

Given the above overview, it was the objective of this research to quantify the amounts of heavy or trace elements in the compost that are readily bioavailable to the plants. This will help provide information on environmental threats that can result from mobilization of heavy metals in biosolids, and may in turn influence uptake of such metals by plants.[24,25,26]

3.3 MATERIALS AND METHODS

3.3.1 Reagents

Nanopure water (18.2 MΩ), KNO_3 (EM Science, Norwood, Ohio), (NaOH, EDTA, HNO_3 from Flinn Scientific Inc., Batavia, Illinois) were used for all extractions. All reagents used were of high analytical purity.

3.3.2 Sequential extraction procedure

Speciation of metals in the composted sludge (biosolids) was conducted using the modified Tessier protocol designed by Urasa and Macha (Table 3.1).[11] Sequential extraction was performed on 2.0000 g dried samples of Soil Therapy Compost (STC), Nacogdoches Wastewater Sludge (NWWS), and Lufkin Wastewater Sludge (LWWS) in 50 mL Beckman polypropylene centrifuge tubes with polyethylene caps. The fractions were fractionated into the exchangeable and adsorbed (KNO_3 and distilled deionized water), organically bound fraction (NaOH), carbonate fraction (EDTA), and sulfide/residual fraction (HNO_3). After carrying out all the chemical extractions, the samples were shaken at 200 rpm using a Thermo Scientific MaxQ 6000 incubated/refrigerated stackable shaker at different temperature and hours as given by the modified Tessier protocol (Table 3.1). The samples were centrifuged using Beckman Coulter Centrifuge at 2,500g for 30 minutes at room temperature (25 °C) using 20-JA rotor. Thereafter, the samples were filtered using a digi-filter with a 0.45 µm hydrophilic teflon filter to get rid of large particles prior to analysis using ICP-OES.

Table 3.1: Summary of the Modified Tessier Successive Extraction Protocol.[11]

STEP/REAGENT	STIRRING TIME (HRS)	METAL EXTRACTION
1. 0.5 M KNO_3	16	Exchangeable
2. 18.2 MΩ Nano-pure H_2O	2 (done 3 times)	Adsorbed
3. 0.5 M NaOH	16	Organically bonded
4. 0.05 M EDTA	6	Carbonate
5. 4 M HNO_3	16 (70-80 °C)	Sulfide/residual

3.4 RESULTS

For ease of discussion, the metals are divided into Group IA, IIA, IIIA, IVA, VA, VIA, and transition metals. Figures 3.1 – 3.13 show the sequential extraction of 26 metals (As, B, Ba, Ca, Cd, Co, Cu, Fe, Hg, K, Mg, Mn, Mo, Na, P, Pb, Se, V, Zn, S, Ni, Al, Cr, Sr, Li, Cs). The metal speciation/fractions are divided into 5 steps, namely, the exchangeable fraction (EXH, step 1), the adsorbed fraction (ADS, step 2), the organically bonded fraction (OB, step 3), the carbonate fraction (CB, step 4), and the sulfide/residual fraction (SR, step 5). The sum of the exchangeable and adsorbed fraction provides information on the mobile fraction or bioavailable fraction.

3.4.1 Group IA Elements

Group IA elements (Li, Na, K, Cs) were extracted from the biosolids into the 5 fractionation steps. Figures 3.1, 3.2 and 3.3 show the sequential extraction of group 1A elements in the biosolid samples. Although group 1A elements are known to be readily soluble, Li was found below detection in the first 4 fractionation steps (EXH, ADS, OB, and CB). Most of Li was found in the sulfide/residual fraction in the biosolid samples (STC (1.92 ± 0.01 ppm, NWWS (1.22 ± 0.01 ppm), and LWWS (0.68 ± 0.01 ppm). Li can occur as lithium sulfate (Li_2SO_4). Ingestion of lithium sulfate can lead to chronic kidney failure and diarrhea.[27] In all biosolid samples, K was the most bioavailable element with the following percents; STC (92%), NWWS (89%) and LWWS (89%). Thus, K is highly bioavailable for plants. Although K is an essential nutrient for both plant and animals, high intake of potassium in humans can result in hyperkalemia; a condition where the kidney lacks the ability to get rid of enough potassium from the body. [28] Na and Cs were more abundant in the organically bonded fraction compared to other fractions. The percentage composition of Na and Cs in the organically bonded fraction in all three biosolid samples are STC (47 - 63%), NWWS (39 - 47%), and LWWS (3.7 - 9.9%).

Thus, Li occurs predominantly in the sulfide fraction, K occurs mostly in the mobile fraction, while Na and Cs are predominant in the organically bonded fraction. in biosolid samples.

3.4.2 Group IIA Elements (Mg, Ca, Sr, Ba)

Figures 3.4, 3.5 and 3.6 show the sequential extraction of group IIA elements (Mg, Ca, Sr, Ba). Mg had the highest concentration in the mobile fraction in all biosolids; STC (31 ± 4%) > LWWS (59 ± 3%) > NWWS (47 ± 12%) while Ba had the lowest percentage, namely, STC (1.10 ± 0.02%) > LWWS (0.34 ± 0.02%) > NWWS (0.10 ±

0.02%). Barium (Ba) has the highest composition in the sulfide/residual fraction in all biosolids, and was present in the sulfide fraction as follows; STC (82 ± 1 %), NWWS (91± 2%), and LWWS (85 ± 1%). Ba exists in various forms including barium sulfate ($BaSO_4$), barium sulfite ($BaSO_3$), and barium hydrogen sulfate $Ba(HSO_4)_2$. Barium sulfate ($BaSO_4$) can persist for a long time if released into the environment. When Ba enters the body through ingestion, it is usually excreted.[29] Ca had the highest percentage in the carbonate fraction: STC (29 ± 14%), NWWS (28 ± 13%) and LWWS (36 ± 15%). The investigation of ^{80}Sr and ^{83}Sr isotopes in the biosolid samples showed similar concentrations in all chemical fractions. In general, most metals in group IIA are dominant in the sulfide fraction.

3.4.3 Group IIIA Elements (Al, B)

Figure 3.7 shows the sequential extraction of B and Al in the biosolids. Boron was readily present in the mobile phase. This implies that it can be readily taken up by plants. Ingestion of boron can affect the stomach, liver, kidney, intestine or even lead to death over a short time. NWWS has a lower percentage of B in the mobile fraction (19 ± 0%) followed by STC (30 ± 0%) and LWWS (31 ± 0%). Except in STC (0.1 ± 1.0%) samples Al concentration was found below detection in the mobile phase in all biosolids. In STC, Al has a higher concentration in the sulfide/residual fraction (52 ± 15%). The boron concentration in NWWS and LWWS was higher in the sulfide fraction. In NWWS, B percent was higher (69.0 ± 0.2%) compared to Al (63 ± 39%). In LWWS B (59.0 ± 0.1

%) was greater than Al (47 ± 2%). Al can exist in forms including aluminum sulfate $Al_2(SO_4)_3$, aluminum sulfite $Al_2(SO_3)_3$, aluminum sulfide Al_2S_3, aluminum thiosulfate $Al_2(S_2O_3)_3$, and aluminum hydrogen sulfate $Al(HSO_4)_3$. High concentration of Al can result in brain and bone disease in children.[30] In conclusion, B and Al were predominant in the sulfide/residual fraction.

3.4.4 Group IVA Elements (Pb,)

Lead (Pb) was the only group IVA element that was fractionated in the biosolid samples. Figure 3.8 shows the sequential extraction of Pb in STC, NWWS and LWWS. Pb was not detected in the bioavailable fraction in all biosolid samples. Pb was found mostly in the sulfide fraction vis-a-vis all other fractions (STC (76 ± 0%), NWWS (86.0 ± 0.1%), LWWS (70.0 ± 0.1%). Pb can occur as lead (iv) sulfate $Pb(SO_4)_2$, lead (iv) sulfite $Pb(SO_3)_2$, lead (ii) sulfate, ($PbSO_4$) and lead (ii) sulfite ($PbSO_3$). Ingestion of Pb can affect the blood, gastrointestinal tract, nervous systems, kidney, cardiovascular blood forming systems. Lead also inhibits red blood cell enzyme systems, and in high concentration can cause anemia.[31,32,33] The lowest concentrations of Pb was found in the organically bonded fraction (STC (1.3 ± 0.0%), NWWS (4 ± 0%), LWWS (4.0 ± 0.1%)). In general, Pb is most dominant in the sulfide fraction in the biosolid samples. Pb was not detected in the mobile fraction which implies that it is less readily bioavailable to plants.

3.4.5 Group VA Elements (P, As)

Phosphorus and arsenic were selectively extracted into different chemical fractions. Figure 3.9 shows the sequential extraction of P and As in STC, NWWS and LWWS. In Soil Therapy Compost (STC), As was in a higher percentage in the mobile fraction (6 ± 0%) compared to P (2.4 ± 1.6%). Similarly, in NWWS, As (33 ± 0%) was found present in the mobile phase compared to P (15 ± 1%). In LWWS, P (10 ± 5%) and As (9 ± 0%) were both present in the mobile fraction in very close concentrations. In Soil Therapy Compost, P was in a higher percentage (42 ± 17%) compared to As (35 ± 0%) in the sulfide/residual fraction. Phosphorus can exist in forms such as diphosphorus pentasulfide (P_2S_5), tetraphosphorus heptasulfide (P_4S_7), diphosphorus trisulfide (P_2S_3), phosphorus sesquisulfide (P_4S_3), tetraphosphorus hexasulfide (P_4S_6), tetraphosphorus pentasulfide (P_4S_5).[34] The NWWS (41 ± 0%) and LWWS (64 ± 0%) had As in the highest concentration in the sulfide fraction. As can occur in forms such as arsenic trisulfide (As_2S_3), tetraarsenic tetrasulfide (As_4S_4), tetraarsenic trisulfide (As_4S_3), arsenic hydrogen sulfate $As(HSO_4)_3$, arsenic (ii) sulfite ($AsSO_3$) and arsenic tetrasulfide (As_2S_4). Arsenic is a known carcinogen in humans. Arsenic can cause urinary bladder cancers, lung cancer, skin and kidney cancer.[35] It can also result in blood and cardiovascular diseases.[35] NWWS had highest concentration of P in the organically bonded fraction (32 ± 10%) while LWWS had the highest concentrations of P in the carbonate fraction (33 ± 2%).

In general, P and As are dominant in the sulfide fraction with percentages of (42.1%, 35%), (40.9%, 30.3%) and (64%, 30%) in STC, NWWS and LWWS, respectively. It is noted that the P and As are less dominant in the mobile fractions with percentages of (2.4%, 5.6%), (15%, 33.3%) and (9.9%, 8.5%) in STC, NWWS and LWWS, respectively.

3.4.6 Group VIA Elements (S and Se)

Figure 3.10 shows the sequential extraction of S and Se in the three biosolid samples. S has the highest concentration in the mobile fraction in STC (44 ± 10.28%) and LWWS (48 ± 14.6%). In NWWS S (29 ± 10%) and Se (28 ± 0.026%) were found in comparable concentration in the bioavailable fraction. STC. The majority of Se was found in the organically bonded fraction in both NWWS (56 ± 0%) and LWWS (95 ± 0%). Selenium is less toxic compared to arsenic, cadmium, and lead when ingested. The gastrointestinal tract and lungs usually absorb selenium readily.[36] In NWWS, Se was determined below detection in the sulfide fraction. In LWWS Se were found below detection in the sulfide and carbonate fraction. This implies that both NWWS and LWWS although quite similar might have some variations. Comparatively, S and Se are dominant in the mobile fraction and organically bonded fractions, respectively.

3.4.7 Transition Elements

Figures 3.11, 3.12, and 3.13 show the sequential extraction of transition elements (Cd, Co, Cr, Cu, Fe, Hg, Mn, Mo, V, Zn, Ni) in STC, NWWS, and LWWS, respectively. Cd, Cr, Co and V were not detected in the bioavailable fraction in STC. Both NWWS and LWWS had Co, Cr and V below detection in the bioavailable fractions. In STC, Mo had the highest percentage in the bioavailable fraction; Mo > (71.36 ± 0.01%) > Hg (59 ± 0%) > Cu (2.4 ± 0.1%) > Mn (1.9 ± 0.1%) > Ni (1.36 ± 0.04%) > Zn (0.58 ± 0.11%) > Fe (0.20 ± 0.34%). In NWWS, Hg had the highest concentration in the mobile phase. Hg > (56 ± 0%) > Mo (50 ± 0%) > Cu (12.0 ± 0.1%) > Ni (9 ± 0%) > Cd (3.0 ± 0.1%) > Mn (1.0 ± 0.5%) > Zn (0.13 ± 0.05) > Fe (0.08 ± 0.06%). Hg (81 ± 0%) and Mo (50 ± 0%) had the highest concentrations in the mobile phase in LWWS.

Cd was detected in the carbonate fraction (1.97 ± 0.13 ppm) and sulfide fractions (1.53 ± 0.02 ppm) in STC. Cd can occur in forms such as cadmium hydrogen sulfate $Cd(HSO_4)_2$, cadmium carbonate ($CdCO_3$), cadmium sulfide (CdS), cadmium sulfite ($CdSO_3$) and cadmium sulfate ($CdSO_4$). The major effect of cadmium, either through digestion or inhalation from aerosols is proteinuria, a condition that affects and may damage the kidneys.[37,38] Compared to other transition metals, plants can readily take up Cd. In STC, Fe showed the highest percent in the sulfide fraction (82 ± 25%). Some compounds of Fe include iron(ii)sulfate heptahydrate ($FeSO_4.7H_2O$), iron (iii) sulfate $Fe_2(SO_4)_3$, iron(ii)sulfate ($FeSO_4$), iron(iii)sulfite ($Fe_2(SO_3)_3$), iron(ii)sulfite ($FeSO_3$) and iron(iii)sulfide (Fe_2S_3).[39] In NWWS, Mn (75 ± 2 %), Ni (61± 0%), Zn (73 ± 2 %), V

(68.0 ± 0.2%), Fe (96 ± 10%), Cu (63 ± 1%) and Cr (97.0 ± 0.2%) were predominant in the sulfide fraction. The element Cu (8.7 ± 0.1%), Hg (13 ± 0%), V (12 ± 0%) and Mo (8 ± 0%) have their lowest concentration in the carbonate fraction.

In LWWS, Cd (7.0 ± 0.1%), Cu (6 ± 1%), Fe (0.1± 0.1%), Zn (0.10 ± 0.03%), and Ni (16 ± 0%) have the lowest concentrations in the mobile fraction compared to other chemical fractions. Cd (79 ± 0%), Co (97 ± 0%), Cr (84.0 ± 0.1%), Cu (68 ± 1%), Fe (88 ± 48%), Zn (53 ± 2%), Ni (45 ± 0%) and Mn (50 ± 2%) were found mostly present in the sulfide fraction. Whereas Cd and Co were determined below detection in the organically bonded fraction, Hg and V were not detected in the carbonate and sulfide fractions. V was below detection in all chemical fractions apart from the organically bonded fraction (4 ± 0 ppm).

In conclusion, most of the transition metals were dominant in the sulfide fraction. While Mn predominant in the sulfide fractions, it was also present in micro-concentrations in the mobile or bioavailable fraction in biosolid samples.

3.5 DISCUSSION

Macroelements

Macroelements (Ca (STC (2168 ± 20 ppm), NWWS (1682 ± 10 ppm), LWWS (7342 ± 11 ppm)), Fe ((STC (25.69 ± 0.34), NWWS (16.99 ± 0.10 ppm), LWWS (7.49 ± 0.07 ppm), K (STC (105140 ± 588 ppm), NWWS (117960 ± 205 ppm), LWWS (109980 ± 110 ppm)), Mg (STC (417 ± 4 ppm), NWWS (1973 ± 12 ppm), LWWS (2722 ± 3 ppm)), P ((STC (259 ± 2), NWWS (3332 ± 16 ppm), LWWS (2424 ± 5 ppm)), S (STC (2690 ± 10 ppm), NWWS (2979 ± 10 ppm), LWWS (9848 ± 15 ppm)) and Na (STC (643 ± 3 ppm), NWWS (2513 ± 5 ppm), LWWS (11750 ± 4 ppm)) were found in the bioavailable fraction in all biosolids samples. Al was the only macroelement below detection in both NWWS and LWWS.

Microelements

Microelements (As (STC (0.19 ± 0.00 ppm), NWWS (2.10 ± 0.01 ppm), LWWS (1.38 ± 0.01 ppm)), B (STC (5.34 ± 0.02 ppm), NWWS (6.82 ± 0.02 ppm), LWWS (15.42 ± 0.04 ppm)), Ba (STC (4.40 ± 0.02 ppm), NWWS (0.34 ± 0.02 ppm), LWWS (1.22± 0.01 ppm)), Hg (STC (4.10 ± 0.00 ppm), NWWS (3.95 ± 0.00 ppm), LWWS (4.07 ± 0.01 ppm)), Mn (STC (14.53 ± 0.10 ppm), NWWS (6.34 ± 0.48 ppm), LWWS (60.1 ± 0.7 ppm)), Mo (STC (1.59 ± 0.01 ppm), NWWS (1.54 ± 0.01 ppm), LWWS (3.80 ± 0.03 ppm), Ni (STC (0.17 ± 0.04 ppm), NWWS (1.69 ± 0.03 ppm), LWWS (3.79 ± 0.01 ppm)), Zn (STC (3.11 ± 0.11), NWWS (0.60 ± 0.05 ppm), LWWS (1.35 ± 0.03 ppm)) and Cu (STC (9.67 ± 0.08), NWWS (28.82 ± 0.11 ppm), LWWS (27.69 ± 0.72 ppm)) were found in the bioavailable or mobile fraction in the biosolid samples, although some were in low concentrations.

Toxic elements

Toxic elements Cr, Pb, Co, and V were not detected in the mobile fraction of the biosolids. Selenium was only detected in the mobile fraction in NWWS and LWWS while Cd was present in the bioavailable fraction in the three biosolid samples.

Comparisons to other studies

A sequential extraction analysis found Pb below detection in the mobile fraction which is similar to what is observed in this study.[36] The non-toxic elements Ca, K, Mg, Na were readily present in mobile fraction, with K being the most readily bioavailable (92% ± 10). Ni which is a contaminant was found present in the mobile fraction, although at a lower concentration.[38] Another analysis recorded that elements Cu, Fe, Cr, Pb, Cd, Ni, Zn and Mn were bioavailable in the composted sludge.[12] Except for Cr and Pb, these elements were found to be bioavailable in the biosolids. Only STC showed no detectable Cd in the bioavailable fraction. The elements Pb, Ni, Cr, and Cd were found at higher concentrations in the sulfide or residual fraction of biosolids in another study.[7] This was also the case with the biosolid samples except for Cd in STC and NWWS. One analysis showed that Zn had low concentrations in the residual fraction of the biosolid, and was found below detection in the mobile fraction.[39] Comparing this with Zn found in the STC, Zn had the highest concentration in its residual fraction (61%) and the lowest concentration in the mobile fraction (0.58%). Both NWWS (73%) and LWWS (53%) showed the highest Zn concentrations in the residual fraction, while the lowest fraction of Zn in NWWS (0.13 ± 0.04%) and LWWS (0.18 ± 0.03%) was found in the mobile fraction. This present analysis showed Cd to be mainly bonded to the carbonates.[37] This is in accordance with the Cd in the soil therapy compost and LWWS.

3.6 IMPLICATION OF SEQUENTIAL EXTRACTION DATA

3.6.1 Readily Soluble Elements

Plants can only absorb an element, if the element is in soluble form. Group 1 elements K, Li, Cs and Na are known to be readily soluble. These readily soluble elements can easily be taken up by plants. From the sequential extraction data, K is most readily soluble element in the biosolid samples. K is an essential macro-nutrient which readily mobile in plants and can be easily leached from the soil. K also enhances the rigidity of stalks in plants.[38]

3.6.2 Uptake of Elements by Plants

The root is an important organ of the plant that enables plants to take up elements from the soil. Elements are present in soils in varying concentrations. The average concentrations of some elements in the soil are given.: Al (72000 ppm), As (7.2 ppm), B (33 ppm), Ba (580 ppm), Ca (24000 ppm), Co (9.1 ppm), Cr (54 ppm), Cu (25 ppm), Fe (26000 ppm), Hg (0.09 ppm), K (15000 ppm), Li (24 ppm), Mg (9000 ppm), Mn (550 ppm), Mo (0.97 ppm), Na (12000 ppm), Ni (19 ppm), P (430 ppm), Pb (19 ppm), S (1600 ppm), Se (0.39 ppm), Sr (240 ppm) and Zn (60 ppm).[39] Since the soil already contain some elements, amendment of the soil will lead to large concentration of the elements in the soil. Continual land use of the biosolids can lead to deposition of very large concentration of toxic metals such as As, Pb and Hg into the soil. The plants can also take up some of these toxic metals. Consumption of such plants can lead several health disorders in humans.

3.7 CONCLUSIONS

In conclusion most of the elements were found predominantly in the sulfide fraction in biosolid samples. K was the most mobile element in the biosolid samples. K is an essential nutrient needed for plant growth, the high concentration of K in the biosolid samples supports their use as soil supplement to boost crop production. Other essential nutrients for plant growth were also present in the mobile fraction (Ca (STC (2168 ± 20 ppm), NWWS (1682 ± 10 ppm), LWWS (7342 ± 11 ppm)), Mg (STC (417 ± 4 ppm), NWWS (1973 ± 12 ppm), LWWS (2722 ± 3 ppm)), P ((STC (259 ± 2), NWWS (3332 ± 16 ppm), LWWS (2424 ± 5 ppm)) and Mn (STC (14.5 ± 0.1 ppm), NWWS (6.3 ± 0.5 ppm), LWWS (60 ± 1 ppm)). The metals Co, Pb, and Cr were found below detection in all the biosolid samples. Toxic pollutants (As, Hg, and Cd) were determined present in the mobile fraction. Although these heavy metals are below the maximum concentration limit, continual land use of the biosolids can result in the accumulation of these toxic metals in the soil. The sequential extraction provides information on the mobility of metals, the determination of the total metal content in the root, shoot and leaves of plants will further help determine the percent of the metals the plant take up.

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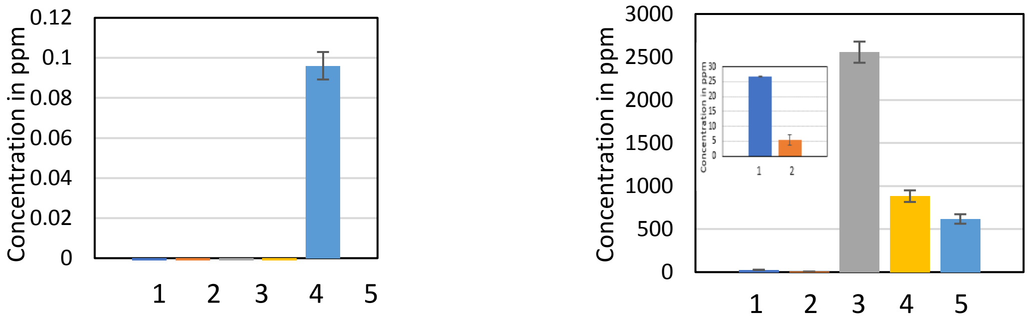
FIGURES

a) Li

b) Na



c) K

d) Cs

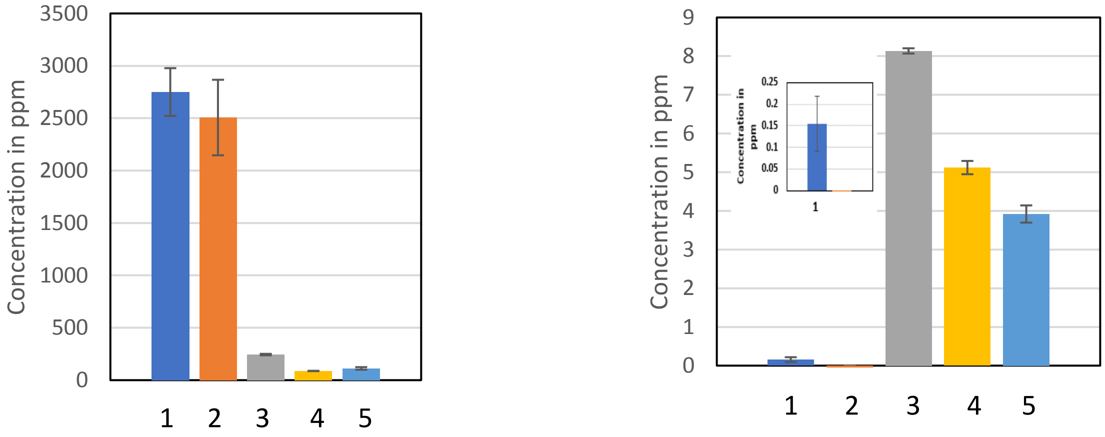
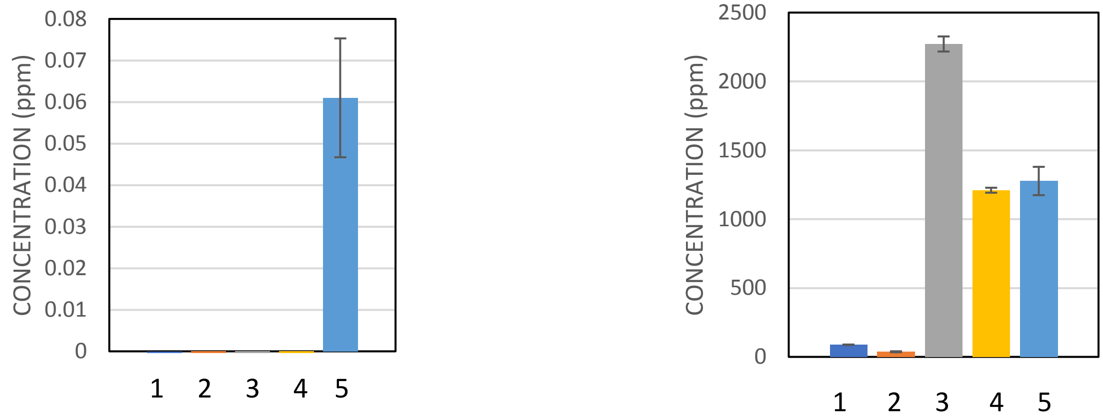
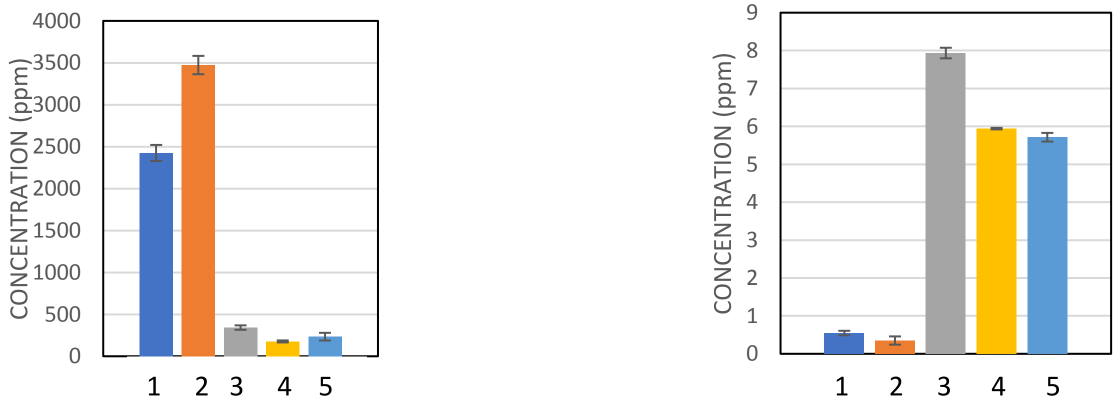


Figure 3.1: Sequential fractionation of Group 1A metals, Li (a), Na (b), K (c), and Cs (d) in soil therapy compost (1 = exchangeable fraction, 2 = adsorbed fraction, 3 = organically bonded fraction, 4 = carbonate fraction, and 5 = sulfide/residual fraction).


a) Li

b) Na


c) K

d) Cs

Figure 3.2: Sequential fractionation of Group 1A metals, Li (a), Na (b), K (c), and Cs (d) in Nacogdoches wastewater sludge (1 = exchangeable fraction, 2 = adsorbed fraction, 3 = organically bonded fraction, 4 = carbonate fraction, and 5 = sulfide/residual fraction).

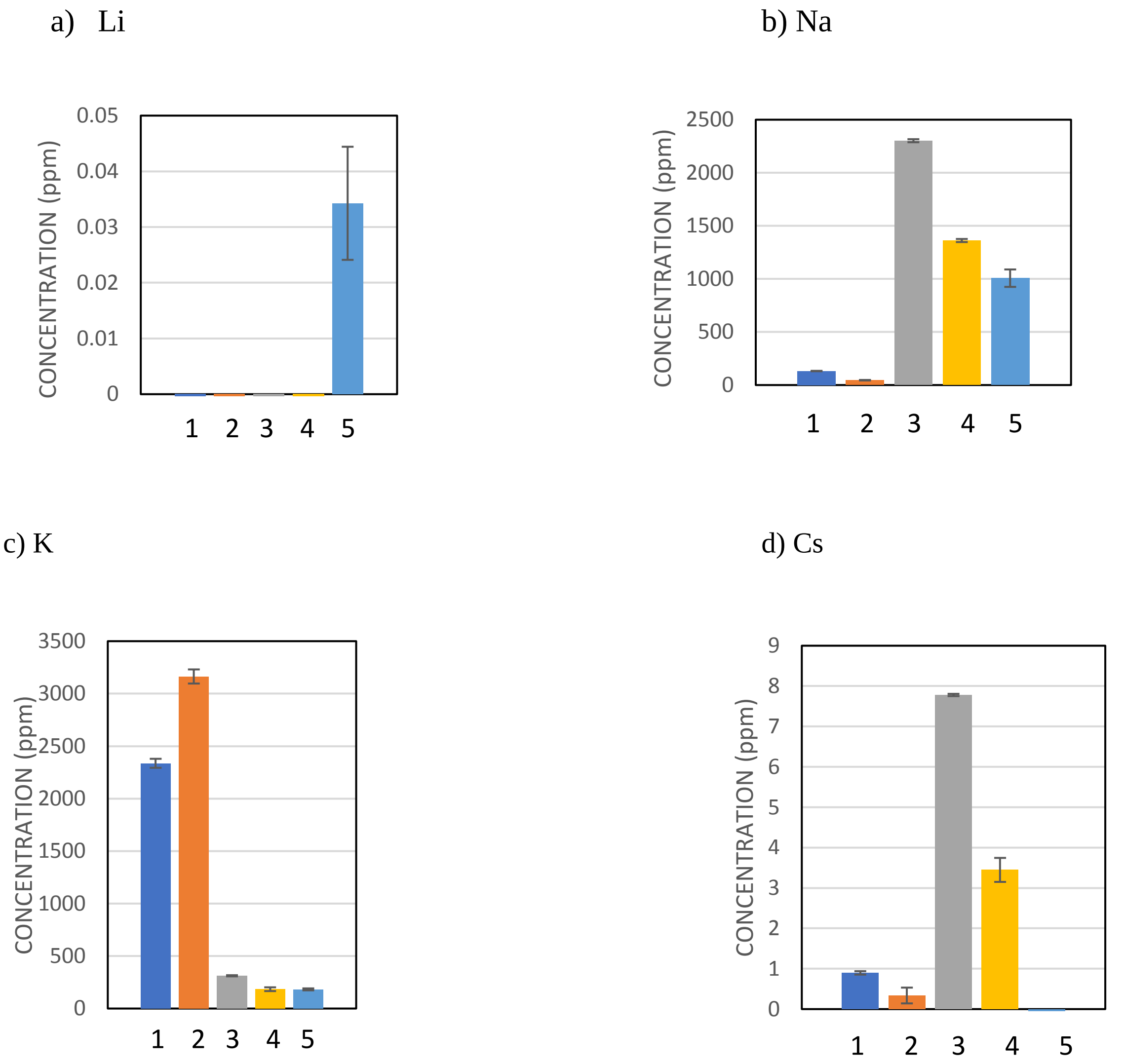
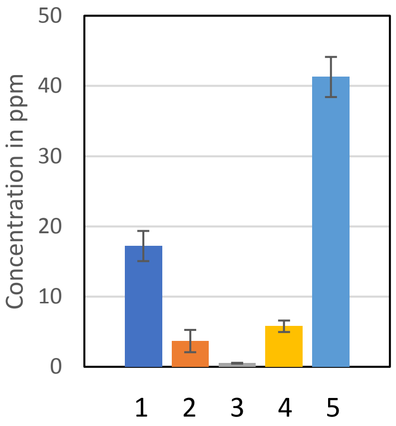
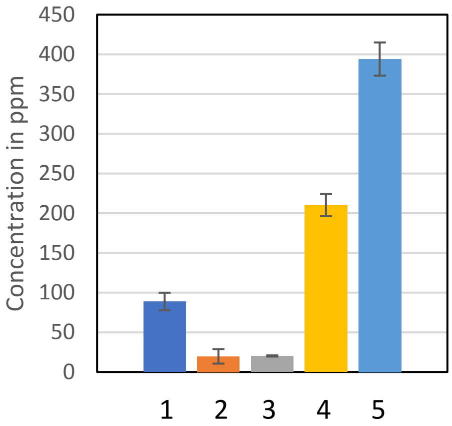


Figure 3.3: Sequential fractionation of Group 1A metals, Li (a), Na (b), K (c), and Cs (d) in Lufkin wastewater sludge (1 = exchangeable fraction, 2 = adsorbed fraction, 3 = organically bonded fraction, 4 = carbonate fraction, and 5 = sulfide/residual fraction).

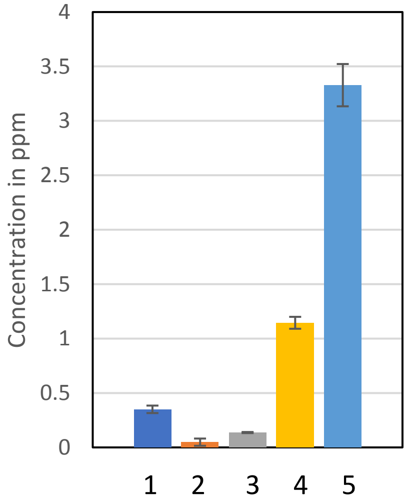
a) Mg



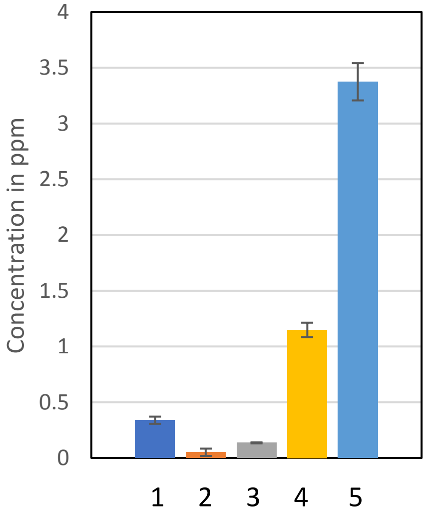
b) Ca



c) ^{83}Sr



d) ^{80}Sr



e) Ba

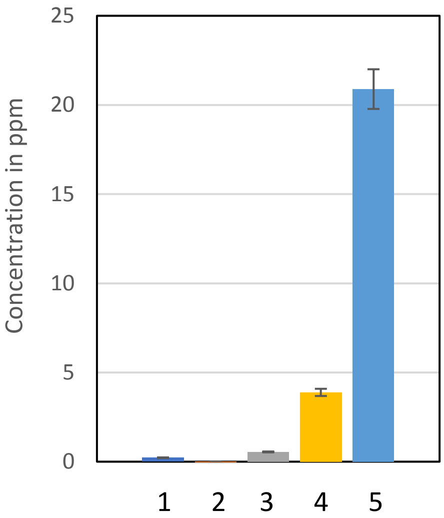
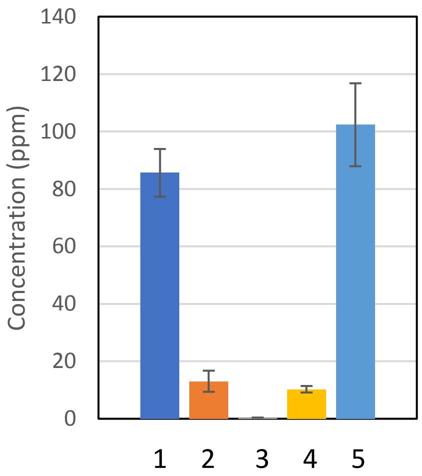
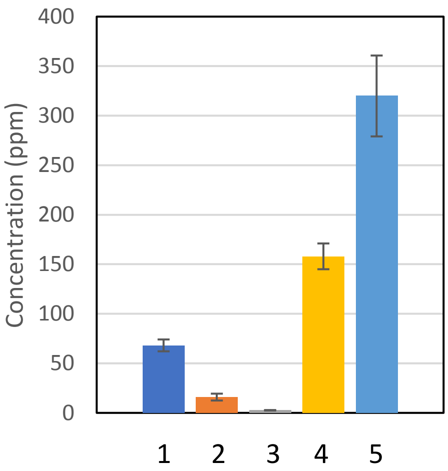


Figure 3.4: Sequential fractionation of Group 2A metals, Mg (a), Ca (b), ^{83}Sr (c), ^{80}Sr (d), and Ba (e) in soil therapy compost (1 = exchangeable fraction, 2 = adsorbed fraction, 3 = organically bonded fraction, 4 = carbonate fraction, and 5 = sulfide/residual fraction).

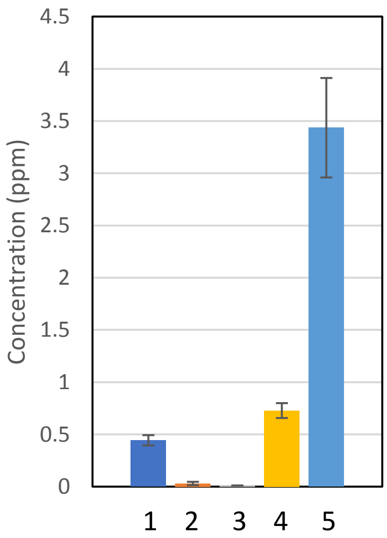
a) Mg



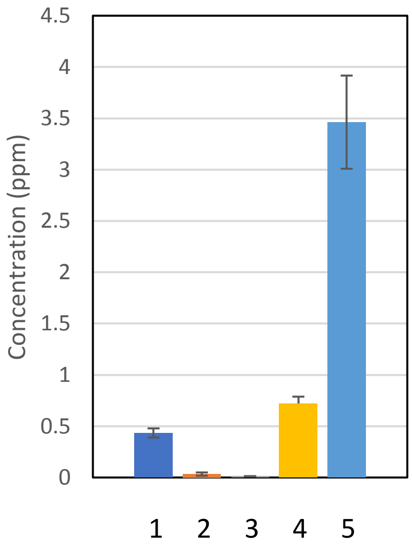
b) Ca



c) ^{83}Sr



d) ^{80}Sr



e) Ba

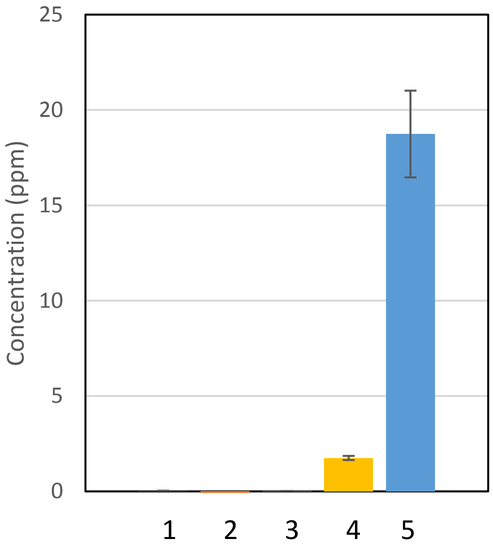
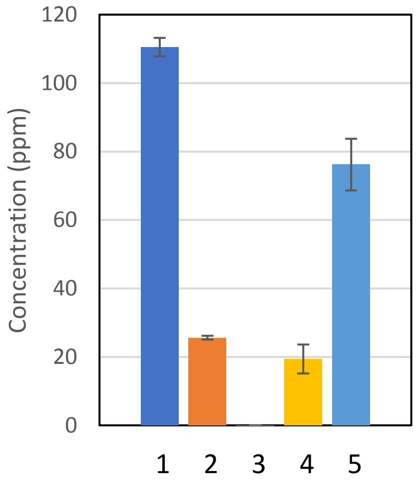
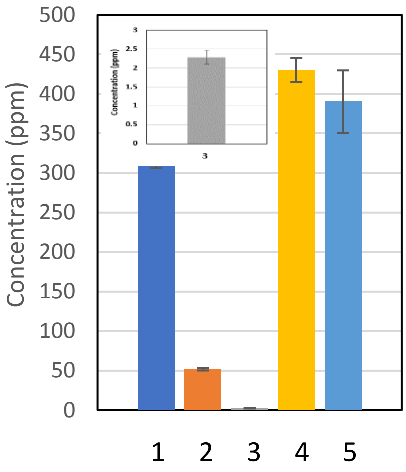


Figure 3.5: Sequential fractionation of Group 2A metals, Mg (a), Ca (b), ^{83}Sr (c), ^{80}Sr (d), and Ba (e) in Nacogdoches wastewater sludge (1 = exchangeable fraction, 2 = adsorbed fraction, 3 = organically bonded fraction, 4 = carbonate fraction, and 5 = sulfide/residual fraction).

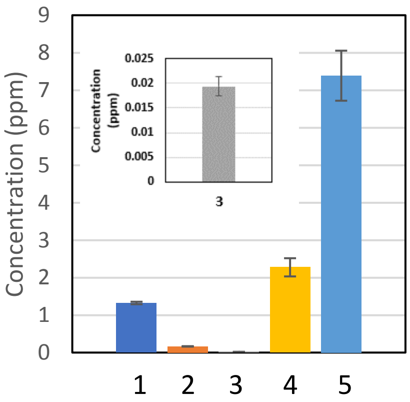
a) Mg



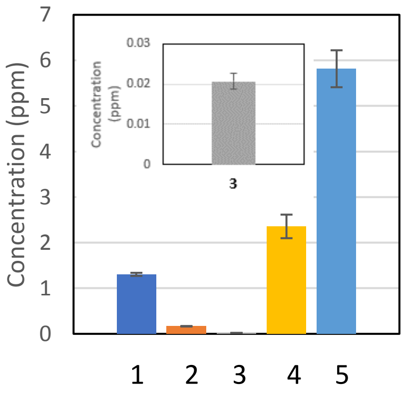
b) Ca



c) ^{83}Sr



d) ^{80}Sr



e) Ba

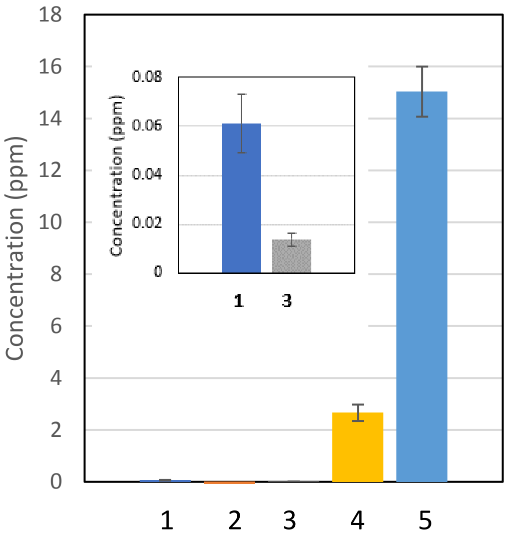
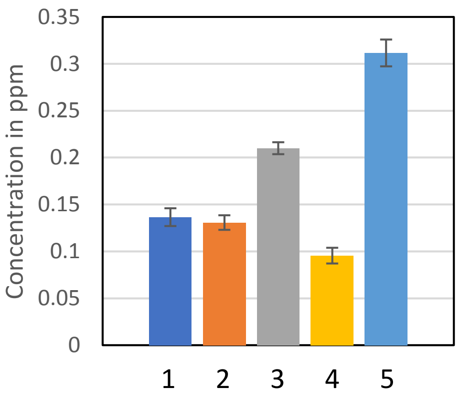


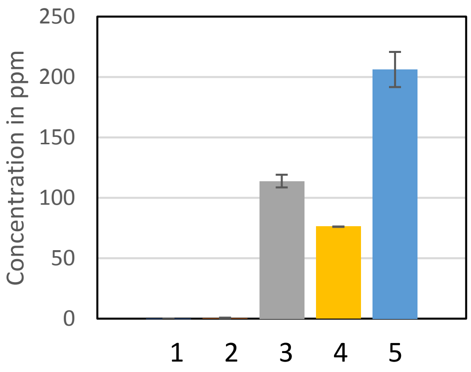
Figure 3.6: Sequential fractionation of Group 2A metals, Mg (a), Ca (b), ^{83}Sr (c), ^{80}Sr (d), and Ba (e) in Lufkin wastewater sludge (1 = exchangeable fraction, 2 = adsorbed fraction, 3 = organically bonded fraction, 4 = carbonate fraction, and 5 = sulfide/residual fraction).

I)

a) B

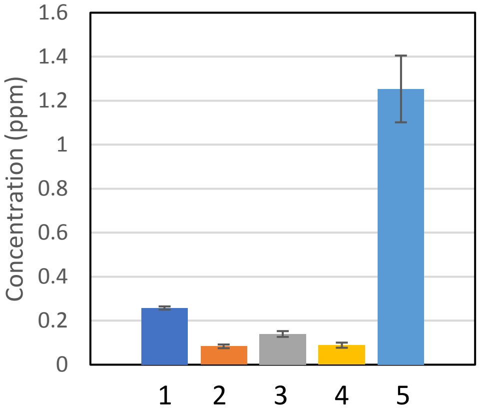


b) Al

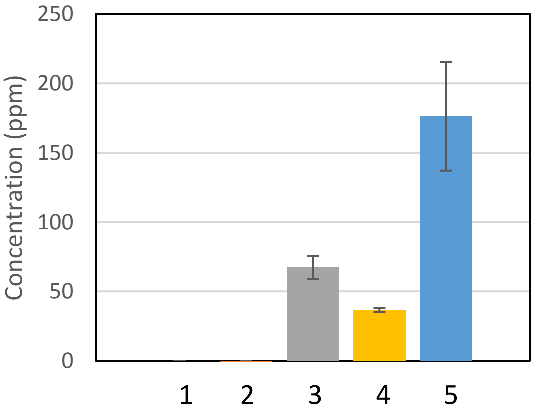


II)

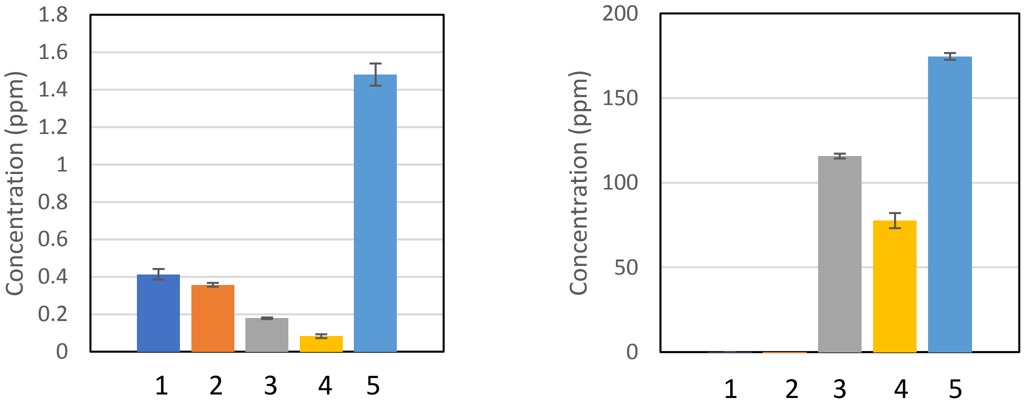
a) B



b) Al



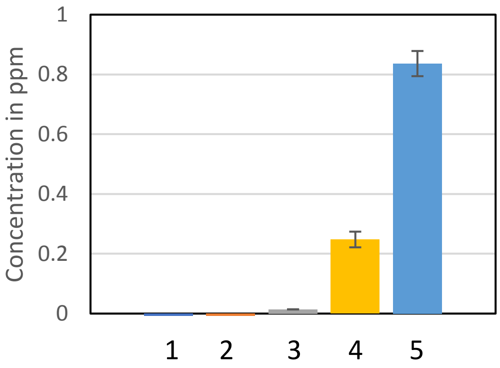
III)


a) B

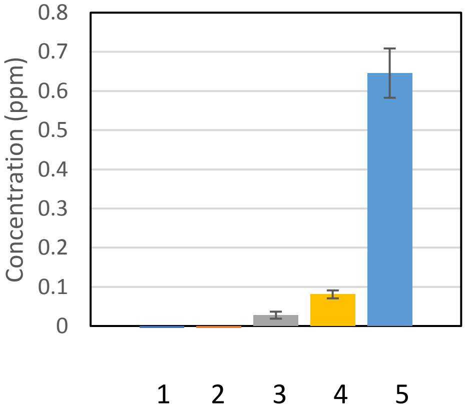
b) Al

Figure 3.7: Sequential fractionation of B and Al in STC (panel I), NWWS (panel II), and LWWS (panel III). (1 = exchangeable fraction, 2 = adsorbed fraction, 3 = organically bonded fraction, 4 = carbonate fraction, and 5 = sulfide/residual fraction).

a) Pb



b)



c) Pb

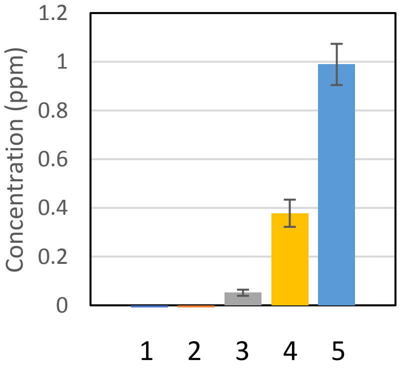
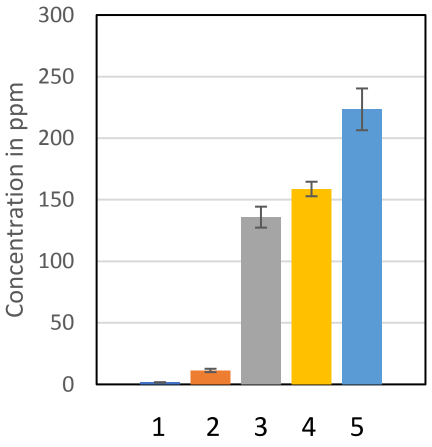


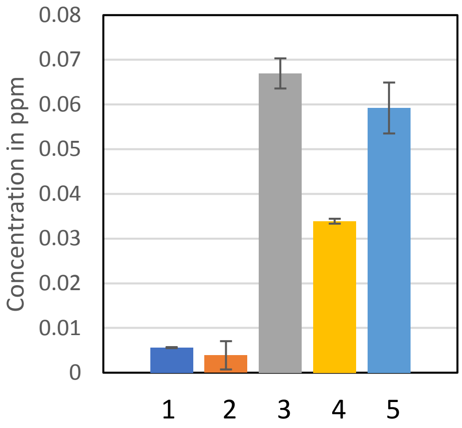
Figure 3.8: Sequential fractionation of Pb in STC (a), Nacogdoches (b), and Lufkin (c) Lufkin wastewater sludge (1 = exchangeable fraction, 2 = adsorbed fraction, 3 = organically bonded fraction, 4 = carbonate fraction, and 5 = sulfide/residual fraction).

I)

a)

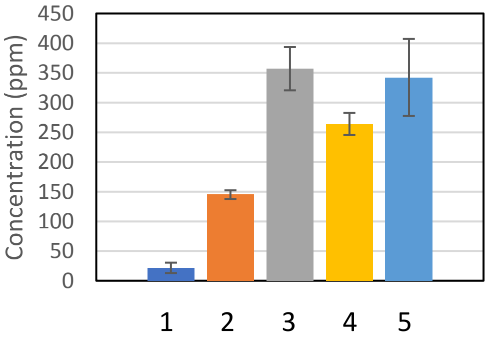


b) As

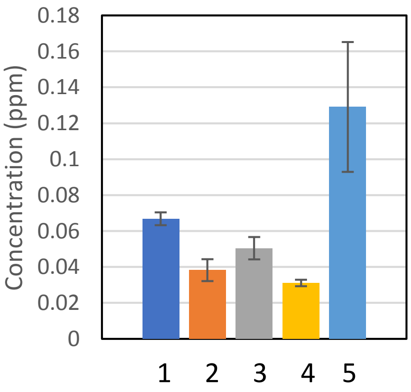


II)

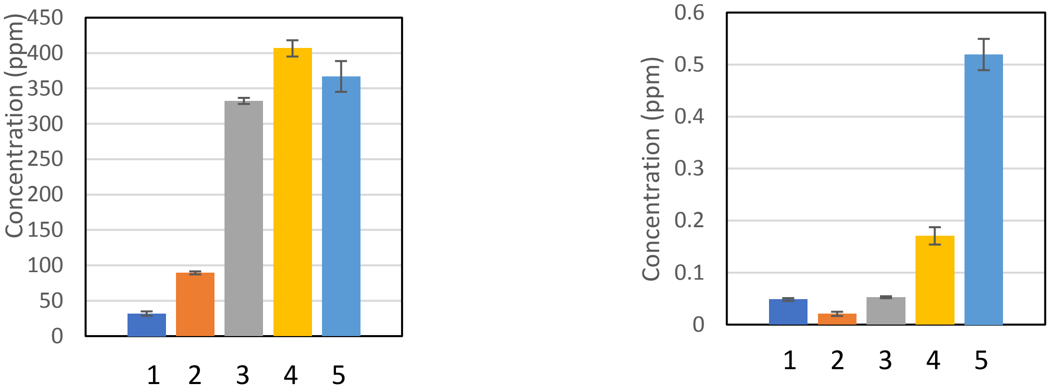
a) P



b) As



III)

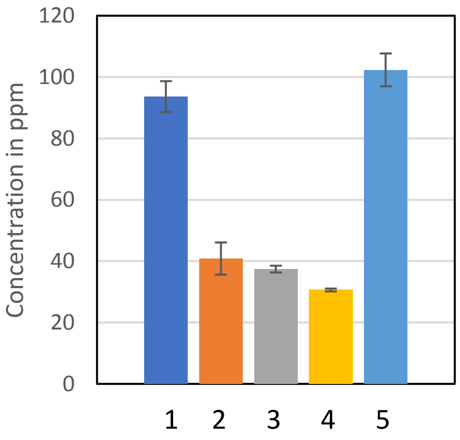

a) P

b) As

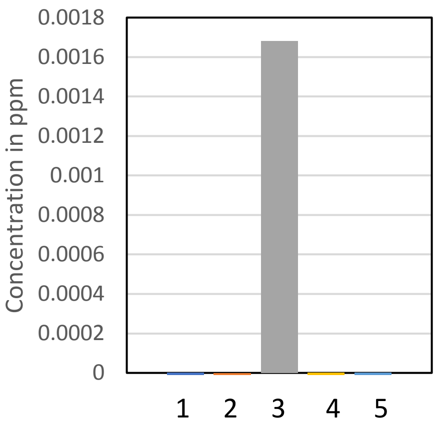
Figure 3.9: Sequential fractionation of (P, As) in STC (panel I), NWWS (panel II), and LWWS (panel III). (1 = exchangeable fraction, 2 = adsorbed fraction, 3 = organically bonded fraction, 4 = carbonate fraction, and 5 = sulfide/residual fraction).

I)

a) S

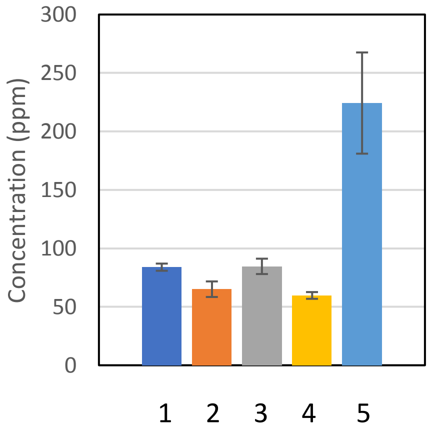


b) Se

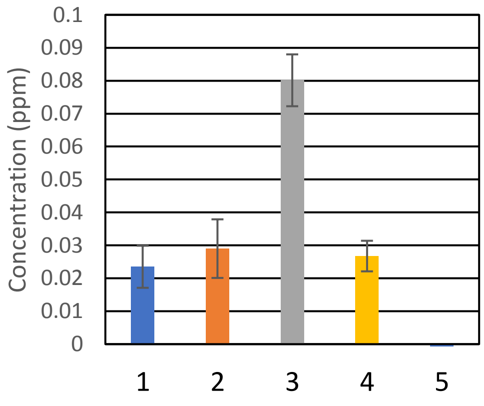


II)

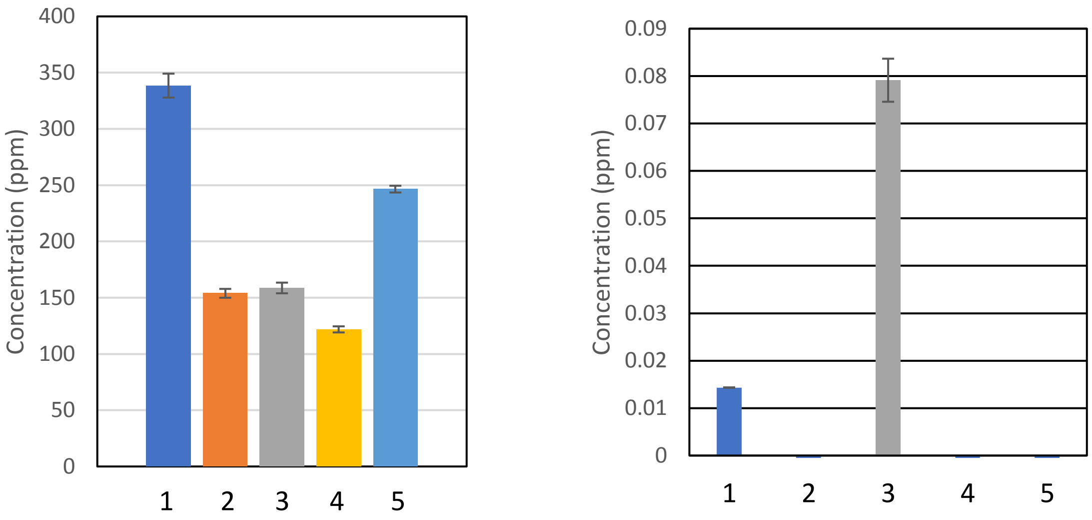
a) S



b) Se



III)

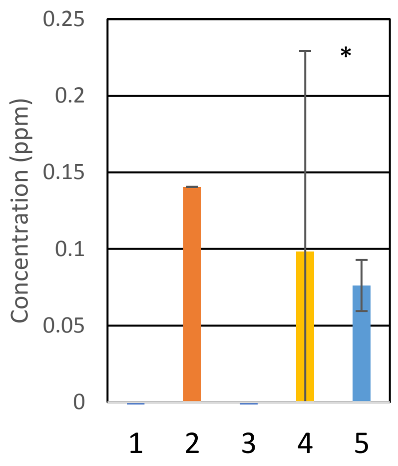


a) S

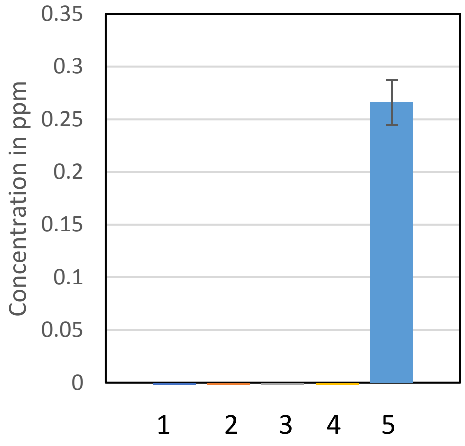
b) Se

Figure 3.10: Sequential fractionation of S, Se in STC (panel I), NWWS (panel II), and LWWS (panel III). (1 = exchangeable fraction, 2 = adsorbed fraction, 3 = organically bonded fraction, 4 = carbonate fraction, and 5 = sulfide/residual fraction).

a) Cd

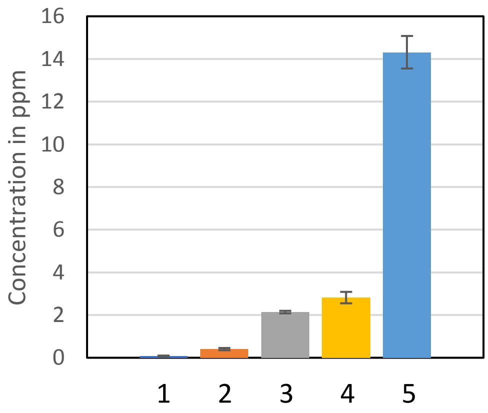


b) Co

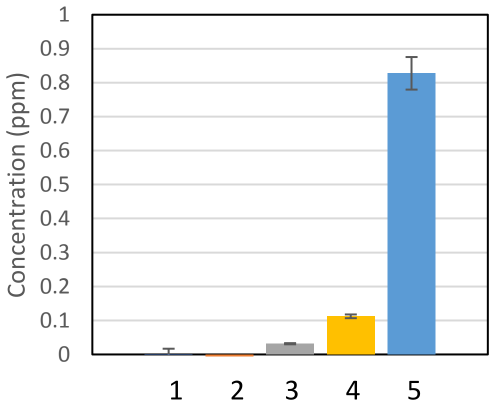


*The error bar on the carbonate fraction of Cd is as a result of the different amount recovered from the triplicate samples during extraction.

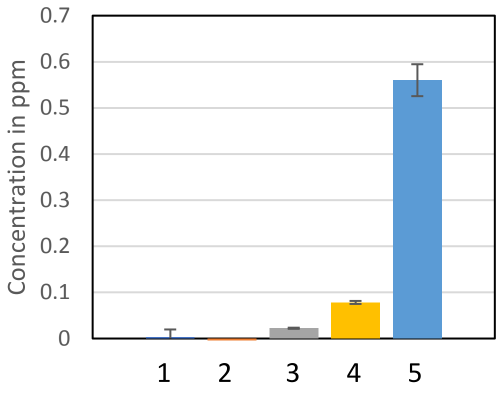
c) Cu



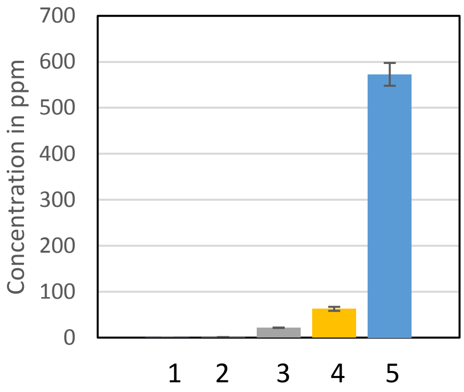
d) ^{119}Cr



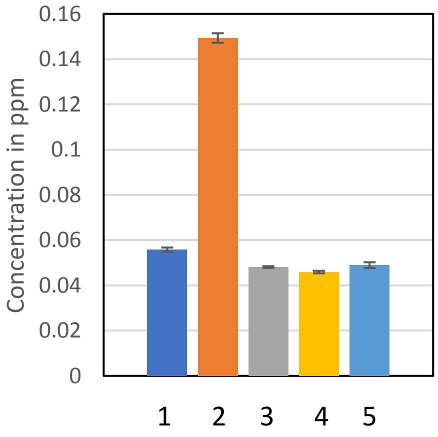
e) ^{126}Cr



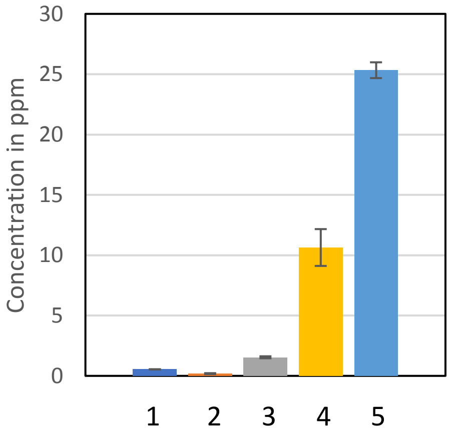
f) Fe

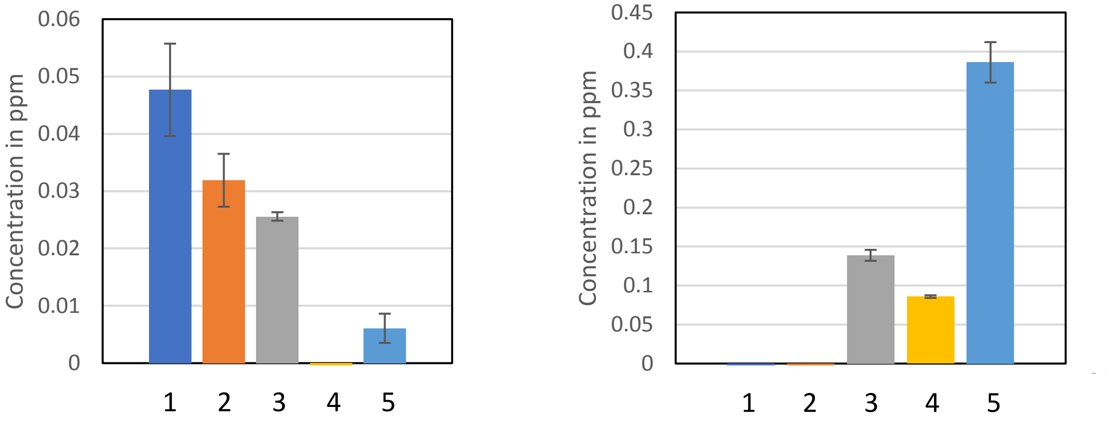


g) Hg

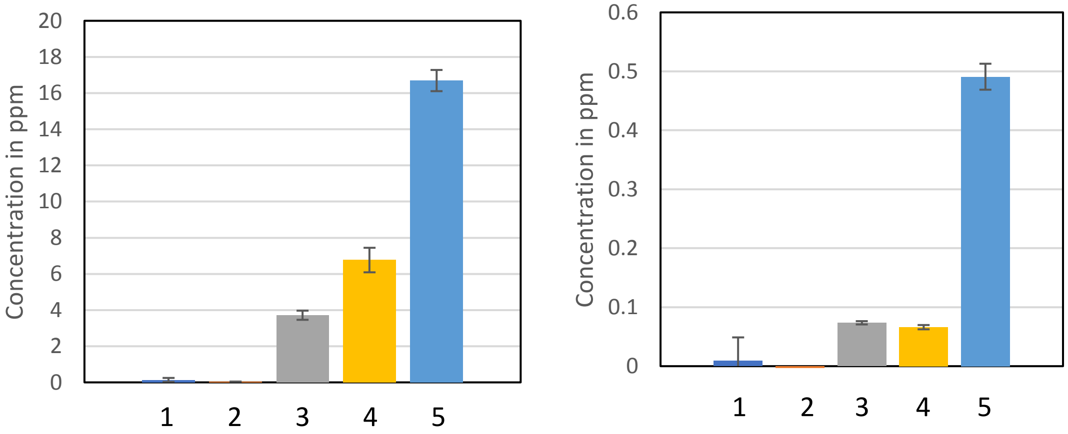


h) Mn




i) Mo

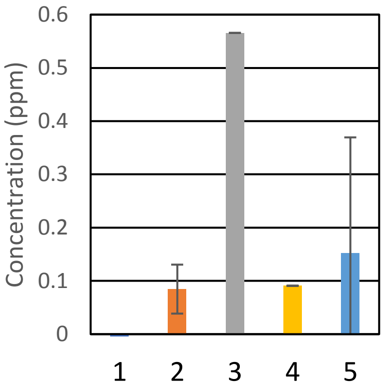
j) V


k) Zn

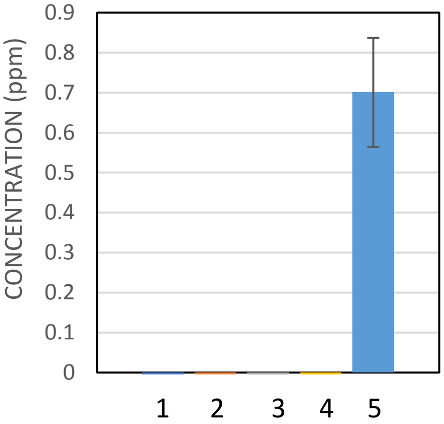
l) Ni

Figure 3.11: Sequential fractionation of Cd (a), Co (b), Cu (c), Cr (119) (d), Cr (126) (e), Fe (f), Hg (g), Mn (h), Mo (i), V (j), Zn (k), and Ni (l) in soil therapy compost (1 = exchangeable fraction, 2 = adsorbed fraction, 3 = organically bonded fraction, 4 = carbonate fraction, and 5 = sulfide/residual fraction).

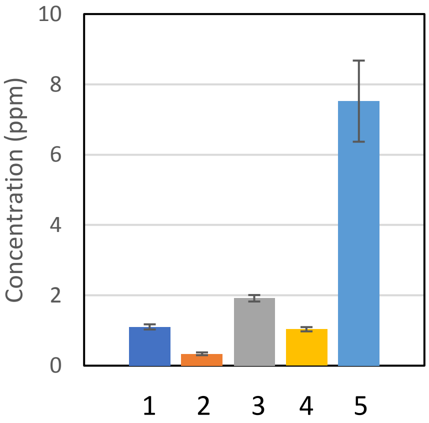
a) Cd



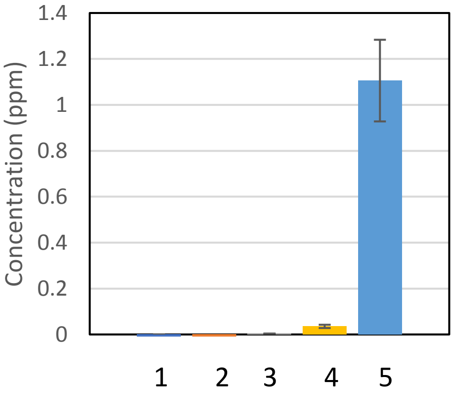
b) Co



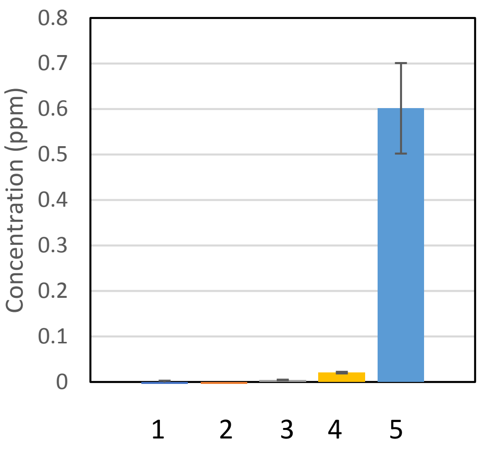
c) Cu



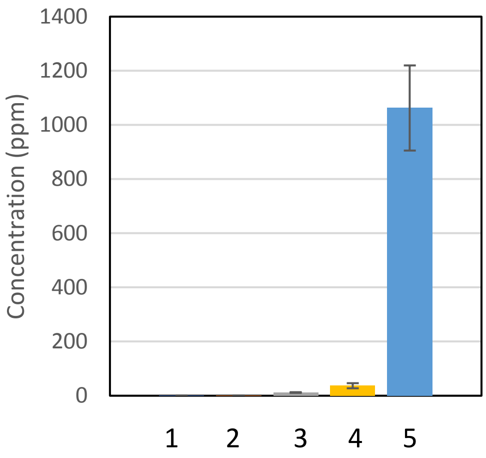
d) ^{119}Cr



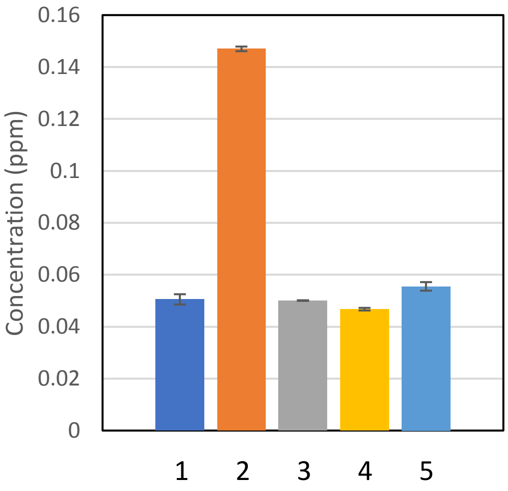
e) ^{126}Cr



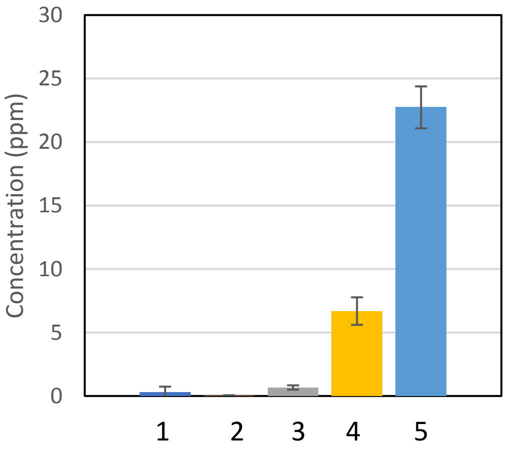
f) Fe



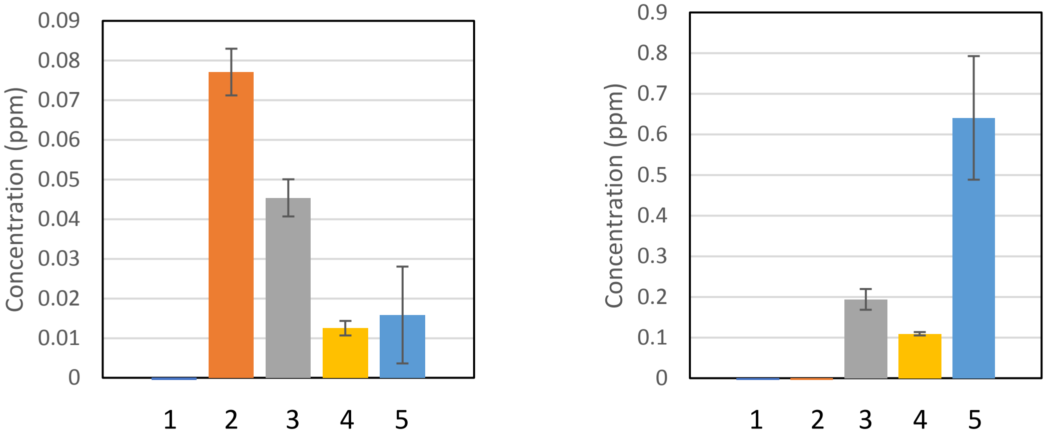
g) Hg



h) Mn



i) Mo j) V



k) Zn l) Ni

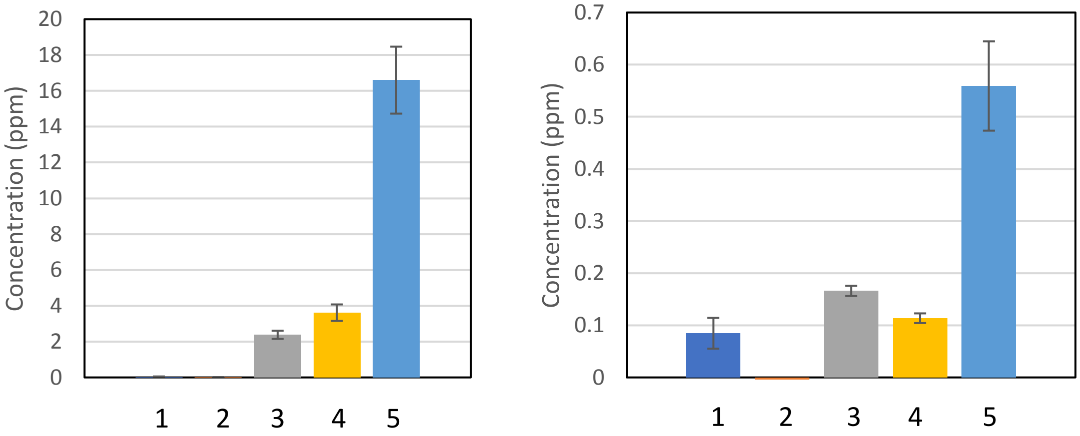
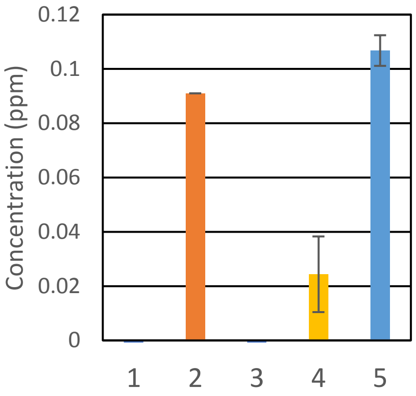
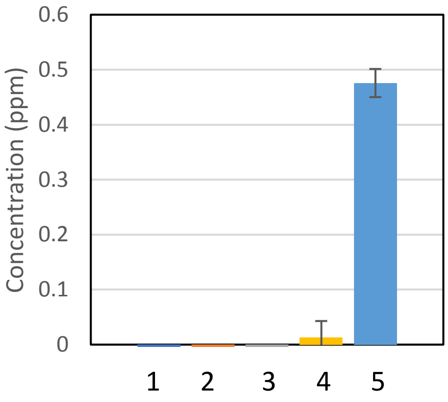


Figure 3.12: Sequential fractionation of Cd (a), Co (b), Cu (c), Cr (119) (d), Cr (126) (e), Fe (f), Hg (g), Mn (h), Mo (i), V (j), Zn (k), and Ni (l) in Nacogdoches wastewater sludge (1 = exchangeable fraction, 2 = adsorbed fraction, 3 = organically bonded fraction, 4 = carbonate fraction, and 5 = sulfide/residual fraction).

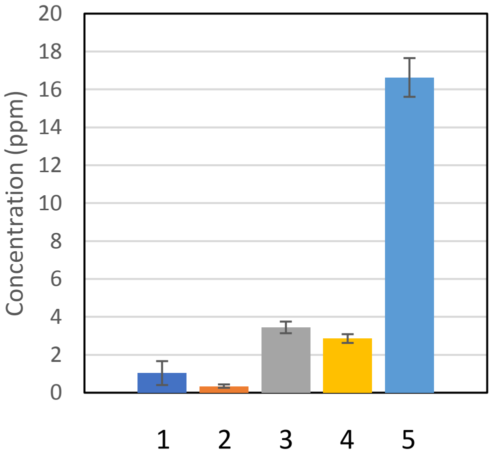
a) Cd



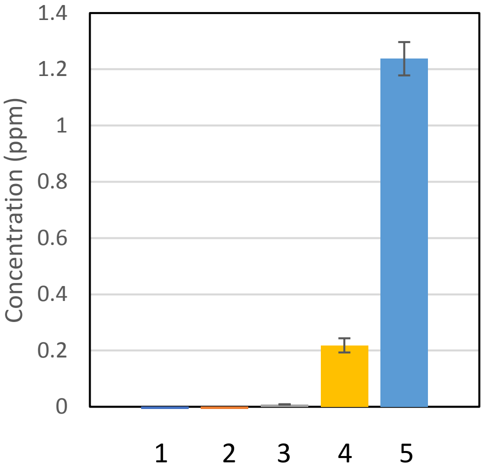
b) Co



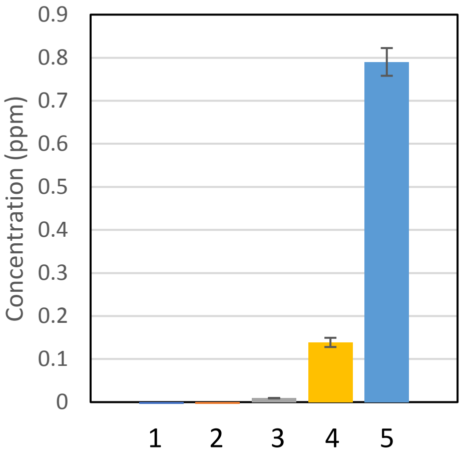
c) Cu



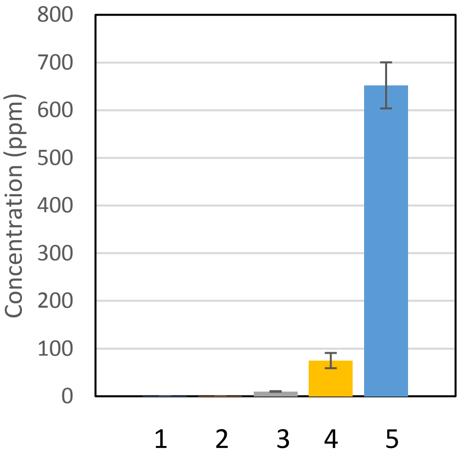
d) ^{119}Cr



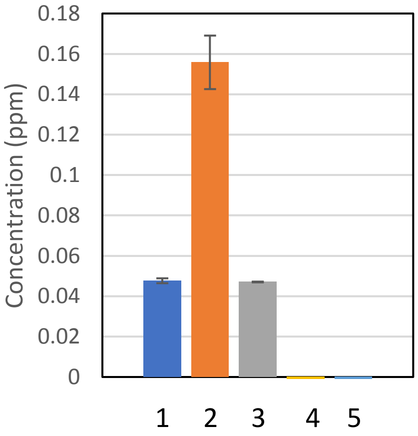
e) ^{126}Cr



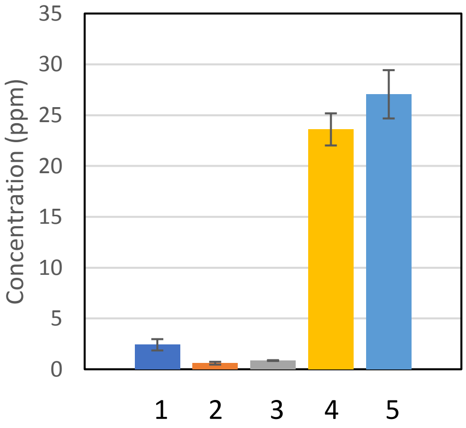
f) Fe

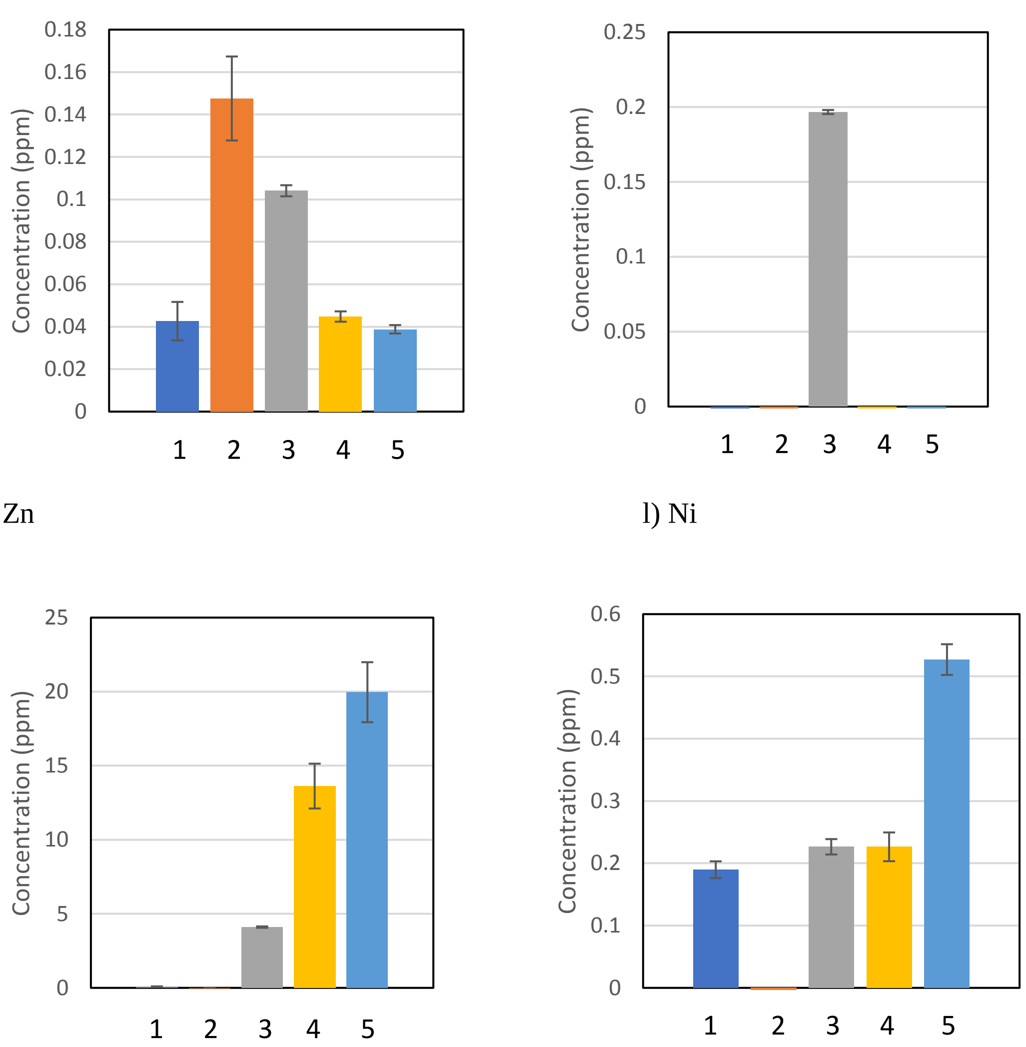


g) Hg



h) Mn





i) Mo

j) V

k) Zn

l) Ni

Figure 3.13: Sequential fractionation of Cd (a), Co (b), Cu (c), Cr (119) (d), Cr (126) (e), Fe (f), Hg (g), Mn (h), Mo (i), V (j), Zn (k), and Ni (l) in Lufkin wastewater sludge (1 = exchangeable fraction, 2 = adsorbed fraction, 3 = organically bonded fraction, 4 = carbonate fraction, and 5 = sulfide/residual fraction).

Table 3.2: Concentration (ppm) of metals in different chemical fractions in Soil therapy compost (STC). (Bd = below detection)

Elements	Exchangeable Fraction (ppm)	Adsorbed Fraction (ppm)	Organically bonded Fraction (ppm)	Carbonate Fraction (ppm)	Sulfide/residual Fraction (ppm)
As	0.11 ± 0.00	0.08 ± 0.00	0.07 ± 0.03	1.3 ± 0.0	1.2 ± 0.0
B	2.70 ± 0.01	2.60 ± 0.01	4.2 ± 0.0	1.90 ± 0.01	6.20 ± 0.01
Ba	4.40 ± 0.02	Bd	11 ± 0	78 ± 0	418 ± 1
Cd	Bd	0.14 ± 0.00	Bd	0.09 ± 0.01	0.08 ± 0.02
Ca	1774 ± 11	394 ± 9	404 ± 1	4206 ± 14	7882 ± 21
Cu	2 ± 0	8.0 ± 0.1	43.0 ± 0.1	56.0 ± 0.3	286 ± 1
Fe	4.0 ± 0.1	22.0 ± 0.3	430 ± 1	1252 ± 4	11454 ± 25
Hg	1.1 ± 0.0	3.0 ± 0.0	0.96 ± 0.00	0.92 ± 0.00	0.98 ± 0.00
K	55000 ± 228	50140 ± 360	4888 ± 7	1760 ± 2	2238 ± 13
Mg	344 ± 2	73 ± 2	10.2 ± 0.1	115 ± 1	826 ± 3
Mn	11 ± 0	3.70 ± 0.04	31 ± 0	213 ± 2	507 ± 1
Mo	0.95 ± 0.01	0.64 ± 0.01	0.51 ± 0.00	Bd	0.12 ± 0.00
Na	536 ± 1	107 ± 2	51120 ± 123	17661 ± 67	12356 ± 55
P	32 ± 0	227 ± 1	2718 ± 9	3174 ± 6	4468 ± 17
Zn	2.5 ± 0.1	0.64 ± 0.01	74.0 ± 0.3	135 ± 1	334 ± 1
S	1873 ± 5	817 ± 5	748 ± 1	620 ± 1	2046 ± 5
^{83}Sr	7 ± 0	1 ± 0	2.70 ± 0.01	23.0 ± 0.1	67.00 ± 0.05
^{80}Sr	6.80 ± 0.03	1.0 ± 0.0	2.70 ± 0.01	23.0 ± 0.1	67.5 ± 0.2
Pb	Bd	Bd	0.28 ± 0.00	4.90 ± 0.03	16.73 ± 0.04
Co	Bd	Bd	Bd	Bd	5.32 ± 0.02
V	Bd	Bd	2.70 ± 0.01	1.700 ± 0.002	7.7 ± 0.03
Se	Bd	Bd	0.034 ± 0.023	Bd	Bd
Ni	0.17 ± 0.04	Bd	1.500 ± 0.003	1.300 ± 0.004	10.00 ± 0.02
Al	Bd	4.0 ± 0.6	2278 ± 5	1522 ± 0	4124 ± 15
^{119}Cr	Bd	Bd	0.630 ± 0.002	2.2 ± 0.0	17 ± 0
^{126}Cr	0.05 ± 0.02	Bd	0.45 ± 0.00	1.5 ± 0.0	11 ± 0
Li	Bd	Bd	Bd	Bd	1.90 ± 0.01
Cs	3.1 ± 0.1	Bd	163 ± 0	102 ± 0.2	78 ± 0

Table 3.3: Concentration (ppm) of elements in different chemical fractions in Nacogdoches wastewater sludge (NWWS). (Bd = below detection)

Elements	Exchangeable Fraction (ppm)	Adsorbed Fraction (ppm)	Organically Bonded Fraction (ppm)	Carbonate Fraction (ppm)	Sulfide/Residual Fraction (ppm)
As	1.3 ± 0.0	0.80 ± 0.01	1.00 ± 0.01	0.6 ± 0.0	2.60 ± 0.04
B	5.20 ± 0.01	1.70 ± 0.01	2.80 ± 0.01	1.80 ± 0.01	25 ± 0
Cd	Bd	0.085 ± 0.05	0.6 ± 0.0	0.1 ± 0.0	0.15 ± 0.22
Ca	1363 ± 6	319 ± 3	53 ± 0	3160 ± 13	6398 ± 41
Cu	22 ± 0	6.8 ± 0.040	38.0 ± 0.1	21.0 ± 0.1	150 ± 1
Fe	1.90 ± 0.04	15 ± 0.059	237 ± 1	738 ± 9	21260 ± 157
Hg	1.0 ± 0.0	2.9 ± 0.001	1 ± 0	0.940 ± 0.001	1.000 ± 0.002
K	48500 ± 96	69460 ± 109	6838 ± 27	3538 ± 12	4774 ± 46
Mg	1712 ± 8	261 ± 4	6.8 ± 0.1	205 ± 1	2048 ± 14
Mn	5.7 ± 1	0.66 ± 0.031	13.0 ± 0.2	134 ± 1	455 ± 2
Mo	Bd	1.5 ± 0.01	0.91 ± 0.01	0.25 ± 0.00	0.32 ± 0.01
Na	1769 ± 1	745 ± 4	45440 ± 55	24200 ± 18	25540 ± 103
P	434 ± 9	2898 ± 7	7142 ± 36	5278 ± 19	6846 ± 65
Se	0.47 ± 0.01	0.58 ± 0.010	2.00 ± 0.01	0.53 ± 0.01	Bd
Zn	0.45 ± 0.03	0.15 ± 0.012	48 ± 0	72 ± 1	332 ± 2
S	1678 ± 3	1301 ± 7	1692 ± 7	1194 ± 3	4486 ± 43
Ni	1.70 ± 0.03	Bd	3.3 ± 0.0099	2.00 ± 0.01	11.0 ± 0.09
^{83}Sr	9 ± 0	0.67 ± 0.016	0.240 ± 0.001	14.0 ± 0.1	69 ± 1
^{80}Sr	9 ± 0	0.67 ± 0.016	0.240 ± 0.001	14.0 ± 0.1	69 ± 1
Cs	11.0 ± 0.1	7 ± 0.11	159 ± 0	119 ± 0	114.0 ± 0.1
Pb	Bd	Bd	0.55 ± 0.01	2.00 ± 0.01	13.0 ± 0.1
Co	Bd	Bd	Bd	Bd	14 ± 0
Ba	0.34 ± 0.02	Bd	0.370 ± 0.002	35,0 ± 0.1	374 ± 2
V	Bd	Bd	4.00 ± 0.03	2.200 ± 0.004	13 ± 0
Al	Bd	Bd	1344 ± 8	733 ± 2	3525 ± 39
^{119}Cr	Bd	Bd	0.066 ± 0.001	0.70 ± 0.01	22.0 ± 0.2
^{126}Cr	Bd	Bd	0.088 ± 0.001	0.410 ± 0.002	12.0 ± 0.1
Li	Bd	Bd	Bd	Bd	1.20 ± 0.01
Co	Bd	Bd	Bd	Bd	14.0 ± 0.1
Ba	0.34 ± 0.02	Bd	0.370 ± 0.002	35 ± 0	375 ± 2

Table 3.4: Concentration (ppm) of elements in different chemical fractions in Lufkin wastewater sludge (LWWS). (Bd = below detection)

Elements	Exchangeable Fraction (ppm)	Adsorbed Fraction (ppm)	Organically Bonded Fraction (ppm)	Carbonate Fraction (ppm)	Sulfide/Residual Fraction (ppm)
As	0.970 ± 0.003	0.41 ± 0.00	1.100 ± 0.002	3.40 ± 0.02	10 ± 0
B	8.30 ± 0.03	7.1 ± 0.0	3.6 ± 0.0	1.70 ± 0.01	29 ± 0
Cd	Bd	0.1 ± 0.0	Bd	0.024 ± 0.014	0.11 ± 0.01
Ca	6310 ± 9	1032 ± 1	46.0 ± 0.2	8602 ± 15	7804 ± 39
Cu	21 ± 1	7.0 ± 0.1	69 ± 0	57 ± 0.23	333 ± 1
Fe	1.00 ± 0.01	6.2 ± 0.1	200 ± 1	1497 ± 16	13038 ± 48
Hg	0.95 ± 0.00	3.1 ± 0.0	0.94 ± 0.00	Bd	Bd
K	46720 ± 43	63260 ± 67	6242 ± 5	3684 ± 18	3652 ± 9
Mg	2210 ± 3	512 ± 1	Bd	388 ± 4	1524 ± 8
Mn	48 ± 1	12 ± 0	17 ± 0	472 ± 2	541 ± 2
Mo	0.85 ± 0.01	3 ± 0	2 ± 0	0.9 ± 0.0	0.78 ± 0.00
Na	2638 ± 2	9112 ± 2	46040 ± 14	27240 ± 15	20140 ± 82
P	637 ± 3	1787 ± 2	6648 ± 4	8130 ± 12	7338 ± 22
Zn	1.00 ± 0.03	Bd	82.00 ± 0.05	273 ± 2	399 ± 2
S	6770 ± 11	3078 ± 4	3174 ± 5	2438 ± 3	4930 ± 3
Ni	4.00 ± 0.01	Bd	5 ± 0	4.5 ± 0.0	11 ± 0
^{83}Sr	26 ± 0	3.30 ± 0.01	0.39 ± 0.00	46 ± 0.24	148 ± 1
^{80}Sr	26 ± 0	3.30 ± 0.01	0.41 ± 0.00	47 ± 0.3	116 ± 0
Cs	18 ± 0	6.7 ± 0.2	156 ± 0	69 ± 0	Bd
Pb	Bd	Bd	1.00 ± 0.012	7.6 ± 0.1	20 ± 0.
Co	Bd	Bd	Bd	0.27 ± 0.03	9.5 ± 0
Ba	1 ± 0	Bd	0.270 ± 0.003	53.0 ± 0.3	301 ± 1
Se	0.3 ± 0.0	Bd	2 ± 0	Bd	Bd
V	Bd	Bd	3.9 ± 0.0	Bd	Bd
Al	Bd	Bd	2315 ± 1	1552 ± 4	3492 ± 2
^{119}Cr	Bd	Bd	0.17 ± 0.00	4.4 ± 0.0	25 ± 0
^{126}Cr	Bd	Bd	0.18 ± 0.00	2.8 ± 0.0	16 ± 0
Li	Bd	Bd	Bd	Bd	0.68 ± 0.01

Table 3.5: Sum of concentrations (ppm) of metals in different chemical fractions and their total metal content in soil therapy compost (STC). (Bd = below detection)

Elements	Sum of fractions (ppm)	Concentration of total metal digestion (ppm)
As	3.4	5.5 ± 0.6
B	18	Bd
Ba	511	308 ± 14
Cd	0.31	0.19 ± 0.07
Ca	14660	11760 ± 1358
Cu	395	338 ± 14
Fe	13162	13352 ± 2731
Hg	7.0	Bd
K	114026	2207 ± 82
Mg	1369	1362 ± 98
Mn	765	794 ± 39
Mo	2.2	3.9 ± 0.9
Na	81780	668 ± 92
P	10619	8623 ± 426
Zn	547	409 ± 14
S	6104	2838 ± 89
^{83}Sr	100	-
^{80}Sr	101	-
Pb	22	17 ± 1
Co	5.3	14 ± 0
V	12	12 ± 2
Se	0.034	Bd
Ni	13	17 ± 0
Al	7928	12424 ± 824
^{119}Cr	19	4.3 ± 0.2
^{126}Cr	13	-
Li	1.9	-
Cs	347	-

Table 3.6: Sum of concentrations (ppm) of metals in different chemical fractions and their total metal content in Nacogdoches Wastewater Sludge (NWWS). (Bd = below detection)

Elements	Sum of fractions (ppm)	Concentration of total metal digestion (ppm)
As	6.3	12 ± 1
B	36	107 ± 21
Cd	0.90	Bd
Ca	11294	18738 ± 1644
Cu	238	386 ± 35
Fe	22252	32890 ± 2695
Hg	7.0	Bd
K	133110	6126 ± 229
Mg	4234	7293 ± 647
Mn	608	1136 ± 102
Mo	3.0	7.3 ± 1
Na	97693	Bd
P	22598	26102 ± 1522
Se	3.2	Bd
Zn	452	810 ± 71
S	10351	8365 ± 331
Ni	18	44 ± 4
^{83}Sr	93	-
^{80}Sr	93	-
Cs	410	-
Pb	15	27 ± 1
Co	14	26 ± 4
Ba	411	563 ± 45
V	19	33 ± 3
Al	5603	15591 ± 1692
^{119}Cr	23	17 ± 2
^{126}Cr	13	-
Li	1.2	-

Table 3.7: Sum of concentrations (ppm) of metals in different chemical fractions and their total metal content in Lufkin Wastewater Sludge (LWWS). (Bd = below detection)

Elements	Sum of fractions (ppm)	Concentrations of total metal digestion (ppm)
As	16.24	20 ± 3
B	50.25	Bd
Cd	0.22	0.20 ± 0.03
Ca	23793	21552 ± 3186
Cu	486	531 ± 78
Fe	14743	15163 ± 2212
Hg	5.0	Bd
K	123558	3328 ± 548
Mg	4633	4116 ± 561
Mn	1090	12623 ± 192
Mo	7.6	7.7 ± 1.3
Na	105170	524 ± 696
P	24540	20855 ± 2594
Zn	755	883 ± 127
S	20390	12116 ± 1784
Ni	23	33 ± 1.2
^{83}Sr	223.39	-
^{80}Sr	193.32	-
Cs	249.21	-
Pb	28	29 ± 3
Co	9.8	25 ± 4
Ba	355	319 ± 44
Se	1.9	Bd
V	3.9	7.8 ± 1.3
Al	7360	12217 ± 1985
^{119}Cr	29	11 ± 2
^{126}Cr	19	-
Li	0.68	-

CHAPTER 4

INFLUENCE OF BIOSOLIDS UPON THE UPTAKE OF Mn and Cd BY RADISH (*Raphanus sativus L.*)

4.1 ABSTRACT

The use of biosolids in agriculture provides an essential source of plant nutrients and organic matter necessary for plant growth. But it can also result in the incorporation of heavy metals into the soil and the uptake by plants. The uptake of metals by plants depends on the nature of metals present in the biosolids. In this study, the influence of biosolids upon uptake of metals by radish was determined by conducting pot experiments with radish (*Raphanus sativus L.*) cultivated in perlite amended with biosolids at different compositions (0, 25, 50, 75, 100% w/w) treated with 100 ppm Mn and 100 ppm Cd. The effect of pH changes upon uptake of metals by plants was determined by cultivating radish (*Raphanus sativus L.*) at pH 6.70 and 7.30. The radish plants were harvested after 6 weeks and analyzed for Mn, Cd and other metals (See Appendix A). The radish cultivated at pH 6.70 showed an increase in plant biomass upon addition of biosolids. Similarly, radish plants cultivated in cadmium treatment at pH 7.30 showed an increase in plant's biomass up to 25% (w/w) biosolid composition followed by a decrease in biomass through 100%. Analysis of metal concentration in the root, shoot, and leaves of radish showed that Mn is accumulated in the leaves. However, Cd was mostly

accumulated in greater amounts in the root of radish plants. Comparatively, the [Cd] and [Mn] were higher in the roots than in the shoots or leaves at pH 6.70 than 7.30. Examination of pH in all pot samples after harvesting radish plants showed a progressive slight decrease in pH in samples treated with from 0% to 100% w/w STC.

4.2 INTRODUCTION

In the previous chapters, the spectroscopic characterization and thermal analysis of biosolids were discussed. In addition, the speciation and bioavailability of some metals in the biosolid samples were investigated. Mn and Cd were found in the bioavailable fraction of the biosolid samples (Mn: STC (2 ± 0%), NWWS (1.1 ± 0.1%), LWWS (6 ± 1 %), Cd: STC (47 ± 0%), NWWS (9.5 ± 0.1%), LWWS (40 ± 0%)).

In this chapter, the influence of biosolids and the effect of pH changes on metal uptake by radish plants (*Raphanus sativus L.*) is examined and discussed. This analysis is important for the assessment of the impact of the land application of biosolids to plants and humans. The effect of biosolids upon uptake of Mn (an essential metal), and Cd (a non-essential toxic metal) was determined by cultivating radish (*Raphanus sativus L.*) in perlite-soil therapy compost mixtures.

4.2.1 Sample studies of Mn treatments and their effects on plant biomass

Manganese is one of the essential nutrients necessary for plant growth.[1] Processes such as photosynthesis, nitrogen metabolism and chloroplast formation in plants depend on manganese.[2] This significant role of Mn in plants is very important. Soils with neutral to high pH usually are usually deficient in Mn. Deficiency of Mn in plants can lead to yellowing of leaves and internal and interveinal chlorosis.[2] The low phloem mobility of Mn results in typical yellowing of leaves which is usually found in younger leaves.

Manganese is an essential element for human health, and is needed for development, metabolism, growth, and antioxidant system.[3] Although Mn is an essential element, chronic exposure or ingestion can lead to manganism, a condition that causes neuronal death with symptoms associated with Parkinson's disease.[3] Different studies have been carried out to determine the influence of biosolids or other organic wastes on the uptake of Mn by plants. Maftoun et al.[4] determined the effect of two organic wastes in combination with phosphorus on growth and chemical composition of spinach and soil properties. The study used four levels of composted waste (0, 1, 2 and 4% v/v), five rates of poultry manure (0, 1, 2, 3 and 4% v/v) and three levels of phosphorus (0, 25, and 50 mg kg^{-1}) as KH_2PO_4. From the study, it was found that the concentration of Mn decreased upon increase in the applied soil phosphorus. Spinach plants cultivated in both organic wastes accumulated more Mn concentration than the control plants. Spinach plants cultivated in soil amended with composted waste had higher concentrations of manganese compared to those cultivated in poultry manure.[4]

Ramachandran and D'Souza[5] investigated the uptake of Mn by maize in soils amended with sewage sludge and city compost. The results obtained showed that Mn concentrations in the maize reduced significantly with increasing amounts of sludge used to amend the soil. The decrease in Mn concentration was attributed to the formation of insoluble organic complexes of Mn (with the organic matter from the biosolids).[5]

Hechman et al.[6] investigated the residual effects of sewage sludge on soybean. In this study, soybean was cultivated in different sludge rate (0, 56, 112, 224, 336 and 448 Mg ha^{-1}) and pH 5.1 – 7.4. The results obtained, showed that the sludges added more Mn to the soil. The Mn content in soybean was affected by the soil's Mn content and the soil pH. The highest Mn concentration were found in control plots with low pH.[6]

Jamil and Bayan[7] investigated the uptake of nutrients and heavy metals in lettuce in response to sewage application rate to calcareous soils. It was found that the concentration of Mn in the shoot of the lettuce was the highest at the two highest sewage sludge application rates (80 and 160 Kg ha^{-1}). No significant difference in Mn concentration were found at other sewage sludge application rates (0, 40, 60 Kg ha^{-1}).[7]

Garcia et al.[10] investigated the translocation and accumulation of heavy metals in the tissues of corn plants grown on sludge-treated strip-mined soils. The study found the highest concentration of Mn in the leaves. In addition, soils at lower pH had more Mn available to the corn plant.[8]

Sample Cadmium Studies, and Uptake by Biosolids

Cadmium is a trace element needed by animals, plants and humans. The concentration of Cd in ores is low. Greenockite (CdS) is the major mineral of cadmium of importance. It is almost related to sphalerite (ZnS).[9] It is also notable that Cd can complex with other organic compounds in soil.[10,11] Cadmium has also been found in atmospheric trace amounts (0.27 – 15.5 ng/m^3).[12,13]

The major effect of cadmium, either through digestion or inhalation from aerosols is proteinuria; a condition that affects the kidney.[14] Chronic exposure to cadmium can lead to renal failure, obstructive lung disease or cancer[15] and bone defects at minimal concentrations.[16] Cadmium is a toxic metal known to be readily bioavailable. [17,18,19] Thus, cadmium can be taken up by plants if the soil is contaminated.

Different studies have been carried out to study biosolids uptake of cadmium. Brown et al.[19] conducted a study to determine the phytoavailability of cadmium in long term biosolids-amended soils managed at low and high pH. The study was started 13 – 15 years before planting. The biosolids used in the study had Cd concentrations of 13.4 and 210 mg kg^{-1}. Included in the study was a Cd salt treatment in which Cd was added to the soil at the same rate with the biosolids with Cd concentration at 100 Mg ha^{-1}. Lettuce (*Lactuca sativa var. longifolia*) were cultivated in all the treatments. It was found that less Cd was taken up by the lettuce cultivated in biosolids-amended soils than those cultivated in soils with treated Cd. It was observed that the concentration of Cd in lettuce cultivated in low Cd concentration biosolids was not quite different from the control.[19]

Chaney et al.[20] studied the relative uptake of cadmium by garden vegetables and fruits grown on long-term biosolids amended soil. The different vegetables (cabbage (*Brassica oleracea*), carrot (*Daucus carota*), potato (*Solanum tuberosum*), navy bean (*Phaseolus vulgaris*), tomato (*Solanum lycopersicum*), maize (*Zea mays*)) were selected from different classes or families cultivated on a long-term amended sludge and reference plot. This study was conducted at low and high pH levels to figure out the cadmium uptake patterns in relation to a reference crop, lettuce (*Lactuca sativa*), which was used as the indicator crop. The potential of a relative uptake index was examined. This relative uptake index can be used to determine the risk associated with transferring food cultivated with a cadmium-polluted soil to the food chain. It was found that in all vegetables, except for navy bean (*Phaseolus vulgaris*), the uptake of Cd was very low.[20]

Lavado et al.[21] studied how treatment of biosolids could affect availability and uptake of toxic elements Cd, Cr, Cu, Ni, and Pb in the soil. In this study, a hypothesis was put forward that non-digested biosolids have more potential toxic elements compared to the digested ones. In order to confirm the hypothesis, field experiments using maize (*Zea mays L.*) was set up using the digested biosolids and non-digested biosolids as treatments and with controls. It was discovered that the concentration of Cd in the maize plants and grains increased in the non-digested biosolids.[21]

Marta and Raúl [22] carried out research to determine the heavy metal content in lettuce plants cultivated in biosolid compost. Varying amounts of compost biosolids 0 - 100% w/w, was used to cultivate lettuce plant in greenhouse conditions. It was observed that the use of composted biosolid resulted in a 20% and 40% increment in the biomass accumulation. It was also discovered that the Cd concentrations in the leaves of the lettuce plants were found below detection in all treatments.[22] Garrido et al.[23] determined the influence of sewage sludge in soils upon the uptake of heavy metals by broad bean seeds (*Vicia faba L*.). The Cd was not detectable in the broad bean seeds, and a conclusion was drawn that the use of biosolids in broad bean crop might not involve any health or environmental risk.[23]

The objective of this study was to determine whether biosolids competitively uptake Mn vis-a-vis and therefore depriving the radish (*Raphanus sativus L.*) of the needed essential nutrient. In addition, the suitability and the risk of using composted wastewater sludge for land use was investigated via determining the concentration of Cd and Mn in the root, shoot, and leaves of radish (*Raphanus sativus L.*). Although the current research investigation was devoted to examining Mn and Cd uptake, other metals were also examined, and are presented in Appendix.

4.3 Effect of pH Changes upon Uptake of Mn and Cd by Radish (*Raphanus sativus L.*)

There are different factors that can affect the uptake of metals by plants, they include; plant species, the properties of medium, the root zone, pH, and addition of chelating agent or fertilizer.[24,25]

In terms of plant species, some plants can hyperaccumulate toxic metals[26] and produce larger biomass.[27] The root zone of plants is also of special interest because they can absorb pollutants and store or use the pollutants for metabolism in the plant's tissue. Notably, pollutants in the soil can be degraded by plant enzymes in the root.[28, 29] In addition, the use of synthetic chelating agents can result in the mobility of heavy metals or pollutants into the soil.[30] In soils with pH above 5.5 – 6, the metal availability to plants decreases, the use of a chelating agent might be needed in such alkaline soils. The uptake of metals by plants is usually affected by the formation of metal-ligand complexes in the soil which results in the mobility of the metals below the root zone changes.[31]

The ability of a soil to retain and supply nutrient depends on the soil's cation and anion exchange capacities and the number of packing spaces for nutrients on the soil particles.[32] The charge of the soil particles and the soil's organic matter determines the cation and anion exchange capacity of the soil. Soils that are rich in organic matter have higher cation exchange capacity.[32] This means they have higher buffering capacity and can bind more to cations such as calcium or potassium. pH affects the uptake of metals by plants because hydrogen ions take up space on the negative charges along the soil surface displacing the metals.[32] The effect of metal uptake depends upon the size and charge of the nutrient molecules and whether they can be displaced through leaching or not. When metals such as Cu, Mn, Fe, Zn are dissolved in water, they produce 2 to 3 positive charges (high size to charge ratio). These metals usually bind strongly to the surface of the soil particles. At a high pH the metal ions are tightly bound to the surface of soil particles and are not readily found in soil solution. The metals ions become less available for uptake by plants. At low pH, the metals can stick to the soil surface, which makes them more readily available for plant uptake. On the other hand, relatively large element such as S and base-forming cations like Ca^{2+}, Mg^{2+}, K^+ and Na^+ do not bind tightly to soil particles. At high pH, these metals easily come off the soil particles and enter the soil solution. At low pH, they may not be available for plant uptake probably because of leaching.[32]

It is found that N, K, Ca S are more available for plants within pH of 6.5 to 8 while boron, copper, iron, manganese, nickel and zinc are readily available for plant uptake within pH of 5 to 7.[33,34]

In this study, the effect of pH upon Mn and Cd uptake by radish (*Raphanus sativus L.*) was investigated at two pHs 6.70 and 7.30. Based on other studies, an increase in pH is expected to lower metal concentrations.

4.4 MATERIALS AND METHODS

4.4.1 Chemical and Reagents

Concentrated nitric acid (HNO_3 15.8 M, Flinn Scientific Inc., Canada) H_2O_2 (Sigma Aldrich, St. Louis, MO), $CdCl_2$ (Sigma Aldrich, St. Louis, MO), NaOH (Flinn Scientific Inc.), ICP standard solution (SCP Science, Clark Graham, Quebec, Canada) were used. Hoagland solution was prepared from KNO_3 (EM Science), $Ca(NO_3)_2 \cdot 4H_2O$ (Frey Scientific), $NH_4H_2PO_4$, KCl (Mallinckrodt / Analytical Chemical Reagent),

$MgSO_4 \cdot 7H_2O$, Fe-EDTA, H_3BO_3, $MnSO_4 \cdot H_2O$, $ZnSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$, H_2MoO_4, EDTA (all from Flinn Scientific Inc.), H_2O_2 (Sigma Aldrich). All reagents used were of of high analytical purity. Hoagland solution, a hydroponic nutrient solution is used for crop cultivation. Table 4.1 shows the chemicals and amount used in preparation of the Hoagland solution used in this study.[35]

Materials: Perlite (Miracle-Gro, CA), Nacogdoches wastewater sludge (NWWS), Lufkin wastewater sludge (LWWS), Soil Therapy Compost (STC) were used in this study.

4.4.2 Instrumentation

The instruments used in this study include atomic absorption spectroscopy (AAS) and inductively coupled plasma – mass spectroscopy (ICP – MS).

Atomic Absorption Spectroscopy (AAS)

Figure 4.1 shows the schematic diagram of atomic absorption spectroscopy. AAS is used to quantitatively determines chemical elements using the absorption of optical light by free atoms in gaseous state ($A = \varepsilon cl$).[36] Atomic absorption spectroscopy can determine over 70 different elements in solution.[36] Flames or graphite tube atomizers are often used to atomize samples[37] while a hollow cathode lamp irradiates the atoms. The radiation moves through a monochromator which differentiates the element-specific radiation from other radiation sources, after which it is detected.[37]

Inductively Coupled Plasma- Mass Spectrometry (ICP-MS)

The inductively coupled plasma – mass spectrometry was used to determine the metal content in the root, shoot and leaves of the radishes cultivated. ICP -MS follows similar principle as in ICP-OES. However, it uses the isotopes of elements.

4.4.3 Plants and Growth Conditions

Pot experiments were set up in the Soil Science greenhouse at Stephen. F. Austin State University in Fall 2018. A total of 150 g of perlite/biosolid was used with increasing amounts of composted biosolids from Angelina-Neches Compost Facility (NCF) at various compositions (0%, 25%, 50%, 75% and 100% w/w). The experiments were set up in triplicates. There were three different set ups; (i) a control experiment (with samples not treated with 100 ppm Mn or 100 ppm Cd) (ii) pot samples treated with 100 ppm Mn at the beginning of experiments, and (iii) pot samples treated with 100 ppm Cd at the beginning of each experiment.

Three seeds of radish (*Raphanus sativus L.*) (from Burpee Garden Products Co., Warminster, PA) were planted per pot. Plants were watered every 3 days with 100 mL Hoagland solution. After six weeks of growth, the radish plants were harvested. The plants were rinsed with 18.2 MΩ water and the fresh and dried biomass of the plants weighed, and masses recorded. The radish plants (*Raphanus sativus L.*) were oven dried at 60 °C for 48 hours.

4.4.4 Determination of Mn and Cd in Radish Plants Using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) and Atomic absorption spectroscopy

The dried plants samples were separated into the root, shoot and leaves and the masses recorded, and ground with mortar and pestle. The USEPA method 3050B was used for plant digestion. The ground powder was digested with 4 mL of 15.8 M HNO_3 (Flinn Scientific Inc) and 1 mL H_2O_2 in aliquot drops (Sigma Aldrich). The digested samples were filtered with 0.45 µm digi-fiter, and standard dilutions were prepared with 18.2 MΩ water. Inductively coupled plasma-mass spectrometry (ICP-MS) and atomic absorption spectroscopy were then used to determine the metal content in the different root, shoot or leaf parts of radish (*Raphanus sativus L.*).

4.4.5 Quality Control and Assurance

The quality control of this analysis was assured by analyzing standard solutions (10 ppm, 25 ppm, 50 ppm, and 100 ppm) in-between samples runs. The limit of detection (LOD) was determined as LOD = 3_{Sbl}/m.

4.5 RESULTS

4.5.1 Effect of Biosolids Composition on Plant Biomass

4.5.1.1 Plants cultivated in biosolids treated with 100 ppm Cd at pH 6.70 and 7.30

Figures 4.2a and 4.2b show the biomass of radish plants cultivated in perlite-compost mixture treated with 100 ppm of Cd at pH 6.70 and 7.30. The best plant growth was observed at 75% (w/w) for radish plants cultivated at pH 6.70 vis-à-vis plants with no Cd treatment. In general, there was an increase in plant biomass upon addition of biosolid in the order 75% > 100% > 50% > 0% > 25% (w/w).

4.5.1.2 Plants cultivated in biosolids treated with 100 ppm Mn at pH 6.70 and 7.30

Figures 4.3a and 4.3b show the biomass of radish plants cultivated in perlite-compost mixture treated with 100 ppm of Mn at pH 6.70 and 7.30. At pH 6.70, the best plant growth was observed at 100%. The pattern of biomass yield is in the order 100% > 75% > 25% > 50% > 0% (w/w).

As observed, the best growth was at 0% (w/w) for pH 7.30. Continuous decrease in the biomass was observed from 25% to 75% (w/w) composition of compost sludge.

4.5.2 Influence of Biosolids composition, and Effect of pH on Cd and Mn uptake by Radish (*Raphanus sativus L.*)

4.5.2.1 Mn Concentrations in plants cultivated in biosolids treated with 100 ppm Mn at pH 6.70 and 7.30

Figure 4.4a shows the concentration of Mn in the root, shoot, and leaves of radish plants cultivated with Mn treatment at pH 6.70. It is notable that the Mn concentration in the root and shoot of radish increased from 0% to 25% w/w. The highest concentration of Mn in the root and shoot was found at 100% (w/w) and 75% (w/w), respectively. The highest Mn concentrations were found in the leaves at biosolid 75% (w/w).

Figure 4.4b shows the concentration of Mn in the root, shoot and leaves of radish plants cultivated in manganese treatment at pH 7.30. The Mn concentration in the root, shoot and leaves of radish plants increased from 0% to 75% (w/w). An increase in the concentration of Mn in the root upon addition of biosolids is observed. The concentrations of the Mn in radish parts were found in the order $[Mn]_{leaf} > [Mn]_{shoot} > [Mn]_{root.}$

4.5.2.2 Cd Concentrations in plants cultivated in biosolids treated with 100 ppm Cd at pH 6.70 and 7.30

Figure 4.5a shows the concentration of Cd in the root, shoot and leaves of radish cultivated in 100 ppm Cd treatment at pH 6.70. The Cd concentration in the root, shoot and leaves of radish plants decreases from 0% to 100% (w/w). The highest concentrations of Cd were found in the root at 0% (w/w) biosolid composition. In general, there was a decrease in the concentration of Cd in the root upon addition of biosolids. Upon increasing the amount of the compost, the Cd concentration decreased in the order $[Cd]_{root} > [Cd]_{shoot} > [Cd]_{leaf}$.

Figure 4.5b shows the concentration of Cd in the root, shoot and leaves of radish plants cultivated in Cd treatment at pH 7.30. The Cd concentration in the roots was found less than 0.3 mg/kg. The concentration of Cd in plant parts decreased in the order $[Cd]_{root} > [Cd]_{shoot} > [Cd]_{leaf}$ with the highest concentration of Cd in the root and shoot at 0% (w/w) compost. The concentration of Cd in the leaves was found below 0.04 mg/kg.

4.5.3 Interelemental Interactions between Mn and Cd

In order to understand the synergistic effect of Mn on the uptake of Cd by plants and vice versa, radishes were cultivated in perlite soil therapy compost mixtures treated with 100 ppm of Cd and 100 ppm Mn.

4.5.3.1 Mn Concentrations in plants cultivated in biosolids treated with 100 ppm Cd at pH 6.70 and 7.30

Figure 4.6a shows the concentration of Mn in the root, shoot and leaves of radish plants cultivated in cadmium treatment at pH 6.70. The Mn concentration in the root of radish plants increases from 0% to 75% (w/w). The highest concentration of Mn in the shoot was found at 50% (w/w). The concentration of Mn in the leaves increased upon addition of biosolids.

Figure 4.6b shows the concentration of Mn in the root, shoot and leaves of radish plants cultivated in Cd treatment at pH 7.30. The Mn concentration in the root decreased upon addition of biosolids up to 25% (w/w). The highest concentration of Mn in the root and shoot was found at 75% (w/w). Concentration of Mn was accumulated most in the leaves. Mn concentration in the leaves increased upon increment in the composition of the sludge from 25% to 100% (w/w). The highest concentration of Mn was found at 100% (w/w). Relatively less amounts of Mn were uptaken by plants when treated with each metal.

4.5.3.2 Cd Concentrations in plants cultivated in biosolids treated with 100 ppm Mn at pH 6.70 and 7.30

Figure 4.7a shows the concentration of Cd in the root, shoot and leaves of radish plants cultivated with Mn treatment at pH 6.70. The Cd concentration in the root, shoot and leaves of radish plants increases notably from 0% to 25% w/w. The highest concentration of Cd in the root was found at 25% (w/w) while the Cd was mostly accumulated in the shoot and leaves at 50% (w/w).

Figure 4.7b shows the concentration of Cd in the root of radish plants cultivated in Mn treatment at pH 7.30. Cd concentration was found at low concentrations in the varying compost composition. The highest concentration of Cd in the root and shoot was found at 50% and 75% (w/w) respectively. The concentration of Cd in the leaves was lesser than the concentrations of Cd in the root and shoot. In general, there was a decrease in the concentration of Cd in the leaves upon addition of biosolids in the order $[Cd]_{root} > [Cd]_{shoot} > [Cd]_{leaf}$.

4.5.4 pH Determinations of Perlite and STC-Perlite mixture after harvesting Radish (Raphanus sativus L.) at pH 6.70 and 7.30

4.5.4.1 pH of Perlite-STC mixture cultivated in 100 ppm Mn at pH 6.70 and 7.30

Figure 4.8a shows the pH of perlite and STC-perlite mixtures treated with 100 ppm Mn at pH 6.70 after harvesting radish (*Raphanus sativus L.*). As observed, there was a decrease in the pH of both 0% (w/w) perlite and biosolids to 5.50 ± 0.06 and 4.85 ± 0.03), respectively.

Figure 4.8b shows the pH of perlite and STC - perlite mixture with Mn treatment (pH 7.30) after harvesting radish (*Raphanus sativus L.*) plants. A similar trend to that observed at pH 6.30 was found here. There was a decrease in the pH of both perlite and biosolids to 5.53 ± 0.33 and 4.96 ± 0.10, respectively.

4.5.4.2 pH of Perlite-STC mixtures cultivated in 100 ppm Cd at pH 6.70 and 7.30

Figure 4.9a shows the pH of perlite and STC – perlite mixture with Cd treatment at pH 6.70 after harvesting radish (*Raphanus sativus L.*). There was an observed decrease in the pH of perlite and biosolids to (5.61 ± 0.01) and (5.18 ± 0.10) respectively.

Figure 4.9b shows the pH of perlite and STC-perlite mixture with 100 ppm Cd treatment at pH 7.30 after harvesting radish (*Raphanus sativus L.*). Similarly, the pH of the perlite and biosolids lowered to 5.09 ± 0.31, and (5.02 ± 0.12), respectively.
The decrease in the pH of the biosolids and perlite observed in all treatments might be attributed to the presence of carboxylic acid in the root.

4.6 DISCUSSION

From literature studies, an increase in the biomass of the plants up to 25% (w/w) of composted sludge is shown.[17] Usually a reduction is expected in the biomass of plants at compost-perlite mixtures (>75% (w/w)).[17]

In terms of the metal uptake by plants, a study done by Maftoun et. al.[4] determined the effect of organic wastes on Mn uptake by spinach. Result showed that spinach cultivated in organic wastes showed higher Mn concentrations than those cultivated in the control. This study showed radish plants cultivated in both Mn and Cd at pHs 7.30 and 6.60 with similar trends, namely, Mn concentration in the root, shoot and leaves increased upon the addition of the composted biosolid. In general, the control (0% perlite) had the lowest Mn concentration in all parts of the radish. In addition, Mn concentration of radish (*Raphanus sativus L.*) cultivated both in Mn and Cd treatment at pH 7.30 had lower metal concentrations compared to those cultivated in a lower pH 6.70. This research is in accord with published data that show the need for the uptake of Mn and its accumulation in the leaves of many plants.

Some other investigations have shown that the uptake of Cd is relatively low in plants cultivated in biosolids-amended soils. Brown et al.[19] observed that the phytoavailabilty of Cd in long term biosolids-amended soils. The Cd concentration was found lower in lettuce (*Lactuca sativa*) cultivated in the biosoilds-amended soils compared to those cultivated in soil. Similarly, Chaney et al.[20] observed that the uptake of Cd was low in navy bean (*Phaseolus vulgaris*) cultivated in long-term biosolids amended soils. Our studies are in agreement with these studies, namely, the Cd concentration in the radish plants cultivated in both Mn and Cd treatment (pH 6.70 and 7.30) showed lower Cd concentration upon addition of biosolids, although increase in the biosolids increased the Cd concentration in the plants.

Marta and Raul[21] and Garrido et al.[22] found Cd undetectable in lettuce and broad bean seeds (*Vicia faba L*.), respectively. This is in agreement with current studies in which Cd concentration in the leaves were relatively low for both 100 ppm Cd and 100 ppm Mn treatments. Koo et al.[23] found that accumulation of Cd was mostly in the shoot and root of corn plants cultivated in biosolids-treated medium. This trend was observed in all studies in the current investigation. Thus, Cd was mostly accumulated in the root and shoot and not the leaves. In addition, the concentration of Cd in both Cd and Mn treatment at pH 7.3 was lower than those cultivated in a lower pH 6.70.

pH changes with increased biosolid compositions

The pH changes in the area of the soil around the plant root (rhizosphere) are the most documented chemical reactions taking place at the soil-root interface.[38,39] Deherain,[40] explained pH changes in the soil by cultivating root of beans on the surface of a marble polished plate.[40] There was an acid secretion in the beans root which was strong enough to dissolve calcium carbonate leaving behind visible imprints on the rock.[41] The acidic secretion of the beans root was attributed to carbonic and organic acids generated by the rhizosphere microflora and roots through root respiration and exudation. Changes in pH of rhizosphere has been attributed to the release of H^+ or OH^- ions.[42] Philippe et al.[43] has shown that the release of charges caused by hydrogen ions (H^+) and hydroxyl ions (OH) which counterbalances for the unbalanced cation-anion uptake at the soil – root interface is the major factor that causes root-induced pH changes in the rhizosphere (the region of soil around the plant roots).[43] In addition, the ions passing the plasma membrane of the root cells such as the organic anions released by plants also play a role in root-induced pH changes.[43]

The different uptake of cations and anions by plant roots is the main source of the flow of H^+ in the rhizosphere.[44,45,46,47] The necessity to compensate for the electrical charges and regulation of cellular pH in the root cell is the major cause of the uptake of cations and anions in the root cell.[46] The pH of the aqueous part of the cytoplasm is usually maintained with a range of values around 7.30 with an efficient pH-stat system.[46] The pH-stat system consists of both biochemical and biophysical (H^+ exchange).[44] The biochemical components involve the generation and utilization of H^+ as a result of carboxylation and decarboxylation of organic acids in the root cell.[44,46] The pH of both the apoplasm or the cystol cannot be controlled by ATPs.[48] The ATPs are considered to mainly act through energizing the transport of ions across the membrane which results in significant changes in pH.[48] The uptake of cations is better understood with the mechanisms of ATPs.[44] When cations are uptaken more than anions, hydrogen ion is released into the apoplasm to balance for the excess positive charges entering the cell. This results in an increase in the pH of the cytoplasm (cytosol).[45,46] For instance, there is a larger uptake of K^+ than $SO^{2-}{}_4$ when a plant is supplied with a K_2SO_4 solution.[45] But if more anions are uptaken than cations, hydroxyl ion, OH^- will be released or hydrogen ion, H^+ will be taken up from the apoplasm to balance for the excess negative charge entering the cell leading to a decrease in the pH of the cytosol.[46] For instance, there is less uptake of Cd^{2+} than Cl^- when a plant is supplied with a $CaCl_2$ solution.[45,46] This results in a strong relationship occurs between H^+ release and cation-anion balance.[49]

Nitrogen plays a vital role in the cation-anion balance, because it taken up my most plant species at a higher rate[46,50] Nitrogen can be taken up as a cation (NH_4^+, ammonium), anion (NO_3^-, nitrate) and as a neutral specie (N_2), as in the case of nitrogen fixing plants such as legumes. A significant amount of nitrogen can be used as amino acids (positively, negatively or neutrally charged) directly by plants.[51] Plants supplied with NO_3^- will compensate for the corresponding excess negative charges by releasing equal amounts of OH^- or $HCO^-{}_3$ into the rhizosphere and thus leads to an increase in rhizosphere pH.[52,53,54,55] On the other hand, plants supplied with NH_4^+ will compensate for the corresponding excess positive charges released by equal amounts of H^+ in the rhizosphere leading to a decrease in rhizosphere pH. Plants such as legumes relying on atmospheric N_2 will take up more cations than anions and thus leads to the release of excess positive charge as H^+ and acidification of the rhizosphere.[56,57,58]

Apart from the ions taken up by plant roots, cation-anion balance also includes all ions that pass the root cell plasma membrane either through efflux or influx.[59] The release of organic anions is a component of cation-anion balance and this can affect the net release of H^+ and OH^-. The possible origin of rhizosphere acidification is organic acids.[60] The level of root exudation by organic acids depends on the species and environmental constraints.[61,62,63] For instance, studies carried out by Petersen and Bottger[64] to determine the role of exuded organic acid in rhizosphere acidification using maize was minimal not exceeding 0.3%.[64] In contrast, studies by Dinkelaker et al.[59] using white lupin showed that cluster roots can release large amounts of organic anions that can precipitate and accumulate in the rhizosphere leading to rhizosphere acidification.[59] The most commonly referred organic acids for their potential effect on rhizosphere acidification are citric acid, oxalic and malic acids.[65] These organic acids are present in large amounts in the root cells where they contribute to buffering of cytosolic pH.[46] Majority of plant species store these organic acids in the root cells vacuole while in some plant species, a significant amount may be exuded in the rhizosphere.[61,22] The dissociation of these organic acids in the cytosol occurs because of low pK values compared to the neutral pH of the cytosol.[44,45]

Consequently, they are released as their conjugate base (organic anions) and not as acids.[44,45] At pH around 7.30, citrate is present mostly as citrate^{3-} (pK for citrate^{2-}/citrate^{3-} is 6.40 and pK for citrate^{-}/citrate^{2-} is 4.76), malate as malate^{2-} (pK for malate^{-}/malate^{2-} is 5.11) and oxalate as oxalate^{2-} (pK for oxalate^{-}/oxalate^{2-} is 4.19).[65]

Substantial amount of CO_2 in the soil is provided by plant root. This arises from the root respiration and root exudation of organic carbon that are degraded by rhizosphere microorganisms. More than half of the carbohydrates translocated from the shoots to the roots are eventually respired and this generally represents 10 – 50% of photosynthates produced daily.[66] It has been observed in some ^{14}C labelling studies that cereals such as maize (*Zea mays L.*) or wheat (*Triticum aestivum L*) have a total of 30 – 50% allotted to the below-ground plants' parts, 10 – 30% accumulated in the roots, 10 – 20% respired in the rhizosphere and 1 – 5% accumulated as organic material and microbial cells in the rhizosphere.[67] According to Helal and Sauerbeck,[68] about 20% of the photosynthates are absorbed by maize roots into the rhizosphere and about three quarters of these absorbed photosynthates are finally converted into CO_2 by microbial respiration.[68]

Some studies have reported fluxes of CO_2 generated by the plant roots to be in the order of 50 – 200 nmolg^{-1} DWs^{-1} in plants such as *Cucumis sativus, Lycopersicum esculentum* and *Holcus lanatus* while some studies have shown a direct relationship between the values of CO_2 fluxes obtained for some acidic root exudates and the rhizosphere acidification.[66,69] Durand and Bellon[70] reported that the fluxes of CO_2 in maize was between 100 – 200 nmol plant^{-1} s^{-1} while another study with maize seedling of similar age by Durand et al. showed H^+ effluxes of about 5 – 10 nmol H^+ plant^{-1} s^{-1} and measured O_2 fluxes of 3 nmol and 0.2 nmol H^+ g^{-1} FWs^1.[71,72] These values show that show that there is a release of CO_2 by the roots at fluxes of an order of magnitude larger than H^+ given off to compensate for an excess of cations over anions.[71] Also, rhizosphere microorganisms allow for an additional release of CO_2 as a result of their respiration being hoisted by the supply C-compounds from the plant roots.[43] Root exudation and rhizosphere microbial respiration should promote substantial amount of changes in CO_2 concentration in the soil and thus in soil pH.[43]

Chemical reactions associated with changes in oxidation state of Fe, Mn and N also imply the generation and utilization of H^+, resulting in a coupling of redox potential and pH.[43] This can be explained with iron transformation in most soils.[73] Reduction of Fe occurs when electrons are supplied, the dissolution of Fe^{3+} bearing minerals such as goethite, FeOOH, and combined reduction of Fe^{3+} into Fe^{2+} is given by equation 4.1.[74]

$$FeOOH + 3H_3O^+ + e^- \longrightarrow Fe^{2+} + 5H_2O \qquad (4.1)$$

The oxidation of organic matter (both microbial and root respiration) often supply electrons. Sposito derived an example of an equation which can be used for organic compound but applies to formate (CHO_2^-).[75]

$$CHO_2^- + H_2O \longrightarrow CO_2 + H_3O^+ + 2e^- \qquad (4.2)$$

Combination of both redox half reactions gives the overall redox reaction that occurs in the soil.[75]

$$2FeOOH + CHO_2^- + 5H_3O^+ \longrightarrow 2Fe^{2+} + CO_2 + 9H_2O \qquad (4.3)$$

This equation illustrates that the reduction of Fe^{3+} contained in Fe oxides should be followed by the oxidation of organic matter and by H^+ consumption which will eventually lead to an increase in soil pH.[76] Contrarily, the oxidation of Fe^{2+} will result in a decrease in soil pH.[76]

Therefore, it can be concluded that the decrease in pH observed in the pot samples may be attributed to the release of charges carried by H^+ and OH^- to counterbalance the unbalanced uptake of cation-anion in the biosolids-root surface.[43] In addition to this, the observed decrease in pH might result from the organic acids exuded by the radish roots.[43] The root exudation and respiration of radish plants could have also contributed to the decrease in pH due to the build-up of CO_2 concentration. Redox coupled reactions in the biosolids could have also resulted in a decrease in pH.[43]

Translocation factors of Mn and Cd

Translocation factor (TF) is the ratio of metal concentration in the shoot to the root. This ratio explains the ability of a plant to translocate heavy metals from the roots to the stem and leaves of the plant. Table 4.7 and 4.8 show the translocation factor for Mn and Cd in radish cultivated in control, and in Mn and Cd treatment experiments at pH 6.70 and 7.30. The translocation factors for Mn in all treatments at both pH values were greater than 1 while Cd TFs were lesser than 1. The high TFs of Mn suggest that there is an efficient transport system of Mn in the radish plants. On the other hand, the low TFs of

Cd show ineffective Cd transfer which implies that Cd in the radish plants was accumulated mostly in the root than the shoots and leaves.

4.7 CONCLUSIONS

The uptake of metals by plants relies upon both edaphic and plant factors.[77,78] Edaphic factors such as amount of metals in the soil, interactions between the metals and the soil surfaces and the pH at the root-soil interface can affect the metal uptake by plants.[79] Plant factors such as the plant species and its life cycle can also play a vital role in the uptake of metals by plants.[80] From results obtained, it can be concluded that the uptake of metals by plants is lower in plants cultivated in higher pH (7.30) compared to those cultivated in lower pH (6.70). In addition, the concentration of Mn in the radish parts increased upon addition of biosolids and Cd concentration reduced in the radish parts upon addition of biosolids (25% w/w) as compared with the control (0% perlite) but further increment in the biosolids increased the Cd concentration. Therefore, the use of biosolids in radish plants (*Raphanus sativus L.*) might not involve any environmental risk associated with cadmium.

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FIGURES

Table 4.1: Chemicals used for the preparation of the Hoagland solution

	COMPOUND	**NUTRIENT**	**MILLIGRAMS OF COMPOUND /L OF SOLUTION**
Macronutrients	KNO_3	K, N	60.00
	$Ca(NO_3)_2.4H_2O$	Ca, N	94.00
	$NH_4H_2PO_4$	N, P	23.00
	$MgSO_4.7H_2O$	Mg, S	24.00
	Fe-EDTA	Fe	0.69
Micronutrients	KCl	Cl	O.37
	H_3BO_3	B	0.15
	$MnSO_4.H_2O$	Mn	0.033
	$ZnSO_4.7H_2O$	Zn	0.57
	$CuSO_4.5H_2O$	Cu	0.012
	H_2MoO_4 (85% MoO_3)	Mo	0.0081

Table 4.2: Average dry masses ± SD (g) of root, shoot and leaf of radish (*Raphanus sativus L.*) plants cultivated in control (without Mn or Cd treatment) pH 6.70 (n = 3)

SAMPLE ID	ROOT (g)	SHOOT (g)	LEAVES (g)
0%	0.0042 ± 0.0018	0.010 ± 0.002	0.029 ± 0.014
25%	0.0041 ± 0.0019	0.0185 ± 0.0078	0.035 ± 0.019
50%	0.0038 ± 0.0011	0.014 ± 0.002	0.027 ± 0.009
75%	0.0028 ± 0.0016	0.0077 ± 0.0048	0.019 ± 0.009
100%	0.0021 ± 0.0007	0.0077 ± 0.0052	0.044 ± 0.048

Table 4.3: Average dry mass ± SD (g) of root, shoot and leaf of radish (*Raphanus sativus L.*) plants cultivated in 100 ppm Mn treatment at pH 6.70 (n = 3)

SAMPLE ID	ROOT (g)	SHOOT (g)	LEAVES (g)
0%	0.0020 ± 0.0015	0.0035 ± 0.0037	0.0126 ± 0.0091
25%	0.0024 ± 0.0018	0.014 ± 0.003	0.016 ± 0.005
50%	0.0033 ± 0.0003	0.0012 ± 0.0006	0.0079 ± 0.0035
75%	0.0032 ± 0.0014	0.012 ± 0.011	0.029 ± 0.031
100%	0.0076 ± 0.0031	0.028 ± 0.010	0.050 ± 0.024

Table 4.4: Average dry mass ± SD (g) of root, shoot and leaf of of radish (*Raphanus sativus L.*) plants cultivated in Cd treatment at pH 6.70 (n = 3)

SAMPLE ID	ROOT (g)	SHOOT (g)	LEAVES (g)
0%	0.0041 ± 0.0007	0.0105 ± 0.0037	0.029 ± 0.015
25%	0.0025 ± 0.0018	0.0073 ± 0.0028	0.018 ± 0.010
50%	0.0075 ± 0.0010	0.027 ± 0.004	0.071 ± 0.009
75%	0.0084 ± 0.0015	0.032 ± 0.005	0.051 ± 0.023
100%	0.0052 ± 0.0011	0.023 ± 0.007	0.035 ± 0.012

Table 4.5: Average dry masses ± SD (g) of root, shoot and leaf of radish (*Raphanus sativus L.*) plants cultivated in Mn treatment at pH 7.30 (n = 3)

SAMPLE ID	ROOT (g)	SHOOT (g)	LEAVES (g)
0%	0.14 ± 0.19	0.057 ± 0.065	0.69 ± 0.74
25%	0.034 ± 0.025	0.073 ± 0.015	0.36 ± 0.15
50%	0.0042 ± 0.0009	0.019 ± 0.010	0.058 ± 0.036
75%	0.0041 ± 0.0019	0.0073 ± 0.0067	0.036 ± 0.041
100%	0.032 ± 0.009	0.10 ± 0.04	0.22 ± 0.10

Table 4.6: Average dry masses ± SD (g) of root, shoot and leaf of radish (*Raphanus sativus L.*) plants cultivated in Cd treatment at pH 7.30 (n = 3)

SAMPLE ID	ROOT (g)	SHOOT (g)	LEAVES (g)
0%	0.010 ± 0.009	0.025 ± 0.028	0.15 ± 0.14
25%	0.021 ± 0.014	0.047 ± 0.029	0.28 ± 0.17
50%	0.0056 ± 0.0037	0.026 ± 0.017	0.11 ± 0.08
75%	0.010 ± 0.000	0.0260 ± 0.0003	0.042 ± 0.009
100%	0.031 ± 0.006	0.099 ± 0.007	0.22 ± 0.04

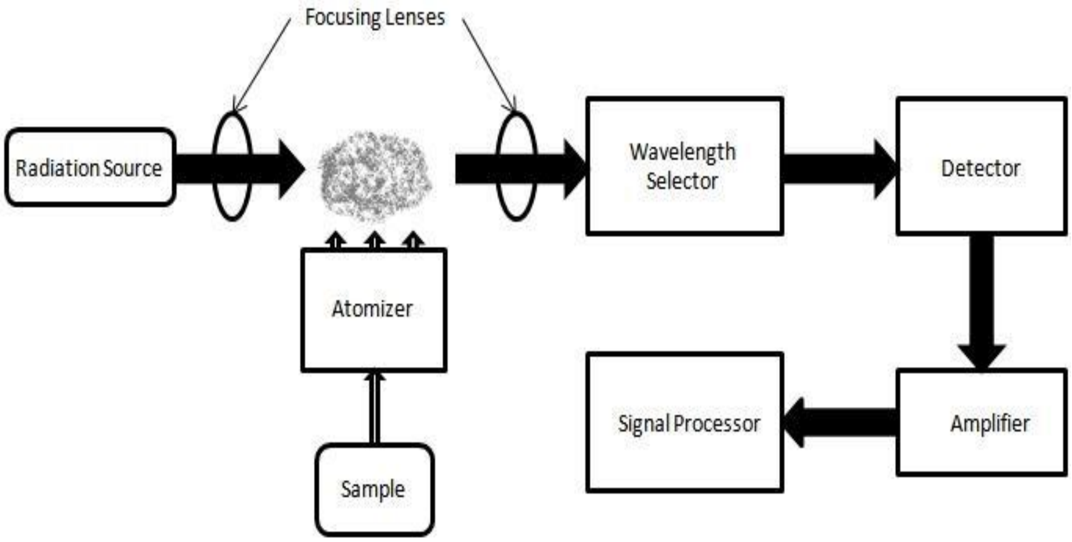
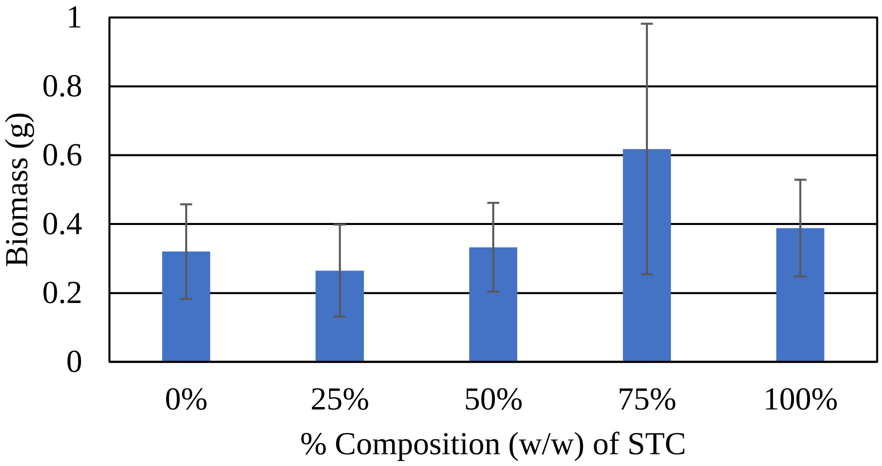


Figure 4.1: Schematic diagram of atomic absorption spectroscopy.[37]

a)



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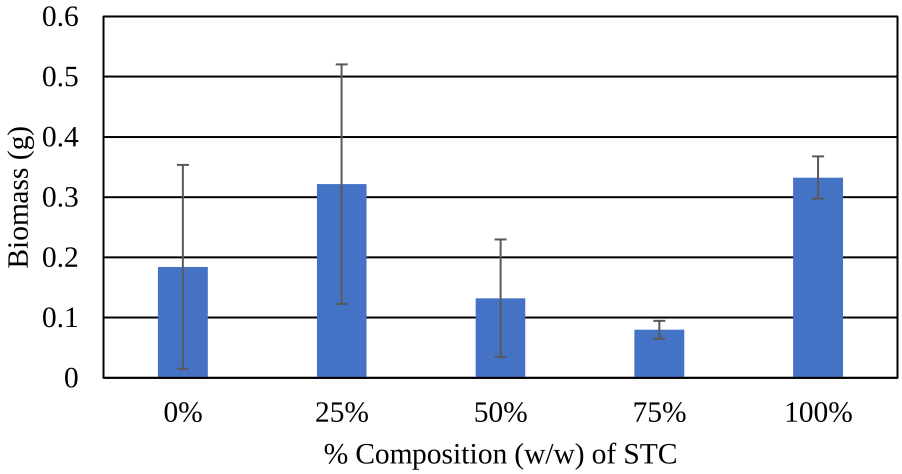
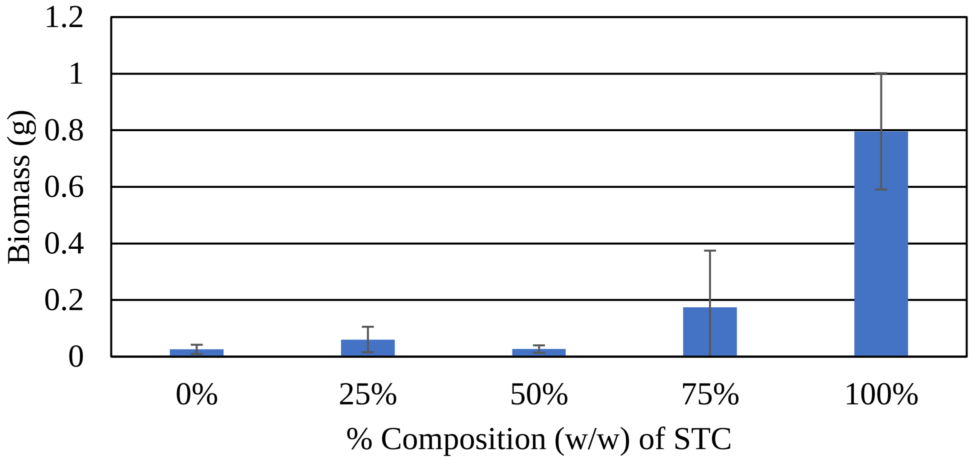


Figure 4.2: **Biomass of radish (*Raphanus sativus L.*) harvested from biosolids treated with 100 ppm Cd at pH 6.70 (a) and pH 7.30 (b)**

(The error bars at 75% (pH 6.70) and 25% (pH 7.30) were as a result of the differences in plant biomass in the triplicate pot samples).

a)



b)

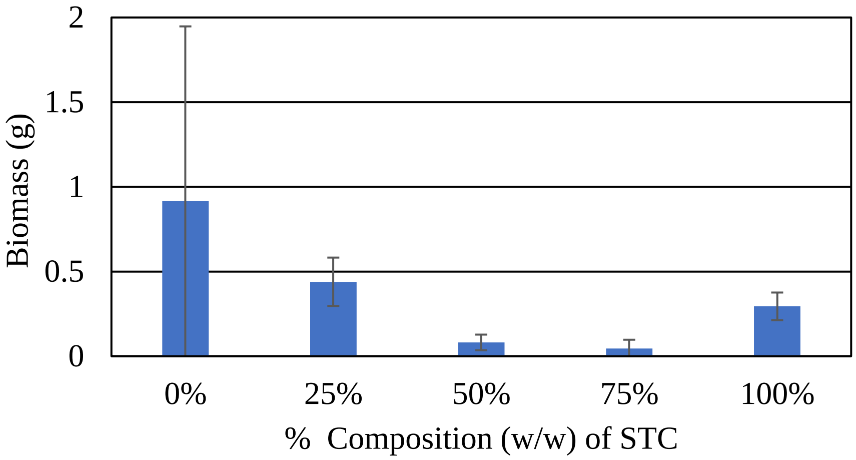


Figure 4.3: **Biomass of radish (*Raphanus sativus L.*) harvested from biosolids treated with 100 ppm Mn at pH 6.70 (a) and pH 7.30 (b).**

(The error bars at 100% (pH 6.70) and 0% (pH 7.30) were as a result of the differences in plant biomass in the triplicate pot samples).

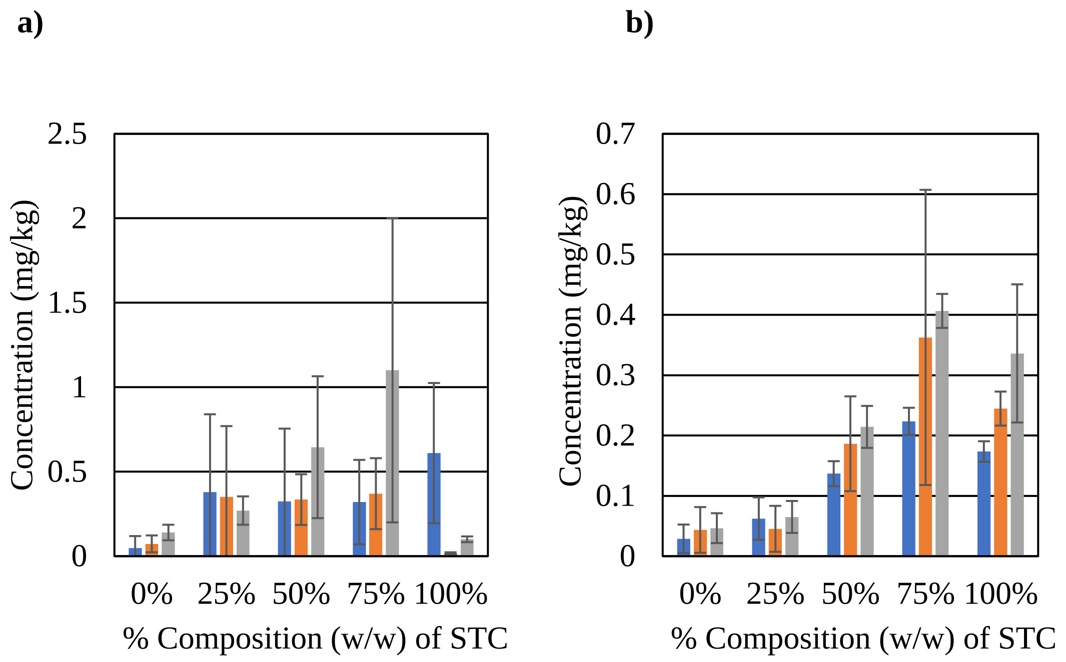


Figure 4.4: **Manganese concentration (mg/kg) in radish (*Raphanus sativus L.*) cultivated with 100 ppm Mn treatment at pH 6.70 (a) and pH 7.30(b)**
🟦 **= root** 🟧 **= shoot** ⬜ **= leaf**

* The Mn concentrations were determined per the biomass of the radishes, since there was an observed difference in the plant biomass for the triplicate samples, it also had effect on the concentration which resulted in the error bars at 75% leaf (pH 6.70) and 75% shoot (pH 7.30).

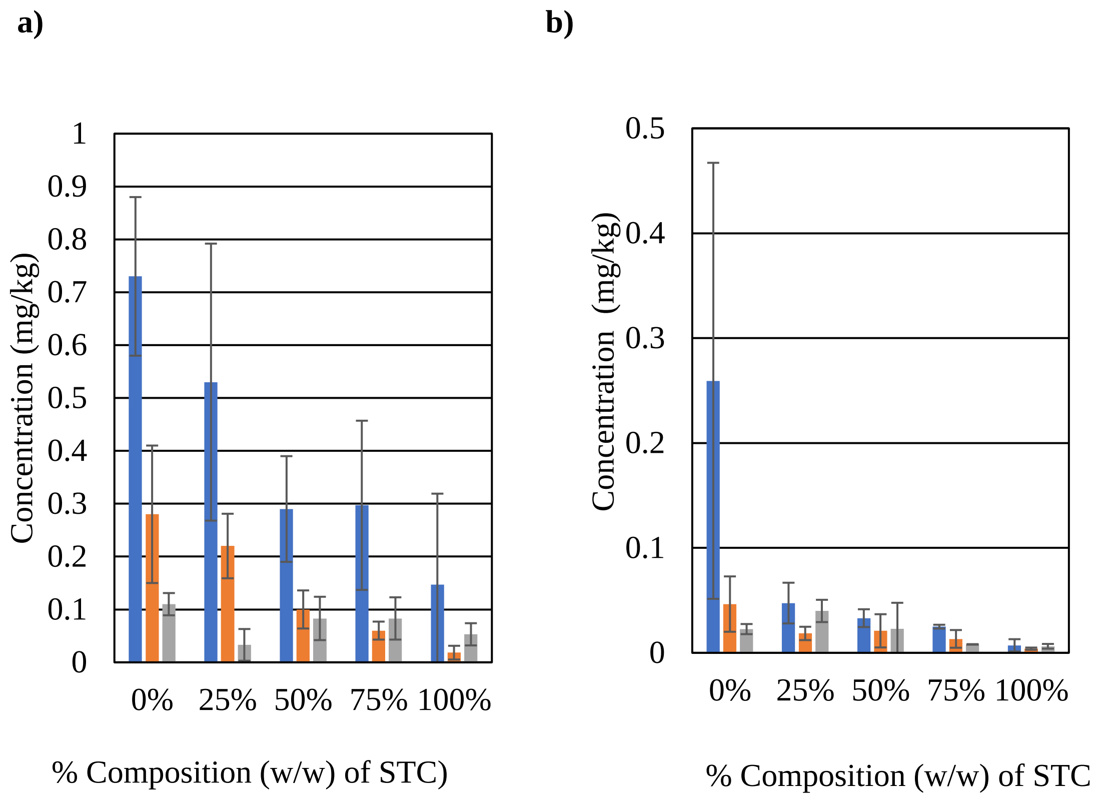


Figure 4.5: **Cadmium concentration (mg/kg) in radish (*Raphanus sativus L.*) cultivated with 100 ppm Cd treatment at pH 6.70 (a) and pH 7.30 (b)**
🟦 **= root** 🟧 **= shoot** ⬜ **= leaf**

*The Cd concentrations were determined per the biomass of the radishes, since there was an observed difference in the plant biomass for the triplicate samples, it also had effect on the concentration which resulted in the error bars at 25% root (pH 6.70) and 0% root (pH 7.30).

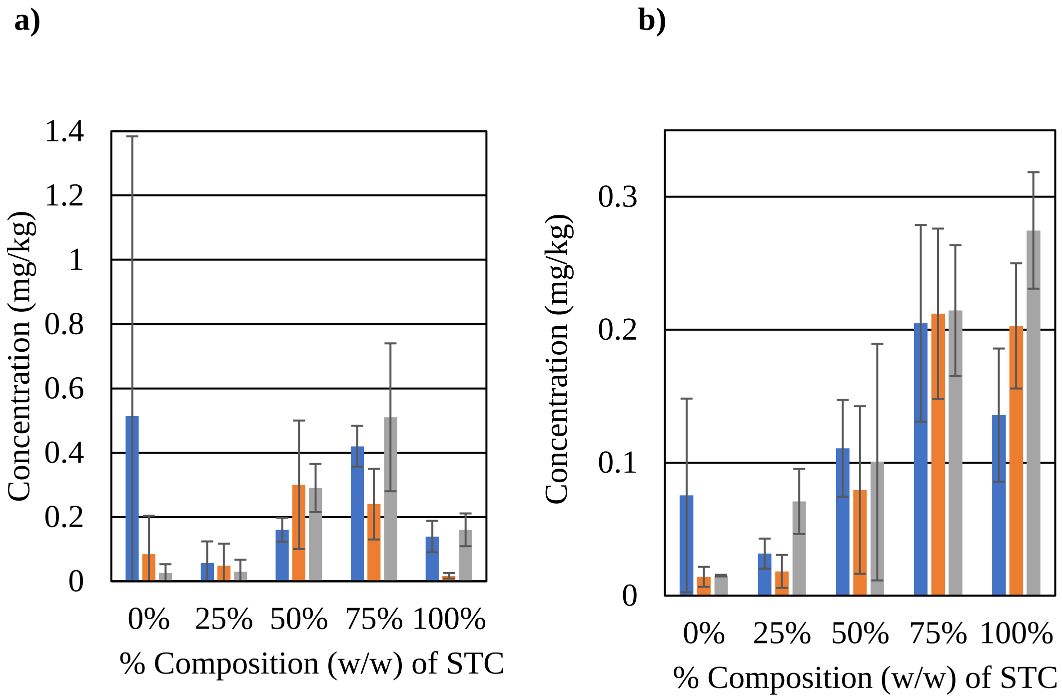


Figure 4.6: **Manganese concentration (mg/kg) in radish (*Raphanus sativus L.*) cultivated with 100 ppm Cd treatment at pH 6.70 (a) and pH 7.30 (b)**
🟦 **= root** 🟧 **= shoot** ⬜ **= leaf**

*The Mn concentrations were determined per the biomass of the radishes, since there was an observed difference in the plant biomass for the triplicate samples, it also had effect on the concentration which resulted in the error bars at 0% root (pH 6.70) and 50% shoot, leaf (pH 7.30).

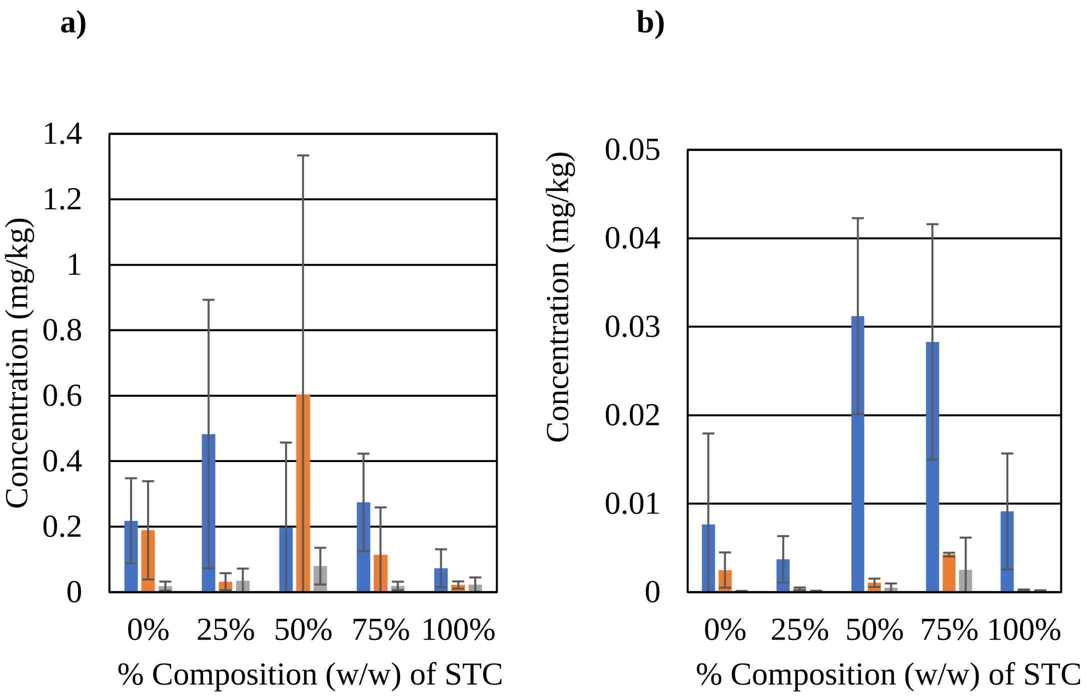


Figure 4.7: **Cadmium concentration (mg/kg) in radish (*Raphanus sativus L.*) cultivated with 100 ppm Mn treatment pH 6.70 (a) and pH 7.30 (b)**
🟦 **= root** 🟧 **=shoot** ▨ **= leaf**

*The Cd concentrations were determined per the biomass of the radishes, since there was an observed difference in the plant biomass for the triplicate samples, it also had effect on the concentration which resulted in the error bars at 25% root, 50% shoot and leaf (pH 6.70).

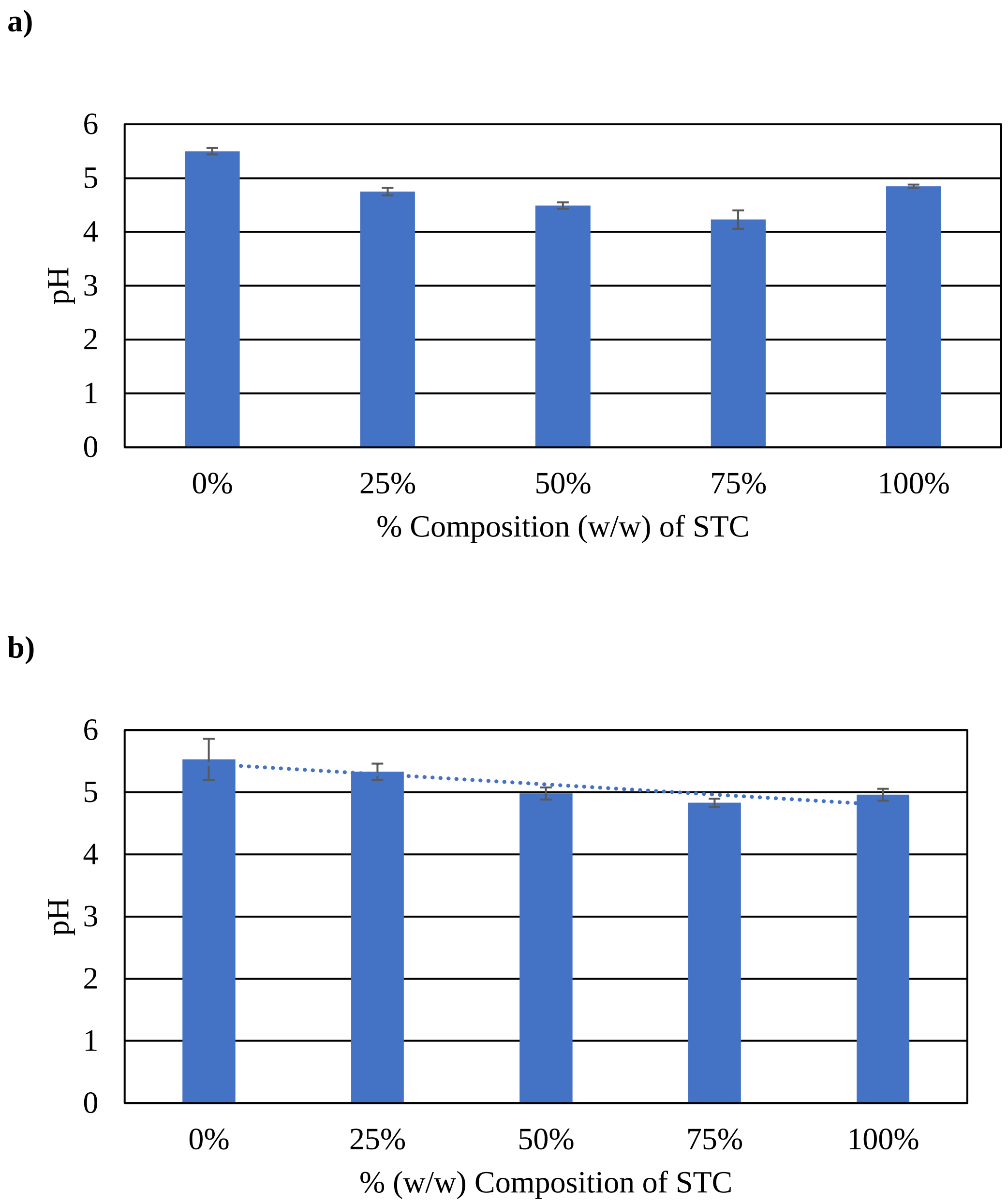


Figure 4.8: pH of perlite and perlite-STC mixtures treated with 100 ppm Mn at pHs 6.70 (a), and 7.30 (b)

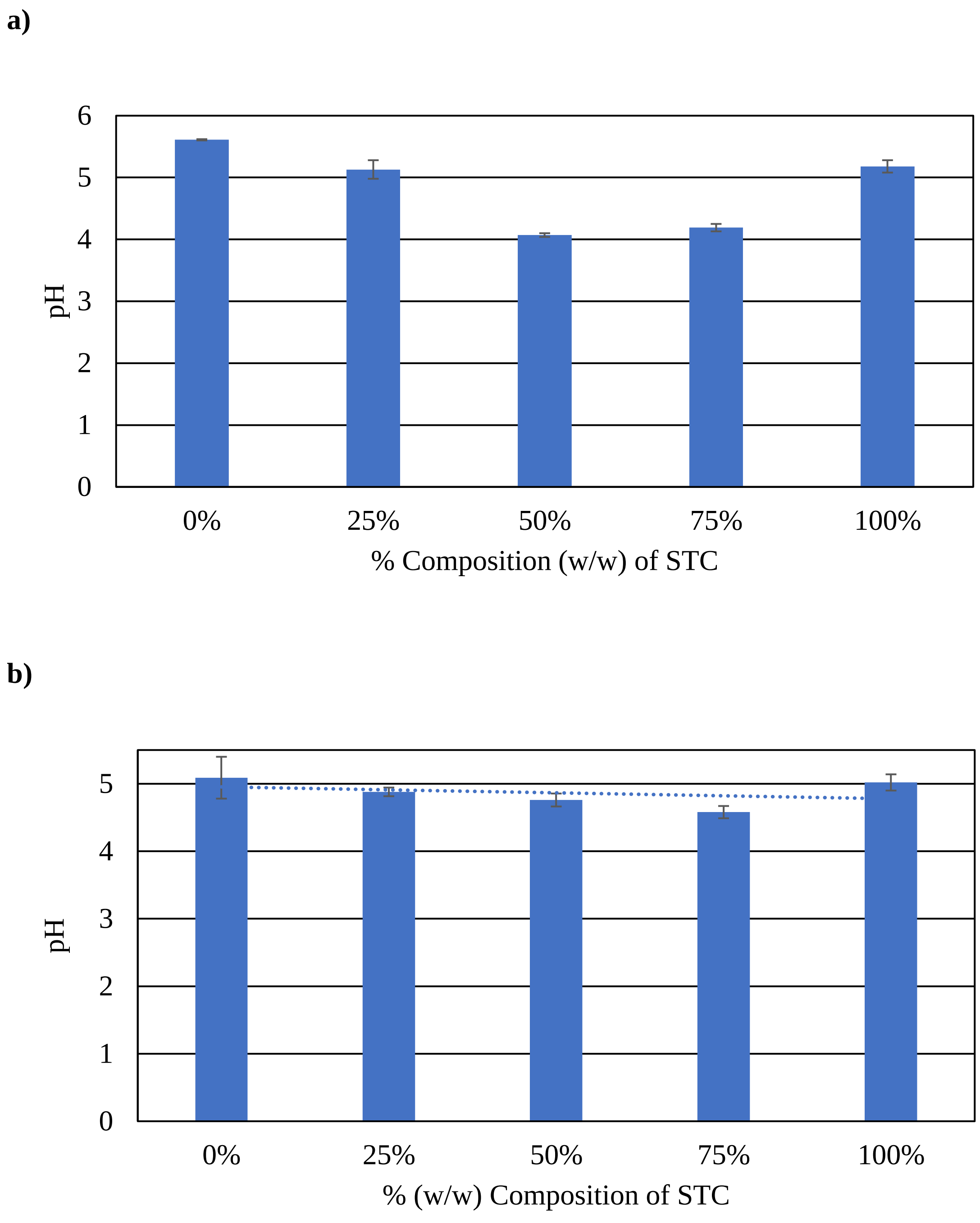


Figure 4.9: pH of perlite and perlite-STC mixture treated with 100 ppm Cd at pHs 6.70 (a), and 7.30 (b)

Table 4.7: Translocation factor of radish plants cultivated in Mn treatment and Cd treatment at pH 6.70

SAMPLE ID	Mn TREATMENT		Cd TREATMENT	
	Mn TF	Cd TF	Mn TF	Cd TF
0%	4.44	0.95	0.21	0.53
25%	1.63	0.14	1.39	0.48
50%	3.02	3.47	3.69	0.63
75%	4.59	0.49	1.79	0.48
100%	0.19	0.62	1.27	0.49

Table 4.8: Translocation factor of radish plants cultivated in Mn and Cd treatment at pH 7.30

SAMPLE ID	Mn TREATMENT		Cd TREATMENT	
	Mn TF	Cd TF	Mn TF	Cd TF
0%	3.13	0.34	0.39	0.27
25%	1.77	0.13	2.82	1.23
50%	2.93	0.05	1.62	1.32
75%	3.44	0.24	2.08	0.85
100%	3.35	0.04	3.52	1.43

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

This study was aimed at evaluating the risks that might be associated with land application of biosolids through the analysis of metal content in biosolids. The speciation and bioavailability of metals in biosolids and determination of the uptake of metals by radish (*Raphanus sativus L.*) cultivated in biosolids was investigated. The metal content of the biosolids was determined using inductively coupled plasma-optical emission spectroscopy (ICP–OES). The heavy metals analyzed in the biosolids had concentrations within the USEPA ceiling limit.

The speciation and bioavailability of the of the biosolids using a modified Tessier sequential extraction protocol showed that most of the metals were predominant in sulfide/residual fractions. The metals Mn and Cd were present in the mobile fraction although at low concentrations (Mn (STC – 2%, NWWS – 1.1%, LWWS – 6%), Cd (STC – 46%, NWWS – 9.5%, LWWS – 40%)). This implies that Mn and Cd are bioavailable in the biosolid sample.

The cultivation of radish (*Raphanus sativus L.*) plants at different percent compositions of biosolids showed that the best growth was observed at 25 – 50% (w/w) of Soil Therapy Compost. The determination of the metal content of the radish (*Raphanus sativus L.*) showed that upon the addition of biosolids the concentration of manganese concentrations increased and accumulated in the leaves. On the other hand, upon increasing biosolids compositions cadmium concentration decreases and accumulated the most in the root of the radish plants. In addition, the lowering the pH on increased the uptake of metals by radish (*Raphanus sativus L.*). Comparatively, more Cd or Mn was uptaken by radish (*Raphanus sativus L.*) at pH 6.70 than 7.30.

The determination of the translocation factor (TF) showed that Mn TFs in all treatments at pH values 6.70 and 7.30 were greater than 1, while Cd TFs were lesser than 1. The high TFs of Mn suggest that the existence of an efficient transport system of Mn in the radish. On the other hand, the low TFs of Cd show that Cd is not easily transported from the root to the shoot of the radish plants.

5.2 RECOMMENDATIONS

I would like to suggest the following recommendations for future work. The study on plant growth in different biosolid compositions should be investigated using a different plant. A plant that grows well throughout the year should be used for comparisons.

Bigger pot samples should be used to prevent pot samples from being waterlogged. Better still, field experiments can be investigated. Although anions concentrations are important, the determination of their effect upon radish plants was not investigated due to instrument downtime. In future study, the anions concentrations should be determined.

APPENDIX A

CONCENTRATION OF OTHER METALS IN PLANT PARTS

Although this research is devoted to analysis of Mn and Cd in plant parts, it was also necessary to determine the concentration of both essential and toxic elements that are uptaken by radish (*Raphanus sativus L.*).

A.1 Plants cultivated in biosolids treated with 100 ppm Mn at pHs 6.70 and 7.30

A.1.1 Analysis of macroelements in plant parts

Figure A.1a shows the metal concentrations of the macroelements Ca, K, Mg, and Na in the root of radish (*Raphanus sativus L.*) cultivated in 100 ppm Mn treatment at 0%, 25%, 50%, 75% and 100% (w/w) of perlite-STC mixtures. An increase in the concentration of the macroelements upon the addition of biosolids is evident. Elements Ca, K, and Na showed similar trends with the root concentration decreasing upon addition of 25% (w/w) biosolid. The highest root concentration for the Ca, K, and Na was observed at 75% (w/w) perlite-biosolid composition. For Mg, on the other hand showed an increase upon addition of compost from 25% to 50% (w/w) biosolid composition. The highest Mg, concentration was found at 100% (w/w) compost ratio.

Figure A.1b shows the macroelements concentrations found in the shoot of radish cultivated (*Raphanus sativus L.*) in Mn treatment. Elements Ca, K, Na, and Mg showed similar trend to what was observed in the root.

Figure A.1c shows the concentrations of macroelements Ca, K, and Na concentrations in the leaves of radish (*Raphanus sativus L.*) cultivated in 100 ppm Mn treatments. The concentrations of Ca and K increases up onto 25%(w/w) compost while Mg concentrations constantly increased in concentration upon increased compost sludge ratios. On the other hand, Na concentrations decreased up to 25% (w/w) compost ratios. The highest concentrations of Ca, K, and Na were determined in the leaves with highest ratios at 75% (w/w) compost, while that of Mg occurred at 100% (w/w).

A.1.2 Analysis of microelements in plant parts

Figure A.2a shows the microelements concentration in the root of radish (*Raphanus sativus L.*) cultivated in the 100 ppm Mn treatment. Elements Ag, Ba, Be, Co, Cr, Cu, Ga, Li, Mo, Ni, Pb, Rb, Sr, Tl, and V were present in the root at concentrations < 0.2 mg/kg. Upon increasing of biosolids ratios increase in concentrations of elements Al, Bi, Cs, Fe, In, and Zn was determined in the roots.

Figure A.2b shows the microelements concentrations in the shoot of radish (*Raphanus sativus L.*) cultivated in 100 ppm Mn treatment. The microelement concentrations of Ag, Ba, Be, Cd, Co, Cr, Cs, Cu, Ga, Li, Mo, Ni, Pb, Rb, Sr, Tl, and V increased in the roots with increase in biosolids. However, a decrease in concentration of Al, Bi, Fe, and In upon the addition of biosolids was observed.

Figure A.2c shows the concentrations of the microelements present in the leaves of radish (*Raphanus sativus L.*) cultivated in 100 ppm Mn treatment. The concentration of elements Ag, Al, Ba, Be, Bi, Cd, Co, Cr, Cs, Cu, Ga, In, Li, Mo, Ni, Rb, Sr, Tl, V, and Zn was relatively lower in the leaves of radish plants at < 0.2 mg/kg. In general, elements Fe and Mn increased in plant parts with the addition of biosolids. The concentration of Pb in the leaves increases upon addition of biosolids up to 75% (w/w).

A.2 Plants cultivated in biosolids treated with 100 ppm Cd at pH 6.70 and 7.30

A.2.1 Analysis of macroelements in plant parts

Figure A.3a shows the concentrations of macroelements in the root of radish (*Raphanus sativus L.*) cultivated in 100 ppm Cd treatment. There was an observed increase in the concentration of Ca and Mg compared to K and Na upon the addition of biosolids. The highest concentrations of Ca, K and Na in the root was found at 50% (w/w) compost amounts, while Mg was found the highest at 100% (w/w) compost.

Figure A.3b shows the concentrations of the macroelements in the shoot of radish (*Raphanus sativus L.*) cultivated in Cd treatment. In general, the concentration of Ca and Mg increases upon addition of biosolids while the concentration of K and Na in the shoot decreased. The highest concentration of Ca, K, Mg, and Na in the shoot was observed at 75%, 0%, 100%, 100% (w/w) respectively.

Figure A.3c shows the concentrations of the macroelements in the leaves of radish (*Raphanus sativus L.*) cultivated in 100 ppm Cd treatments. Notably, an increase in the concentration of Ca, K, Mg upon addition of biosolids was observed while the concentration of Na decreases. The highest concentration of K and Na in the leaves was found at 25% (w/w).

A.2.2 Analysis of microelements in plant parts

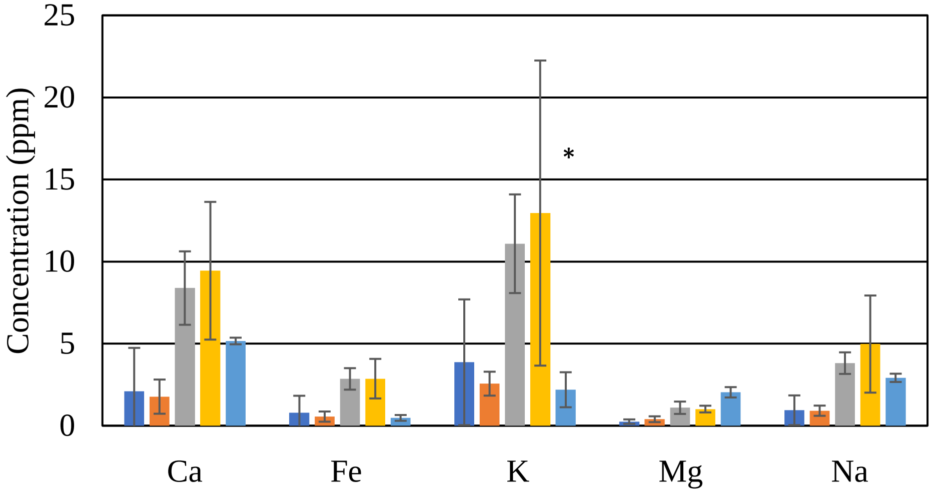
Figure A.4a shows the concentrations of the microelements in the radish (*Raphanus sativus L.*) roots cultivated in 100 ppm Cd treatments. The concentration of microelements Ag, Ba, Be, Cd, Co, Cr, Cu, Ga, Li, Mn, Ni, Pb, Rb, Sr, Tl, V, and Zn in the root were determined below 0.5 mg/kg. Upon increasing biosolid amounts, the concentrations of Al, Bi, Cs, Fe, In, and Mo decreased.

Figure A.4b shows the concentrations of the microelements in the shoot of radish (*Raphanus sativus L.*) cultivated in 100 ppm Cd treatments. Concentrations of Ag, Al, Ba, Be, Cd, Co, Cr, Cu, Ga, Li, Mn, Mo, Ni, Pb, Rb, Sr, Tl, V, and Zn was determined below 0.2 mg/kg in the shoot. An observed decrease in the concentration of Bi, Fe, and In showed upon addition of biosolids amounts.

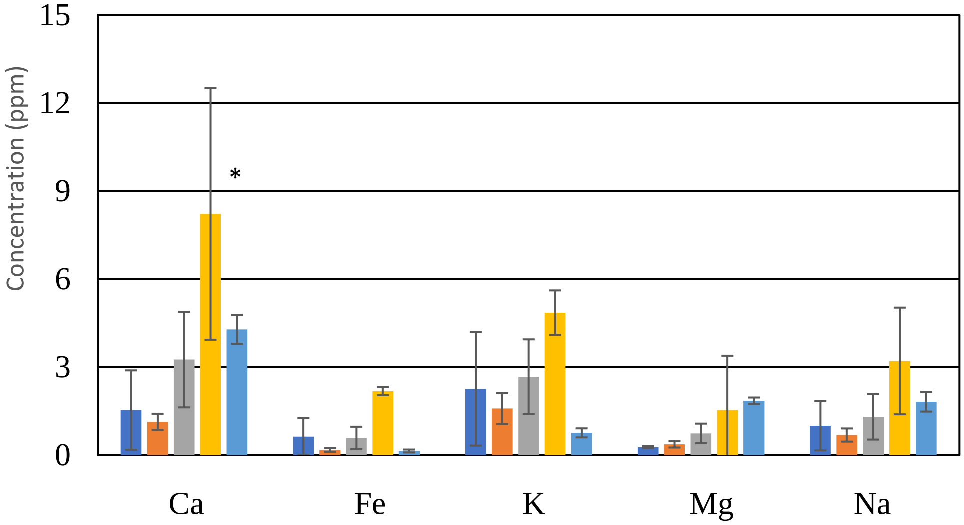
Figure A.4c shows the concentrations of microelements in the leaves of radish (*Raphanus sativus L.*) leaves cultivated in 100 ppm Cd treatments. The concentration of Ag, Al, Ba, Be, Cd, Co, Cr, Cu, Ga, Li, Mo, Ni, Pb, Rb, Sr, Tl, V and Zn was very determined < 0.1 mg/kg in radish leaves. With increase in sludge amounts, the Bi concentrations decreased, while the concentration of Fe amounts in the leaves increased.

Appendix Figures

a)



b)



c)

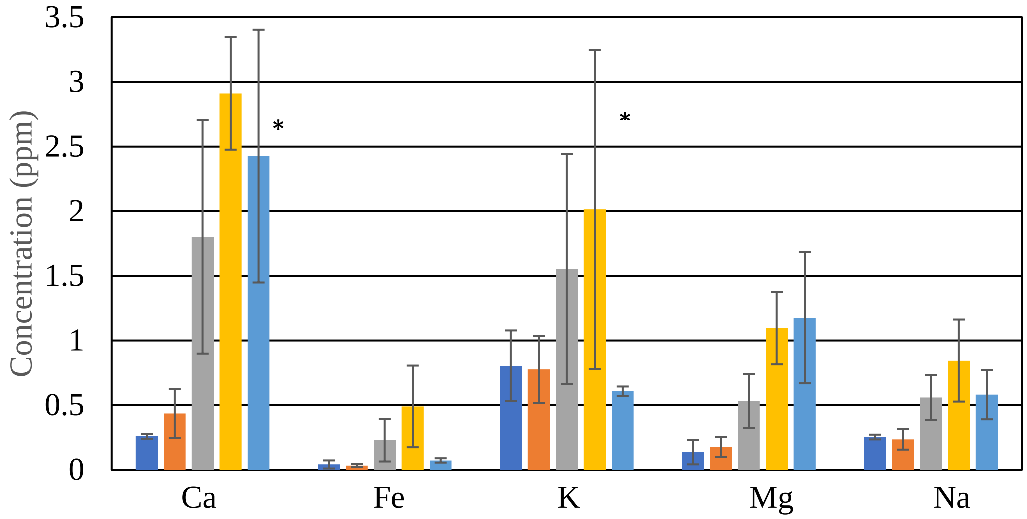
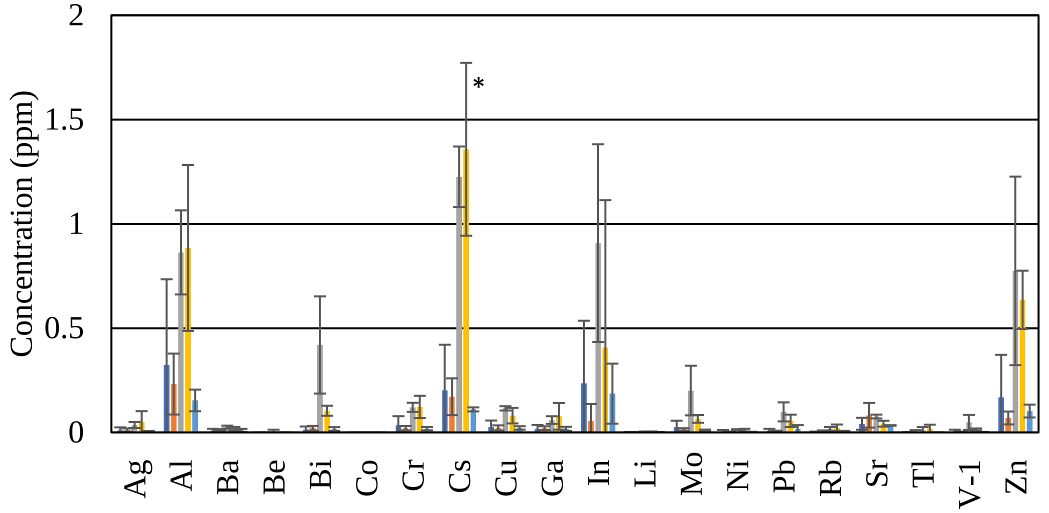


Figure A.1: **Concentration of macroelements (mg/kg) in Radish (*Raphanus sativus L.*) cultivated with 100 ppm Mn treatment at root (a), shoot (b) and leaves (c)**

🟦 = 0% 🟧 = 25% ⬜ = 50% 🟨 = 75% 🟦 = 100%

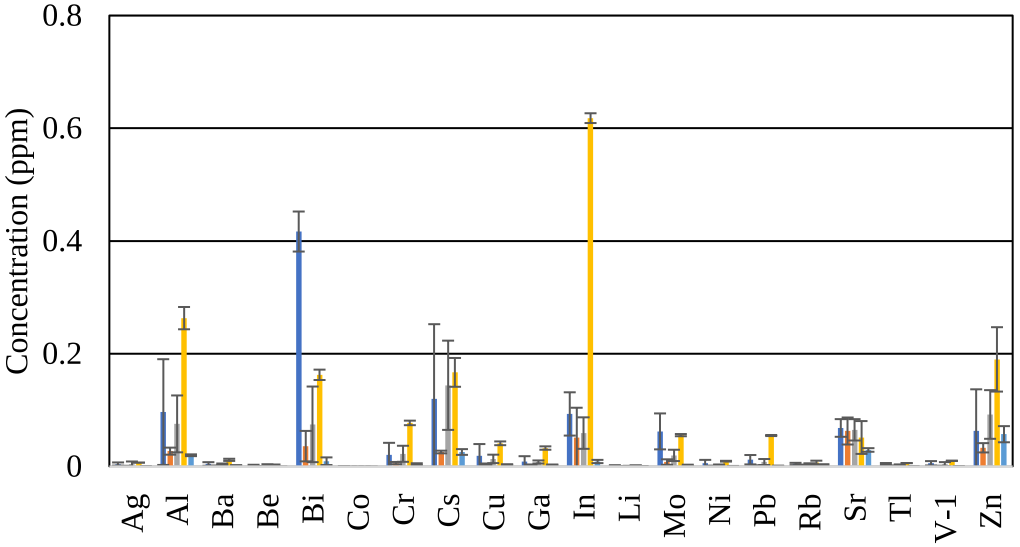
*****The metal concentrations were determined per the biomass of the radishes, since there was an observed difference in the plant biomass for the triplicate samples, it also had effect on the concentration which resulted in the error bars at K (0%, 75% root), Ca (0%, 75% shoot), Ca (50% leaf) and K (50% leaf).

a)



Concentration (ppm)
0
0.5
1
1.5
2
*
Ag
Al
Ba
Be
Bi
Co
Cr
Cs
Cu
Ga
In
Li
Mo
Ni
Pb
Rb
Sr
Tl
V-1
Zn

b)



Concentration (ppm)
0
0.2
0.4
0.6
0.8
Ag
Al
Ba
Be
Bi
Co
Cr
Cs
Cu
Ga
In
Li
Mo
Ni
Pb
Rb
Sr
Tl
V-1
Zn

c)

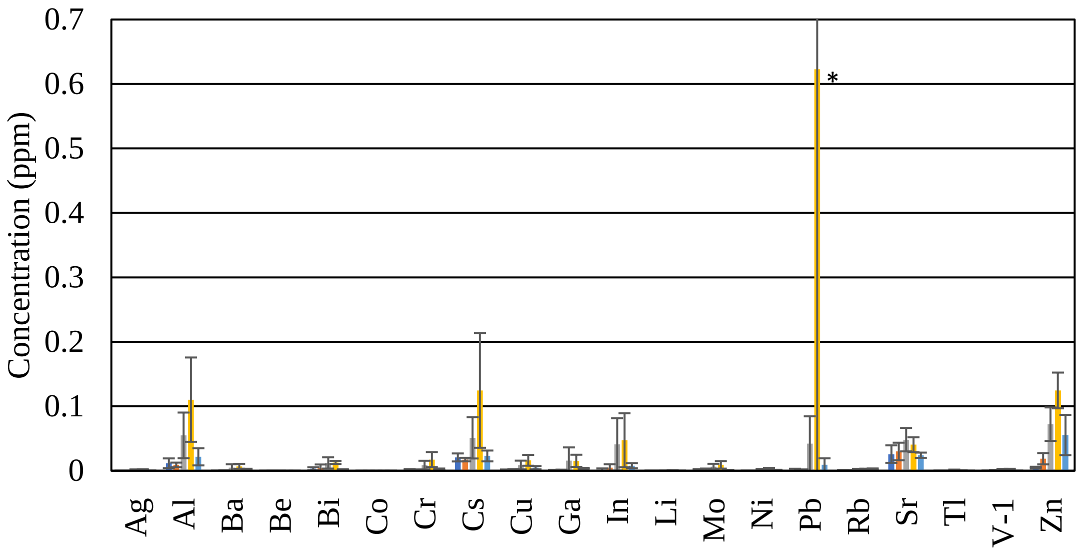
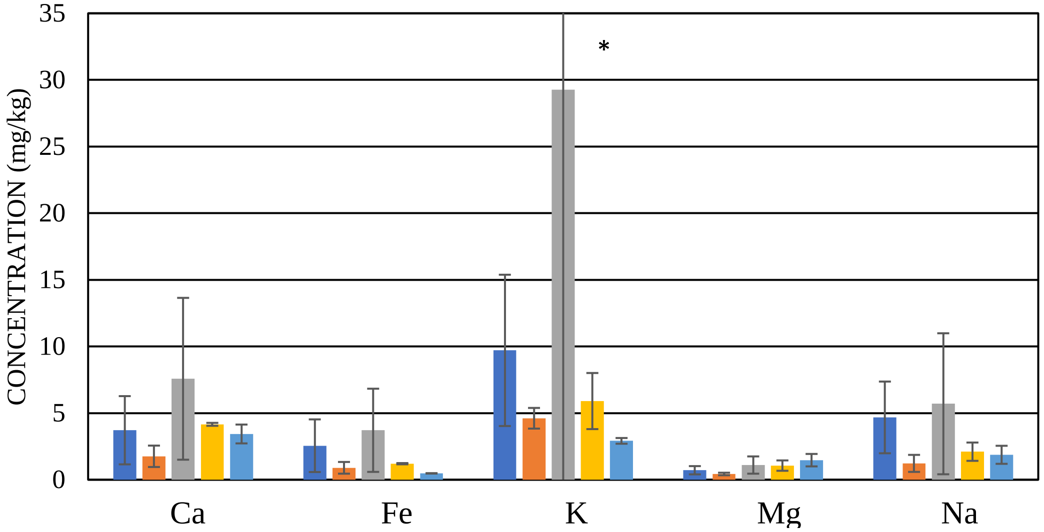


Figure A.2: **Concentration of microelements (mg/kg) in Radish (*Raphanus sativus L.*) cultivated with 100 ppm Mn treatment at root (a), shoot (b), and leaves (c).**

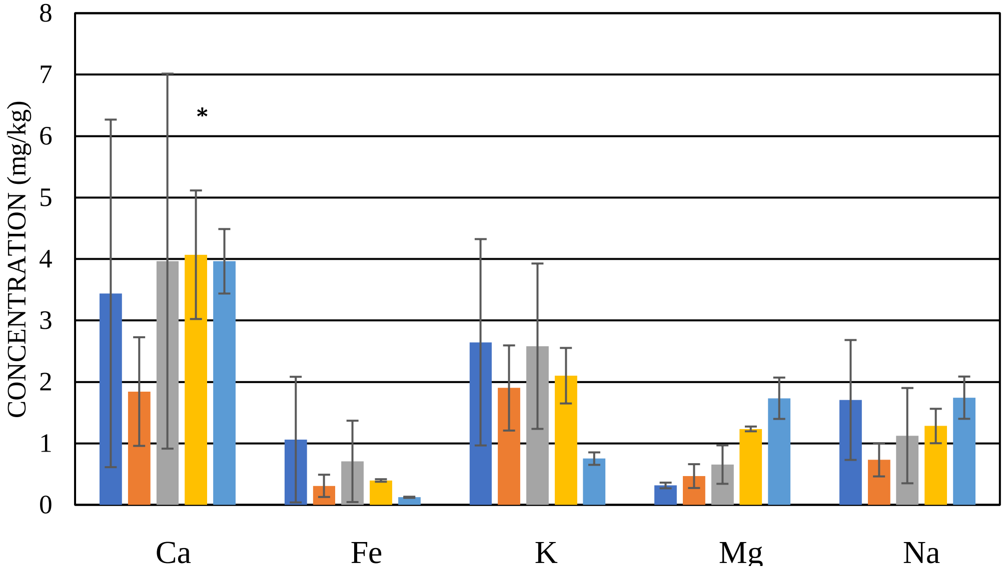
🟦 **= 0%** 🟧 **= 25%** ⬜ **= 50%** 🟨 **= 75%** 🟦 **= 100%**

*****The metal concentrations were determined per the biomass of the radishes, since there was an observed difference in the plant biomass for the triplicate samples, it also had effect on the concentration which resulted in the error bar at Pb (75% leaf).

a)


35
30
25
20
15
10
5
0
CONCENTRATION (mg/kg)
*
Ca
Fe
K
Mg
Na

b)


8
7
6
5
4
3
2
1
0
CONCENTRATION (mg/kg)
*
Ca
Fe
K
Mg
Na

c)

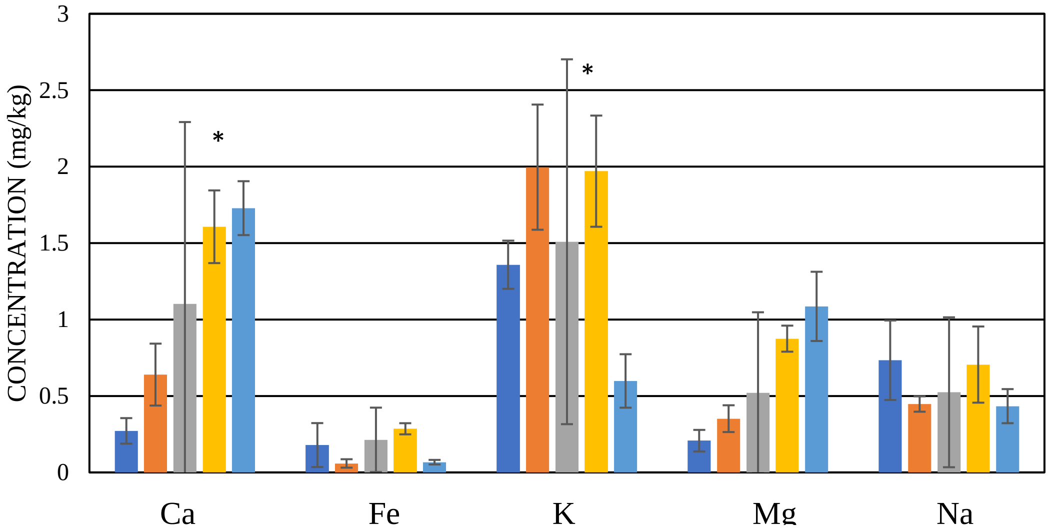
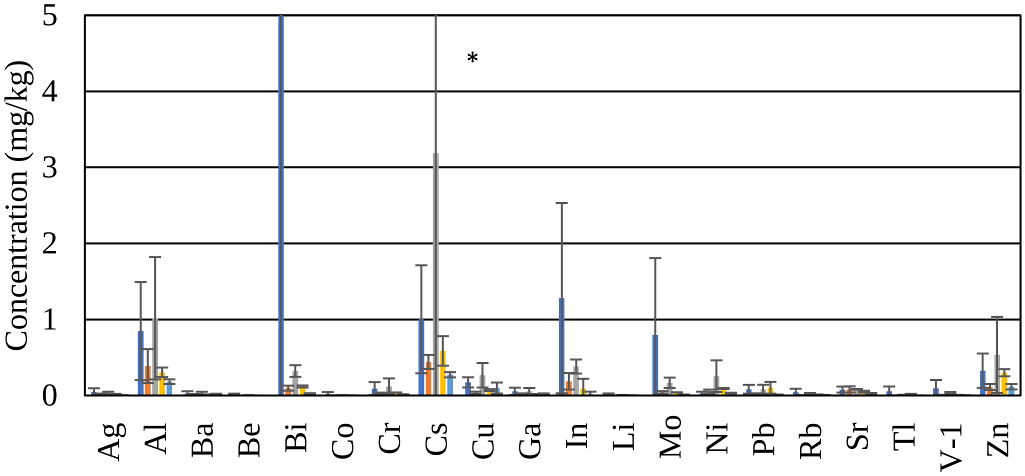


Figure A.3: **Concentration of macroelements (mg/kg) in Radish (*Raphanus sativus L.*) cultivated with 100 ppm Cd treatment at root (a), shoot (b), and leaves (c)**

🟦 = **0%** 🟧 = **25%** ⬜ = **50%** 🟨 = **75%** 🟦 = **100%**

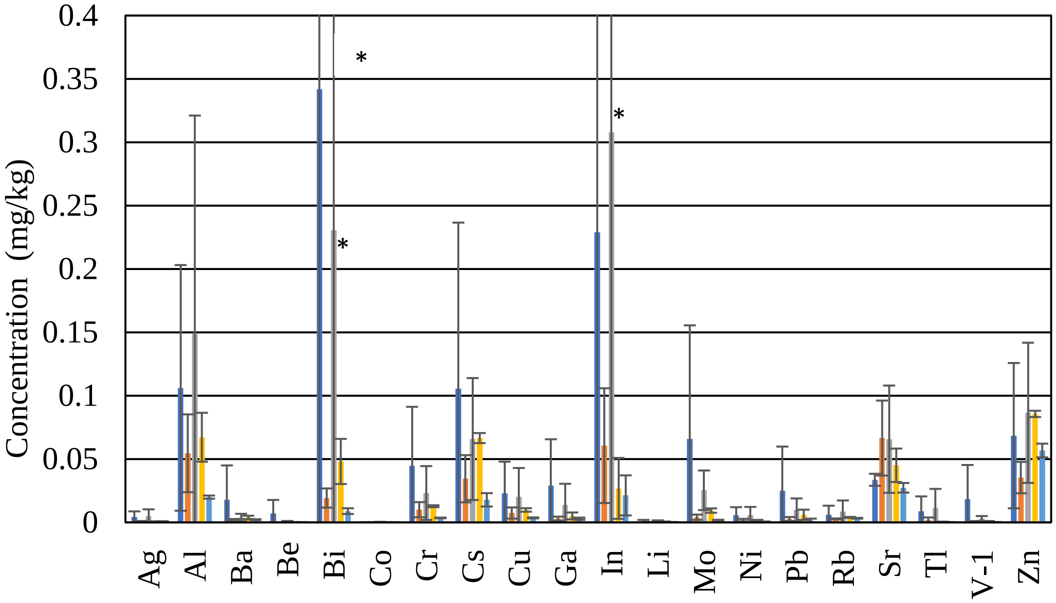
*****The metal concentrations were determined per the biomass of the radishes, since there was an observed difference in the plant biomass for the triplicate samples, it also had effect on the concentration which resulted in the error bars at K (50% root), Ca, Fe (0%, 50% shoot), Ca, Fe, K, Mg, Na (50% leaf).

a)



5
4
3
2
1
0
Concentration (mg/kg)
*
Ag
Al
Ba
Be
Bi
Co
Cr
Cs
Cu
Ga
In
Li
Mo
Ni
Pb
Rb
Sr
Tl
V-1
Zn

b)



0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Concentration (mg/kg)
*
*
*
Ag
Al
Ba
Be
Bi
Co
Cr
Cs
Cu
Ga
In
Li
Mo
Ni
Pb
Rb
Sr
Tl
V-1
Zn

c)

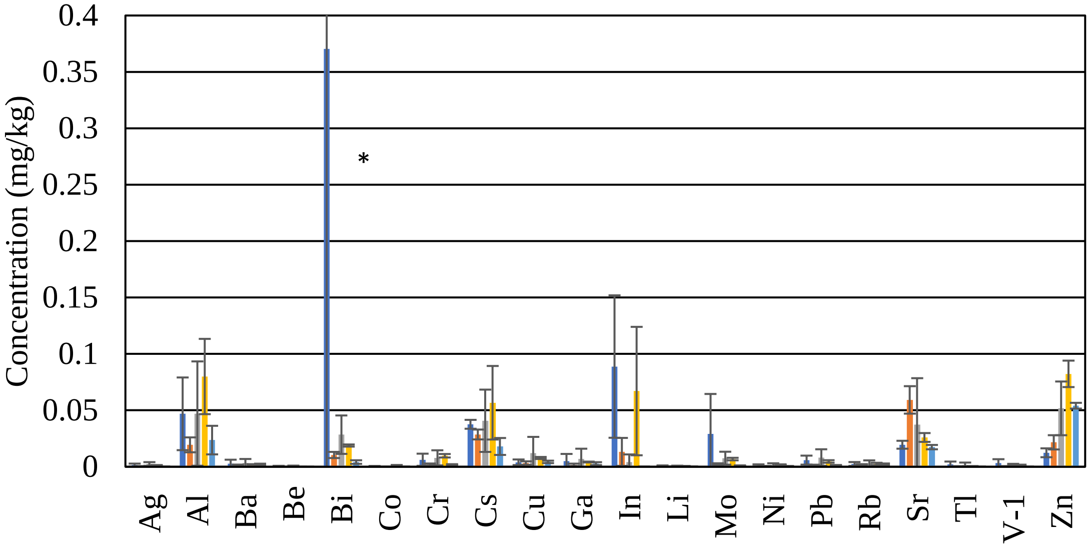


Figure A.4: **Concentration of microelements (mg/kg) in Radish (*Raphanus sativus L.*) cultivated with 100 ppm Cd treatment at root (a), shoot (b), and leaves (c).**

🟦 **= 0%** 🟧 **=25%** ⬜ **= 50%** 🟨 **= 75%** 🟦 **= 100%**

*****The metal concentrations were determined per the biomass of the radishes, since there was an observed difference in the plant biomass for the triplicate samples, it also had effect on the concentration which resulted in the error bars at Bi (0% root), Cs (50% root), Bi, Al, In (0%, 50% shoot), and Bi (0% leaf).

VITA

After completing her work at Taidob College, Ogun State, Nigeria, in 2011, Oluwadamilola Fateru entered Bowen University at Osun State, Nigeria. During the summer break of 2014, she was an intern in Lafarge cement industry. She received the degree of Bachelor of Science from Bowen University in July 2015. During the following two years, she worked as a teaching assistant in St. Ann’s group of schools. In January 2018, she entered the Graduate School of Stephen F. Austin State University, Nacogdoches, Texas and received the degree of Master of Science in December of 2019.

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The reference style used was American Chemical Society (ACS).

This thesis was typed by Oluwadamilola O. Fateru.