REMEDIATION OF CONTAMINATED DUMPSITE SOIL:

AN EVALUATION OF SOIL AMENDMENTS IN COMBINATION WITH PHYTOREMEDIATION

by

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Sasha Jones, July 29, 2022

ABSTRACT

REMEDIATION OF CONTAMINATED DUMPSITE SOIL:
AN EVALUATION OF SOIL AMENDMENTS IN COMBINATION WITH PHYTOREMEDIATION

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Contamination of soils and water from open dump site leachate is a frequent problem in developing nations. Water supplies are threatened when water percolates through the waste, bringing with it a variety of substances, such as metals, bacteria, viruses, flammables, organic chemicals, and other toxics. Metals in particular are complex in terms of the pathways they can choose in the environment; unlike organics, they do not degrade within the environment. Due to increases in population and affluence, the generation of waste has increased, meaning that leachate treatment will become an increasing problem.

The overarching research goal was to evaluate a low-cost method of removing heavy metals from leachate in developing countries, using a combination of phytoremediation and soil amendments. The Perseverance Dump Site in Grenada was used as a case study. Specific objectives of the research were:

1. To assess the potential of locally available amendments (fish bone meal, fertilizer, and fruit peels) to bind/adsorb heavy metals in soil contaminated with leachate from open dumpsites (Zn, Cu, Pb, Cr, Mn, Ni, V, As, Cd, Fe).

2. To assess the efficiency of a plant species native to Grenada (Vetiver grass), in removing heavy metals from soil contaminated with dumpsite leachate, with and without amendments.

The research hypotheses were:

- One of the fishbone amendment concentrations will perform better than the others.
- Banana peel will have a higher concentration removal than the other fruit peels.

To address Obj. 1, a batch study was conducted. Metals in synthetic leachate, at concentrations typical of dumpsites, were applied to clay loam soil (dominant soil type in Grenada) and tested with 11 application rates of amendment (5 concentrations of fishbone; fertilizer; banana, lemon, and orange fruit peels; and no amendment as a control), along with duplicates. Metals in the soil were quantified using a modified sequential extraction procedure (Tessier, 1979), and the resulting liquid samples were analyzed by Shimadzu ICPMS-2030.

To address Obj. 2, amendments with the highest removal efficiencies in the batch study (3% w/w fishbone and banana peel) were selected for bench-scale phytoremediation experiments. Vetiver grass was grown in 10-gallon reactors with clay loam soil mixed with synthetic leachate, amended with fishbone, banana peel, or nothing (control). The experiment ran for 28 days, with sampling on days 14 and 28. Vetiver grass was analyzed for heavy metals by EPA Method 200.7 (1994) using Agilent 7800 ICP-MS.

Metals that enter solution during phases 0-2 of the sequential extraction are water soluble, exchangeable, and bound to carbonate metals; these are easily leached back into the environment. However, metals that enter during phases 3-5 are not readily transferred to the environment; they are bound to iron & manganese oxides, organic matter and crystalline latices. In other words, metals remaining in the soil during phases 0-2, and entering solution only during phases 3-5, are well stabilized by the soil/amendment.

Fishbone was the better-performing amendment in the bench-scale tests (amendments and plants). 3% fishbone amendment with plants increased the amount of metals stabilized in fraction 3-5, compared to the control reactor (plants alone). Fractions 3-5 represent the more stable binding of metals in nature; in these fractions the metals are not easily leached into the environment. The increase was statistically significant, to at least a 70% level of confidence, for 9 of 10 metals near the plant, and for 6 of 10 metals farther away from the plant. Banana peel amendment with plants increased the amount of metals stabilized in fraction 3-5, compared to the control reactor (plants alone). The increase was statistically significant, to at least a 70% level of confidence, for 8 of 10 metals near the plant, and for 5 of 10 metals farther away from the plant.

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Dumpsites receive around 40% of waste worldwide, serving 3.5-4 billion people (ISWA, 2015). Contamination of soils and water from open dump site leachate is a frequent problem in developing nations. Water supplies are threatened when water percolates through the waste, bringing with it a variety of substances, such as metals, bacteria, viruses, flammables, organic chemicals, and other toxics. Metals in particular are complex in terms of the pathways they can take in the environment; unlike organics, they do not degrade within the environment. Due to increases in population and affluence, the generation of waste has increased, meaning that leachate treatment will become an increasing problem. In developing countries, there is a lack of landfills and having an open dumpsite is common practice. Rainwater seeps through the waste, producing large quantities of leachate that is highly toxic and is potentially harmful to ground and surface water and surrounding ecosystems (Igbal et al., 2021).

Removal of heavy metals in particular, remains a challenge in treating leachate, and contaminated surface and ground water. The most frequent hazardous heavy metals in the waste industry are lead (Pb), cadmium (Cd), zinc (Zn), copper (Cu), nickel (Ni) and chromium (Cr) (Mehdipour et al., 2015). The most dangerous of these heavy metals is lead, due to the side effects it has on humans. Lead toxicity increases the risk of skeletal disorders by interacting with calcitropic hormones (Rana, 2014). Damage is caused to important organs such as the brain, liver, kidney, and blood due to their potential toxicity. Development of neurological, physical and muscular degenerative processes can mimic diseases such as multiple sclerosis, Parkinson's disease, Alzheimer's disease and muscular dystrophy with long-term exposure. Repeated long-term exposure to various metals and their compounds risks the development of cancer (Jaishankar et al., 2014).

The preferred leachate treatment strategy is difficult to assess due to the variability in its characteristics and composition. Leachate quality is dependent on but not limited to the following: rainfall, age of the waste, rate of permeability, compaction, and the rate of decomposition (Jones et al., 2005). Developing cost effective, efficient methods for removing heavy metals from leachate is a critical need.

Natural treatment systems are treatment systems that use natural processes involving vegetation, soils, and their associated microbial assemblages to improve water quality. They are perceived as self-decontamination ecosystems with high productivities. Leachate treatment using plants can also be called leachate phytoremediation, where the principle of the treatment is to, "use the potential of the natural or actively managed soil–plant system to detoxify, degrade and inactivate potentially toxic elements present in the leachate" (Jones et al., 2005). Phytoremediation is being recognized as an integrated economically viable technology using green plants for the removal, degradation, and detoxification of chemical pollutants from contaminated soils, sediments, or waters (Clayton, 2007). Constructed wetlands in particular have been observed in various studies to remove 70-100% of various heavy metals like Pb, Zn, Cr, aluminum (Al), Cd, Iron (Fe) (Ibrahim et al., 2012; Mishra and Tripathi, 2009; Mitra et al., 2014; Zheng et al., 2016), which can be transferred into the biomass and immobilized. Phytoremediation reduces metal mobility and leaching into ground water and reduces metal bioavailability for entry into the food chain (Garba et al., 2018).

Plants that can tolerate a high concentration of metals that are otherwise toxic to other organisms are referred to as hyperaccumulators. Plants that are hyperaccumulators can tolerate higher concentrations of available metals. The threshold for different metals and metalloids in dried foliage is "100 µg/g for Cd, Se and Tl; 300 µg/g for Co, Cu and Cr; 1,000 µg/g for Ni, Pb and As; 3,000 µg/g for Zn; and 10,000 µg/g for Mn, with plants growing in their natural habitats" (Van Der Ent et., 2013). The process of using phytoremediation is a new technology and is still being developed. Phytoremediation of soil or water requires that the contaminants be within the zone of influence of the plant roots. Few projects have been conducted using wetland phytoremediation and results are site specific. A phytoremediation study conducted in Thailand using Vetiver grass demonstrated in both greenhouse and field experiments the ability of the plants to remove contaminants from soil (Phusantisampan et al., 2016).

Rates of metal uptake by plants, however, can be slow. Soil amendments containing phosphorous, such as fish bones and fertilizer, have been shown in previous studies to stabilize heavy metals (Freeman, 2012). In addition, a review of various fruit peels to remove copper, cadmium, and lead from wastewater suggest that using fruit peels as adsorbents has equal or greater capacities compared to activated carbon. Soil amendments containing phosphorous, as well as fruit peel adsorbents, could be used in combination with phytoremediation to stabilize the metals while plants are accomplishing the slow process of phytoextraction.

The overall goal of this research is to facilitate low-cost removal of heavy metals from soil in developing countries, utilizing a combination of phytoremediation and soil amendments. The introduced plant species Vetiver grass will be tested, in combination with soil amendments (fish bones, fertilizer, and fruit peels), for their ability to remove ten heavy metals (zinc, copper, lead, nickel, manganese, chromium, arsenic, cadmium, iron and vanadium) from leachate at an open dumpsite in Grenada. Grenada was chosen as a case study because there was a need to create a long-term, economically viable solution to the waste management system. Preliminary analysis of the soil in multiple areas surrounding the open dumpsite indicated that there is a need to protect the surrounding water systems and the health and well-being of the citizens. Grenada is part of the group Small Island Developing States (SIDS) which shares similar sustainable challenges where waste disposal is a commonality. Doing this research will enable other islands to adopt this safe practice to minimize the pollution. The population is increasing, and the availability of space and resources are becoming limited. Waste generation is influenced by the human development index (HDI), GDP, population growth and tourism. The considerable number of wastes emanating from the tourism industry advocates for sustainable methods to be adopted (Mohee etal., 2015).

While previous studies have examined the ability of vetiver grass to remove metals individually, none has focused on using it to remove the ten heavy metals in combination. In addition, no study has tested the efficacy of phytoremediation in combination with fish bone stabilization. This research will fill these gaps, to facilitate removal of metal pollutants from leachate at open dump sites.

1.2 Research Objectives

The overarching research goal is to evaluate a low-cost method of removing heavy metals from leachate in developing countries, using a combination of phytoremediation and soil amendments. The Perseverance Dump Site in Grenada will be used as a case study. Specific objectives of the proposed research are:

1. To assess the potential of soil amendments (fish bone meal, fertilizer, and fruit peels) to bind/adsorb heavy metals in leachate from open dumpsites, (Zn, Cu, Pb, Cr, Mn, Ni, V, As, Cd, Fe).
2. To compare the efficiency of a plant species introduced to Grenada (Vetiver grass) in removing heavy metals from dumpsite leachate, with and without soil amendments.

The hypotheses were:

- One of the amendment concentrations for the fishbone will perform better than the other.
- The banana peel will have a higher removal of metals than the other fruit peels, based on the efficiency of heavy metal removal using food waste by Massimi et al, 2018.

1.3 Dissertation Organization

Subsequent chapters of this dissertation are organized as follows:

- Chapter 2 provides background, a review of the relevant literature, and the background information on the dumpsite located in Grenada.

- Chapter 3 describes the methodology, including the process of collecting and preparing the soil and plant samples for the batch test and the bench scale reactors.
- Chapter 4 discusses the experimental results obtained for the soil, amendments, and plants and compares them to the existing literature.
- Chapter 5 summarizes the main conclusions of the current research work and provides recommendations for future studies.
- The appendices include raw data.

CHAPTER 2

LITERATURE REVIEW AND BACKGROUND

2.1 Grenada Solid Waste Management

Grenada is an island located in the Caribbean approximately 100 miles north of Trinidad and Tobago. It is predominantly volcanic with some sedimentary rock. The central axial mountains create a radial drainage pattern for the rivers. The soils of Grenada are dominated by clay loams (84.5%), along with clays (11.6%) and sandy loam (2.9%) The three major types of clay loam are the Woburn, Capitol, and Belmont, which together constitute 77.8% of the island's soil (Ternan et al., 1989).

The population of Grenada was 111,454 as of 2018, and the per capita waste generation rate is 1.13 kg/person/day (Elgie et al., 2020). Approximately one-third of the population is found in the capital of St. George's, located in the southwestern part of the island. The island's population is concentrated along the coastal regions in each of the six parishes, with St. George having the highest population of 38,000 (Census, 2011). St. George is the parish where Grenada's only dumpsite is located.

GDP per capita in Grenada averaged $12,380 USD from 1990 until 2019, reaching an all-time high of $17,242 USD in 2019. The GDP per capita is relatively low, so implementing an inexpensive method of treating leachate is needed.

Solid waste disposal is managed by the Grenada Solid Waste Management Authority (GSWMA), which was established by parliament in 1995 to improve and manage the solid waste's storage and disposal methods. GSWMA collects 98% of waste on the island. Since there is only one open dumpsite on the entire island (Perseverance Dump Site), more emphasis is placed more on reducing, reusing, and recycling. The government's main objective is to promote the sustainable economic and social development of Grenada (Medium-term Economic Strategy Paper (1996-1998)) and this research project will align with the key environmental issues. The

Government of Grenada's (GoG) strategic priority 1 is to enhanced national capacity for Biodiversity Conservation and Sustainable Use by building capacities of farmers, fisher folks, community activists and leaders in soil and water conservation, biodiversity knowledge, food and nutrition security, sustainable livelihoods and sustainable production and consumption practices (Biodiversity Strategy & Action Plan, 2016-2020). Another priority is to institute environmental protection programs, to ensure that economic and social development is physically and institutionally sustainable (Biodiversity Strategy & Action Plan, 2000).

2.1.1 Perseverance Dump Site

The Perseverance Dump Site (Fig. 2.1) is located on the western side of the island approximately 600 meters inland from the Caribbean Sea. (Fig. 2.2) The site receives approximately 200cm of rainfall per year. The area is rich in clayey soils with the Salle River that runs between both sites eventually emptying into the bay. (OECS EAS, 2013) Adjacent to the dumpsite is an operational quarry and asphalt-producing plant that contribute to waste production in the area. (Fig. 2.3). There is a total estimate of 44,508 tons of waste generated or 1.13 kg/person/day (GoG, 2018). The waste characterization constitutes 29% organic, 14% plastics, 14% paper and cardboard, 8% street sweeping, 8% glass, 6.5% metals with the other 20% consisting of construction, special care, textiles, and other hazardous waste. This site was constructed and became operational in February 2001. This site replaced three open dumpsites (Telescope, Perseverance and Dumfries, Carriacou). The dump does not have methane gas or leachate management, or daily site covering to prevent material leakage. There are ongoing issues with fires due to methane build up (Fig.2.4) (Elgie et al., 2020). The old site reached full capacity and the new location was set up as a functioning landfill in 2001. Six months after operation began, heavy rains caused a landslide and the eventual closure for repairs; the old site was reinstated. Both old and new site are now open dumpsites (CHARIM, 2016).

Figure 2.1. Location of Grenada and the Perseverance Dump Site

Figure 2.2. Aerial View of Perseverance Dump Site

Figure 2.3. Aerial View of Perseverance Dump Site showing the locations of preliminary sample collection, asphalt manufacturing plant, bay area, and Salle River.

In the initial development of the new site, a leachate management pond (polishing pond) was created to collect the excess leachate when rain fell. Organic contaminants reaching the pond were to be treated with the aid of sunlight breaking down the biota contained in the leachate (Fig. 2.5). After the landslide, this practice no longer continued. With no methane gas management, leachate management, or daily trash covering to prevent the trash from being blown away the site poses an environmental and human hazard. There is a track loader (bulldozer) on site to move and compact the trash (Fig. 2.6). Approximately 15 informal workers are on site removing valuable recyclables. The Solid Waste Authority does not provide sanitation or health and safety services for these workers (Elgie et al., 2020). The tires at the dumpsite are shredded or repurposed; they represent a high volume of waste content at the site (Fig. 2.8). Most of the metals are compacted and exported, while the rest are removed by informal workers (Fig. 2.9).

Figure 2.4. View of Old Dumpsite burning since 2016

Figure 2.5. Polishing pond

Figure 2.6. View of New Dumpsite

Figure 2.7. Track loader operation


Figure 2.8. Tire Shredding Area


Figure 2.9. Metal Compacting Area

2.1.2 Prior metals data collection at the Perseverance Dump Site

In December 2017, soil samples were collected from four areas (bay area, loading area, old site, and stream new site) around the Perseverance dump site (Fig. 2.10). The samples were analyzed using Shimadzu EDX-7000 (energy-dispersive X-ray fluorescence) spectrometer to produce qualitative results for the metal species (Table 2.1). Generally, the pH of leachate at young dumpsites (<10 years) is more acidic than atmature or older sites. This is due to biological decomposition of organic N into ammonium N (Chen, 1996). pH has a major effect on the solubility of free metals such as Zn, Cu, Ni, Cd and Pb. The lower the pH the higher the solubility of the metals.

Figure 2.10. Aerial View of Perseverance Dump Site showing the locations of preliminary sample collection.

Of the metals measured at the site, Zn, Cu, Pb, Cr, Mn, Ni, V, As, Cd, and Fe were chosen for further testing (fish bone binding and phytoremediation, as described in Ch. 3) because of their prevalence in Table 3, and because a literature review of metals in dumpsites listed them as prevalent for tropical regions like Grenada (Vaccari et al. 2019). In addition, these metals were chosen because of the effect they have on the human body. The brain is particularly susceptible to prolonged exposure to these environmental toxicants, which can cause cognitive dysfunction (Karri et al., 2016). Exposure to these potentially toxic metals (PTMs) can also lead to severe consequences like kidney and liver failure, damage to the nervous and immune systems, and disruption of hemoglobin synthesis. As these PTMs accumulate in soil and plant systems, their removal from soil and water is the most concerning issue for researchers (Raj et al., 2020).

Table 2.1. Elemental Weight Percentage of Metals Identified at Perseverance Dump Site Soil

Sample A	**Bay area**		**Sample B**	**Loading area**		**Sample C**	**Old site**		**Sample D**	**stream new site**		**Typical Concentrations found in the Soil**
	Result	**Approx**		**Result**	**Approx**		**Result**	**Approx**		**Result**	**Approx**	
Metal	Wt.% +/- 15%	ppm	Metal	Wt.% +/- 15%	ppm	Metal	Wt.% +/- 15%	ppm	Metal	Wt.% +/- 15%	ppm	mg/kg
Al	12.198	121,980	**Al**	14.41	144,100	**Al**	12.08	120,800	**Al**	14.493	144,930	
Br	0.004	40	Br	0.009	90	Br	0.038	380	Br	0.026	260	
Ca	37.492	374,920	**Ca**	13.488	134,880	**Ca**	5.75	57,500	**Ca**	12.963	129,630	
Cl	0.504	5,040	**Cl**	0.208	2,080				**Cl**	0.881	8,810	
Cr	0.116	1,160	**Cr**	0.126	1,260	**Cr**	0.158	1,580	**Cr**	0.077	770	5 to 1,500
Fe	16.435	164,350	**Cu**	0.165	1,650	**Cu**	0.636	6,360	**Cu**	0.147	1,470	2 to 250
Ga	0.005	50										
			Fe	30.67	306,700	**Fe**	52.023	520,230	**Fe**	27.663	276,630	2,000 to 550,000
			Ir	0.016	160	Ir	0.018	180	Ir	0.033	330	
K	1.718	17,180	**K**	2.887	28,870	**K**	1.102	11,020	**K**	4.482	44,820	
Mg	1.668	16,680	**Mg**	0.907	9,070				**Mg**	1.078	10,780	
Mn	0.369	3,690	**Mn**	1.043	10,430	**Mn**	0.715	7,150	**Mn**	1.12	11,200	20 to 10,000
Ni	0.022	220	**Ni**	0.045	450	**Ni**	0.057	570	**Ni**	0.047	470	2 to 750
			P	0.062	620	P	0.136	1,360	P	0.122	1,220	
Rb	0.012	120										
			Pb	0.142	1,420	**Pb**	0.199	1,990	**Pb**	0.03	300	2 to 300
S	0.148	1,480	**S**	0.225	2,250	**S**	0.58	5,800	**S**	0.844	8,440	
Si	27.133	271,330	**Si**	32.922	329,220	**Si**	23.331	233,310	**Si**	33.343	333,430	
Sr	0.925	9,250	Sr	0.291	2,910	Sr	0.14	1,400	Sr	0.398	3,980	
Ti	1.154	11,540	**Ti**	1.657	16,570	**Ti**	1.637	16,370	**Ti**	1.68	16,800	

V	0.052	520	V	0.139	1,390	V	0.115	1,150	V	0.106	1,060	3 to 500
Y	0.008	80										
Zn	0.039	390	Zn	0.499	4,990	Zn	1.216	12,160	Zn	0.284	2,840	
			Zr	0.089	890	Zr	0.068	680	Zr	0.096	960	1 to 900

Red: Waste Disposal **Green: Abundant in the earth's crust** **Blue: Metallurgic (Tar) Industries**

2.2 Phytoremediation

Phytoremediation is the use of plants and associated soil microbes to reduce the concentrations or toxic effects of contaminants in the environment (Ali et al., 2013). The plants extract the contaminants (e.g. heavy metals) making the bioavailability in the soil lower. (Yan et al., 2020). There are many kinds of phytoremediation approaches that can remove heavy metals from soil, including (1) phytostabilization – reduces the bioavailability of heavy metals in soil using plants, (2) phytoextraction – use of plants to remove heavy metals from soil, (3) phytovolatilization – plants uptake the contaminant, convert it to a gaseous stage, and release it into the atmosphere, (4) phytofiltration – using hydroponically cultured plants to absorb or adsorb heavy metals in ground and aqueous water, (5) phytodegradation –plants break down organic chemicals. (Yan et al, 2020) and (6) phytodesalination – removal of salt from salt affected soils to enable them to support normal plant growth (Ali et al., 2013). Phytoremediation can be used to treat various pollutants, including metals, pesticides, solvents, explosives, crude oil, polycyclic aromatic hydrocarbons, and landfill leachate (US Environmental Protection Agency, 1999).

Factors that adversely affect phytoremediation efficiency are water deficiency, low bioavailability of metals, salinity or sodicity and low fertility of soil (Razmi et al., 2021). Advantages of phytoremediation are cost effectiveness, being a highly accepted method for waste removal, and suitability for sites with shallow contaminants. Some disadvantages are accumulation of pollutants in the edible parts of the plant, low biomass production, slow treatment, and not being applicable to all compounds (Farraji et al., 2016). Phytoremediation should be reconsidered if the introduction of a species is invasive, the contaminants are deeper than the root system of the plant, or phytoremediation is otherwise not effective.

2.3 Plant Species Introduced to Grenada with Phytoremediation Potential

Phytoremediation plant species introduced to the island of Grenada include **vetiver grass** (*Chrysopogon zizanioides),* or colloquially named razor grass, **wild coffee** *(Senna* *occidentalis)*, and **nut grass** *(Cyperus rotundus)*. Vetiver is a known accumulator of metals: due to its high biomass production. It has a long shoot system that can grow from 3 m tall and a massive, deep, fast growing root system. The root system can reach 3-4 meters in year one and a total length of 7 meters in 36 months (Danh et al., 2009). The plant can accumulate 46.5 kg of Pb, 144.9 kg of Zn, and 4 kg of Cd per hectare of land (Attinti et al., 2017; Assefa et al. 2018). It is a noninvasive (with sterile seeds) species, retains soil moisture, prevents soil erosion, can survive in harsh conditions, and stabilizes soil. Currently, the Small Grants Programme (SGP) of the Global Environment Facility (GEF) implemented by the United Nations Development Programme (UNDP) is funding a project in Grenada to raise awareness and encourage the utilization of vetiver grass. When planted in accordance with the Vetiver System (VS), vetiver grass becomes a low-cost, readily available, easy to use, effective and long-lasting green technology, suitable to be incorporated into many initiatives designed to combat erosion and mitigate climate change (Mondal et al., 2020). The current project is geared to be beneficial to farmers. Since the species is already being studied, it can be implemented at the open dumpsite to absorb heavy metals from the leachate. Table 2.2 summarizes the series of glasshouse experiments proved that vetiver has high tolerance to a wide range of heavy metals in soils due to its high threshold levels of these metals in soils (Danh et al., 2009).

Table 2.2 Tolerance levels of vetiver grass growth based on single experiment (Troung, 1999b)

Heavy metals	Threshold to growth of most Threshold to vetiver vascular plants (mg/kg)		Threshold to vetiver growth (mg/kg)	
	Hydroponic level (Bowen, 1979)	Soil level (Baker & Eldershaw, 1993)	Soil Level	Shoot level
Arsenic	0.02-7.5	2	100–250	21–72
Cadmium	0.2-9.0	1.5	20–60	45–48
Copper	0.5-8.0	NA	50–100	13–15

Chromium	0.5-10.0	NA	200–600	5–18
Lead	NA	NA	>1500	>78
Mercury	NA	NA	>6	>0.12
Nickel	0.5-2.0	7–10	100	347
Selenium	NA	2–14	>74	>11
Zinc	NA	NA	>750	880

Senna occidentalis (wild coffee) is a tropical and subtropical plant approximately 0.8-1.5m tall. The seeds are used as a coffee substitute. It is mainly used for liver detoxification and to treat internal bacterial and fungal disorders. It enhances immunity and promotes perspiration. It grows as a weed in disturbed forest areas, on waste land, fields, roadsides and around villages and farms. It is especially abundant in ditches and seasonally wet depressions. Although it is resistant to dry conditions, it grows best in a moist environment and reduces the heavy metal in soils (Guo et al., 2016).

Cyperus rotundus (nut grass) grows in all types of soils and can also survive high temperatures. *C. rotundus* can be found in a wide variety of habitats including cultivated fields, waste areas, roadsides, pastures, riverbanks, sandbanks, irrigation channels, river and stream shores and natural areas.

For this research, specimens of Vetiver grass were obtained for the experiment. Although wild coffee and nut grass were good candidates for the research, it proved difficult to acquire the plants within the United States. Germinating the seeds to a mature level was unsuccessful. Vetiver grass was purchased locally be grown successfully in an indoor setting and so was used in subsequent experiments.

2.4 Phytoremediation Studies Using Vetiver Grass

Vetiver grass originated in the sub-continent of India. Found mostly in flood plains and stream banks but can also be found throughout tropical and subtropical regions (Danh et al., 2009). In various parts of the world, it is being used as a soil erosion prevention method. Very high removal rates of Fe (81%) and Pb (81%) and low removal of Ni (38%), Zn (35%), SO_4^{2-} (28%), Mn (27%), Cr (21%), Al (11%) and Cu (8.0%) this was done over a one-year experimental period to determine the effectiveness of vetiver grass for treating acid mine drainage. Vetiver removal efficiency for heavy metals in water was in the order of Fe>Pb>Cu>Mn>Zn. It was determined that Vetiver grass with longer root and higher root density was more effective in removing heavy metals such as Cu, Fe, Mn, Pb, and Zn. Vetiver tolerates a wide range of pH (3.5-11.5), salinity and heavy metals such arsenic, cadmium, copper, chromium, lead, mercury, nickel, selenium and zinc (Darajeh et al., 2019).

Table 2.3 summarizes prior studies of vetiver grass for the removal of heavy metals. Zinc is the most frequently tested metal, followed by copper and lead.

Table 2.3. Previous studies of heavy metal removal by vetiver grass showing removal efficiency in lab and field studies

Article #	Author, Year	Title	Zn	Cu	Pb	Mn	Ni	V	Cr	As	Cd	Fe
1	Anning et al., 2017	Potted experiment for removal of Hg, As, Pb, Cu and Zn. A higher removal efficiency of metals in vetiver than in cattail with amended soil. For vetiver, EDTA was more effective at enhancing Zn removal but less for As, Cu and Hg compared with $Al_2(SO_4)$ and the control. On the other hand, Al2(SO4)3 increased RE of Cu than did the EDTA and the control.	X	X	X					X		
2	Darajeh et al., 2016	Floating wetland by Chrysopogon zizanioides (L.) using response surface methodology										
3	Darajeh et al., 2019	Vetiver survived and grew in all metal (Fe, Zn and Mn) concentrations and removed 85% to 99% of the metal ions at the different concentrations.	X			X						X
4	Fasani et al., 2019	Phytoremediatory efficiency of Chrysopogon zizanioides in the treatment of landfill leachate	X			X	X					
5	Kiiskila et al., 2017	A preliminary study to design a floating treatment wetland for remediating acid mine drainage impacted water using vetiver grass (Chrysopogon zizanioides)	X	X	X		X					X
6	Kiiskila et al., 2019	Remediation of acid mine drainage-impacted water by vetiver grass (Chrysopogon zizanioides): A multiscale long-term study	X	X	X	X	X		X			X
7	Love et al., 2012	Assessment of oxidative stress markers and concentrations of selected elements in the leaves of c growing wild on a coal fly ash basin			X	X	X			X		X
8	Mudhiriza et al., 2015	Removal of nutrient and heavy metal loads from sewage effluent using vetiver grass, Chrysopogon zizanioides (L.)	X			X	X					
10	Phusantisampan et al., 2016	Phytostabilization potential of two ecotypes of Vetiveria zizanioides in cadmium-contaminated soils: greenhouse and field experiments	X	X		X	X					
11	Suelee et al., 2017	Phytoremediation Potential of Vetiver Grass (Vetiveria zizanioides) for Treatment of Metal-Contaminated Water	X	X	X	X						X
12	Vargas et al., 2016	Phytoremediation of Cu and Zn by vetiver grass in mine soils amended with humic acids	X	X								

2.5 Availability of Soil Amendments in Grenada

Fish bones, fertilizer, and fruit peels were chosen as soil amendments because of their success in binding metals, demonstrated in prior studies, as well as their availability in Grenada, discussed below.

Fish bones: Grenada is a fishing nation and is one of the best sportfishing locations in the Caribbean (Genter et al., 2018). Fish bone waste is a readily available waste material that can be used to stabilize heavy metals in leachate. Landings in Grenada are dominated by pelagic fish species (80%), followed by reef fish (18%), and shellfish (2%). Total annual production from this sector was 1,183 tons (GoG, 2018). The fishing sector contributes 1.4 percent to Grenada's GDP. Grenadians have traditionally relied on agriculture as a significant contributor to the island economy, particularly in rural parishes.

Fertilizer: In Grenada, the sub-optimal fertilizer used are inorganic and fossil fuel based. No data has been gathered regarding the use of synthetic fertilizer. Most of the agricultural emissions are from synthetic (nitrogen) fertilizers (75%) (World Bank. 2014)

Fruit peels: Grenada had a banana production of 25,000 tons in 2019 (Knoema, 2021). Green bananas are a staple part of the Grenadian diet. Prior to Hurricane Ivan in 2004, Grenada was an exporter of bananas to United Kingdom (ICCA, 2020). The export of bananas is on the decline, but banana peels are available in surplus. The citrus crops cultivated prior to Hurricane Ivan were predominantly oranges, grapefruits, and mandarins. Though production has declined, they are still available throughout the island.

2.6 Studies Using Fish Bones for Binding Metals

Fish bones are made of the phosphate mineral apatite, which readily combines with lead to form pyromorphite, a stable crystalline mineral that cannot be absorbed by the human digestive system (Freeman, 2012). Fishbone apatite is an effective additive for removal of divalent heavy metal ions from aqueous solutions (Admassu et al., 1999). The reaction between the fishbone (apatite) and metals is very rapid, allowing the treatment to take immediate effect (Wright et al., 1996) and immobilize the metal. The heavy metal is converted into pyromorphite, a microcrystalline solid that is harmless when consumed.

Mu et al. (2017) investigated fishbone waste as a natural source of hydroxyapatite (HAP) for heavy metal (mainly Pb) stabilization of municipal solid waste incineration (MSWI) fly ash. The experiment using ground *Lates niloticus* fishbones found metal adsorption efficiency to depend mainly on the content of the natural hydroxyapatite (HAP) (Rezk et al., 2018) and the contact times with the heavy metals (Tay et al. 2015) examined fishbone meal as a biosorbent for the removal of lead under acidic conditions with a contact time of sixty minutes, resulting in an 85% removal efficiency. Chen et al. (2020) found that there is a great potential for remediating multi-metal contaminated soil using fish bones. No study, however, has tested the efficacy of phytoremediation in combination with fish bone stabilization for removal of heavy metals.

Table 2.3 represents previous studies that experimented with fishbones and heavy metal removal at different concentrations and contact times. To maintain consistency and parallels with the current study, similar concentrations and contact times will be used. In terms of fishbone applications in wet or dry medium, studies were conducted using wet medium via leaching, which gave quick results and high removal efficiency.

Table 2.3 Fishbone application dosages in previous studies

Article	Metal Type	Fishbone Types	Fishbone dosage	Method	Contact time	Removal efficiency
Admassu et al, 1999	Pb	Mix fishbones	30 mg & 60 mg fishbone, 200 ml aqueous metal solution at 200 & 500 ppm at pH 4 & 7	coupled plasma atomic absorption	1.2 hr	NA
Chen et. al, 2019	Cd, Pb & Zn	HAP/CSH and biochar	(800 g) soil mixed with HAP/CSH and biochar	water spinach phyto-remediation pot experiment	42 days	Removal efficiency Cd/56%, Pb/50% and Zn 54%. 4:6 w/w HAP/CSH
Lim et al, 2012	Zinc $Zn(NO_3)_2 \cdot 6H_2O$	Mix fishbones	1.0 g -1.8 g	100 mL Zn^{2+}	5, 10, 15, 20, 25, 30, 60, 120, 240, 480 720 min.	98% at 12 hr using 1.8g/100ml solution
Mua et al, 2016	Pb (Fly Ash)	Japanese horse mackerel	10 g fly ash, each added to fishbone/fly ash ratios (w/w) 0/0%, 0.5/5%, 1.0/10%, 1.5/15%, 2.0/20% g	Leaching process 100ml distilled water	3, 6, 24, 72 hr	20% concentration had 24.76% at 72 h
Mu et al, 2017	Pb	lizardfish	10 g fly ash, each added to fishbone/fly ash ratios (w/w) 0, 5,10,15, 20, 30, 50, 70, and 100%	Leaching process 100ml distilled water	3, 6, 72 hr	100% concentration 59.31% after a 72 h
Nag et. al, 2000	Pb & Zn	Mix fishbones	20g fly ash added to 2g fishbone	Leaching process 200ml distilled water	6, 12, 24, 672 hr	86% Pb removal & 62.67% Zn removal after 28 days
Tay et. al, 2015	Pb	Lutjanus erythrop-terus fish bone meal	at pH 5 10-60mL was added to a 0.005 - 0.6g of fishbone sorbent	fishbone biosorbent	1 - 75 minutes	85% efficiency in 60 minutes with 0.09 g biosorbent and 50mg/l lead concentration

2.7 Studies Using Fruit Peel for Binding Metals

Using different fruit peel waste, such as banana, kiwi and tangerine, for the removal of toxic metals such as cadmium, chromium and zinc has been investigated (Al-Qahtani, 2015). The main advantage to this method is the availability of the materials and their low cost. Table 2.4 shows a review of the various fruit peels to remove copper, cadmium, and lead from wastewater suggest that fruit peels have equal or greater adsorption capacities compared to regular activated carbon. Effectiveness of the adsorbent is dependent on various parameters such as pH, temperature, contact time and particle size (Abd-Talib et al., 2020).

Table 2.4. Fruit peel application dosages and contact times in previous studies

Article	**Type of Peel**	**Metal Adsorbed /Removed**			**Peel Conc. or size**	**Contact time (Weeks)**	**Notes/Other Important Information**	**pH**
		Cr	**Cu**	**Pb**				
Al-Hiyaly et al., 2013	Orange powder		34.5	56.7	200 g	-	-	5
Al-Hiyaly et al., 2014	Orange powder		71.3	72.5	200 g	1,2,3,4	-	5
	fresh Lemon peels		-	70.9	200 g	1,2,3,4	Optimum 5 and 40C° for pH and temperature, respectively	5
	Orange peels		29.6	48.7	200 g	1,2,3,4	-	5
	Dry lemon peels		57.1	58	200 g	1,2,3,4	-	5
	Dry orange peels		23.7	37.2	200 g	1,2,3,4	-	5
Ghosh et al. (2013)	Orange		92	-	2 mm	90 min	Dried OP was treated with 0.05 m CaOH solution for 18 h	3.5
Lugo-Lugo et al. (2009)	Orange		-				Natural, formaldehyde treated (acetic acid) and copolymer (poly acrylamidecometacrylic acid)-grafted	5
Memon et al. (2008a)	Banana	95			0.125 mm	30 min	Esterification was achieved by adding 9 g of washed and dried BP in 633 ml of 99.9% methanol, to which 5.4 ml of conc. HCl was added.	4

							Then solution was heated at 60°C and for 48 h.	
Pablo Garcia-Chevesich et al., 2020	Lemon peel		-	90.91	500 g	35 min	25 C°	5
*1,2,3,4 - This means that regular readings were taken during each week, up to 4 weeks.								

OP: Orange peel
BP: Banana peel

2.8 Studies Using Fertilizer for Binding Metals

pH has a major effect on the solubility of the free metals such as Zn, Cu, Ni, Cd and Pb. The lower the pH the higher the solubility of the metals. (Chen, 1996). Lower pH limits the bioavailability of phosphorus for plants which in turn reduces the crop production. Pierzynski et al. (2018) investigated the mobility and availability of phosphorus in three different soil types with three different forms of fertilizers monoammonium phosphate (MAP), diammonium phosphate (DAP), and ammonium polyphosphate (APP). The outcome indicated that there is more movement of P in the liquid treatment than granular application. Phytostabilization combined with soil amendments decreases contaminant bioavailability and promotes the health of the contaminated soil to support plant growth (Alasmary et al., 2021). Hydroxyapatite [$Ca_5(PO_4)_3OH$] has the potential to immobilize Pb in solution by forming hydroxypyromorphite. The formation of lead phosphates in contaminated soils with phosphorus and lead may be responsible for immobilizing lead and reducing bioavailability (Hettiarachchi et al., 2002).

2.9 Goals & Objectives of the Research

As mentioned before, contamination of soils and water from open dumpsites is a frequent problem in developing nations. Removal of heavy metals remains a challenge in treating leachate, contaminated surface water, and ground water; an economical method of removal is especially needed.

Phytoremediation using green plants is one potential economical strategy for the removal of heavy metals from soil and water associated with open dump sites. While previous studies have examined the ability of Vetiver grass to remove metals individually, none has focused on using it to remove the ten heavy metals to be addressed in this study in combination. In addition, no study has tested the efficacy of phytoremediation **<u>in combination</u>** with fish bone or fruit peel stabilization. This research will fill these gaps.

The overarching research goal is to evaluate a low-cost method of removing heavy metals from leachate at open dump sites in developing countries, using a combination of phytoremediation and soil amendments. The Perseverance Dump Site in Grenada will be used as a case study. Specific objectives of the proposed research are:

1. To assess the potential of soil amendments (fish bone meal, fertilizer, and fruit peels) to bind/adsorb heavy metals in leachate from open dumpsites, (Zn, Cu, Pb, Cr, Mn, Ni, V, As, Cd, Fe).
2. To compare the efficiency of a plant species introduced to Grenada (Vetiver grass) in removing heavy metals from dumpsite leachate, with and without soil amendments.
3. One of the amendment concentrations for the fishbone will perform better than the other. Banana peel would have a higher concentration removal than the other fruit peels.

CHAPTER 3

MATERIALS & METHODS

In this chapter, the experimental design for the batch study, along with the creation of the synthetic leachate, will be discussed. The batch study was conducted to determine the best amendment concentration (fishbone, fruit peels and fertilizer) for the lab scale study. The bench scale study determined the potential for phytoremediation of metals for the best-performing amendments, in conjunction with vetiver grass.

3.1 Methods to accomplish Obj. 1

To assess the potential of amendments (fish bone meal, fertilizer, and fruit peels) to bind/adsorb heavy metals in soil contaminated with leachate from open dumpsites, (Zn, Cu, Pb, Cr, Mn, Ni, V, As, Cd, Fe).

3.1.1 Experimental Design

The purpose of the batch study was to evaluate the effectiveness of amendment quantities to remove heavy metals from soil contaminated with synthetic leachate. As shown in Table 3.1, metals in synthetic leachate (concentrations 1 and 2) were applied to soil and tested with 11 application rates of amendment, along with duplicates, giving 2 leachate concentrations * 11 amendment application rates * 2 duplicates = 44 bottles. Since some of the fishbone may bind to the soil rather than to metals in the leachate, fishbone concentrations of 1.5, 2, 3 and 5 times stoichiometric were tested. Fishbone was also be added at a soil remediation mix at a rate 3% (w/w) of soil. The ratio 4:6 w/w mixture had the highest removal efficiency for Cd, Pb, and Zn (Chen et al., 2020). The control has soil without amendments. The fruit peel concentrations were determined based on a range of the highest removal efficiency of the metals from the literature of Al-Hiyaly et al. (2014) and Abd-Talib et al. (2020).

Table 3.1. Batch experimental design

	Amendment application rate		**Synthetic Leachate Concentration**	
No.	**Amendment Category**	**Value**	**Conc 1**	**Conc 2**
1	Fishbone	1 X Stoichiometric	Bottle A1	Bottle B1
2		1.5 x Stoichiometric	Bottle A2	Bottle B2
3		2 x Stoichiometric	Bottle A3	Bottle B3
4		3 x Stoichiometric	Bottle A4	Bottle B4
5		5 x Stoichiometric	Bottle A5	Bottle B5
6		3% (w/w) of soil	Bottle A6	Bottle B6
7	None (control – as soil)	N/A	Bottle A7	Bottle B7
8	Fertilizer	1 X Stoichiometric	Bottle A8	Bottle B8
9	Banana Peel	5 g/L	Bottle A9	Bottle B9
10	Orange Peel	5 g/L	Bottle A10	Bottle B10
11	Lemon Peel	5 g/L	Bottle A11	Bottle B11

3.1.2 Synthetic Leachate

The concentrations of metals tested in the synthetic leachate for the batch experiment are shown in Table 3.2. ConcentrationConcentration 1 represents the the mean and Concentrationconcentration 2 represents the median value of metals in leachate from dumpsites in tropical regions, according to a review article by Vaccari et al (2019). Tropical regions represent the location of Grenada. Equation 1 was used to determine the mass of each element within its salt to create a uniform concentration. Calculations of mass to add to create synthetic leachate are give in Appendix B.

$$Mass\ Salt(mg) = volume(l)\ X\ Conc.needed\ \left(\frac{mg}{L}\right) X \frac{1}{MM\ cation\ (\frac{mg}{mmol})}\ X\ MM\ of\ salt\ \left(\frac{mg}{mmol}\right) X\ stoichiometry$$

(1)

Table 3.2 also shows the stochiometric amounts of hydroxyapatite (fish bone) needed to bind with each metal in the leachate (right-hand columns), along with amounts of diammonium phosphate (DAP) to be added via fertilizer, as discussed in further detail in Section 3.1.4. The bottom row of Table 3.2 shows the total amount of hydroxyapatite and fertilizer (DAP) needed to bind with all metals in the leachate.

Table 3.2 Synthetic leachate composition, along with stoichiometric concentrations of fishbone and fertilizer (diammonium phosphate, DAP) to be added to bind metals

Element	Molar mass (g/mol)	Concentration of metals in synthetic leachate				Moles of phosphate needed per mole of metal	Stoichiometric concentration of amendment			
		Conc. 1 (mg/L)	Conc. 2 (mg/L)	Conc. 1 (mol/L)	Conc. 2 (mol/L)		Fishbone phosphate to react with Conc. 1 (mg/L)	Fishbone phosphate to react with Conc. 2 (mg/L)	DAP to react with Conc. 1 (mg/L)	DAP to react with Conc. 2 (mg/L)
As	74.922	0.55	0.60	7.34E-06	8.01E-06	1	3.69	4.02	0.97	1.06
Cd	112.41	0.29	0.07	2.58E-06	6.23E-07	3/5	0.26	0.06	0.20	0.05
Cr	51.996	1.20	0.36	2.31E-05	6.92E-06	3/5	2.32	0.70	1.83	0.55
Cu	63.546	0.70	0.54	1.10E-05	8.50E-06	3/5	1.11	0.85	0.87	0.67
Fe	55.845	34.07	5.45	6.10E-04	9.76E-05	3/5	61.29	9.80	48.34	7.73
Mn	54.938	3.91	1.06	7.12E-05	1.93E-05	3/5	7.15	1.94	5.64	1.53
Ni	58.693	0.90	0.42	1.53E-05	7.16E-06	3/5	1.54	0.72	1.22	0.57
Pb	207.2	1.00	0.49	4.83E-06	2.36E-06	3/5	0.48	0.24	0.38	0.19
Zn	65.38	4.10	1.66	6.27E-05	2.54E-05	3/5	6.30	2.55	4.97	2.01
V	50.94	0.139	0.039	2.73E-06	7.66E-07	1 2/3	0.76	0.21	0.60	0.17
TOTAL							**84.90**	**21.09**	**65.62**	**14.70**

3.1.3 Soil

Soil was purchased from a local landscaping company (Whiz-Q Stone). The soil type purchased was “Screen Select Fill” (Figure 3.2), which most resembles the clay loam type found in Grenada. There are various textures that falls within the loamy category. Clay loam is 27-40% clay to 20-45% sand ratio. Loam is almost equal parts sand and silt (40%) and approximately 20% clay. These ratios were the determining factor for selecting the brand “Screen Select Fill” soil for the project. Soils in Grenada are mostly clay loam (84.5%), with clays (11.6%) and sandy loam (2.9%). The three major types of clay loam constituting 77.8% of the islands soil are Woburn, Capitol and Belmont (Ternan et al., 1989). A soil texture analysis (Figure 3.3) was conducted using the Soil Triangle method. The soil texture triangle (Figure 3.4) is used to convert particle size distribution into a recognized texture class based on the relative amounts of sand, silt and clay as a percentage. Details of the Triangle method are described in Appendix A.

Figure 3.2. “Screen Select Fill”

Figure 3.3. soil texture analysis

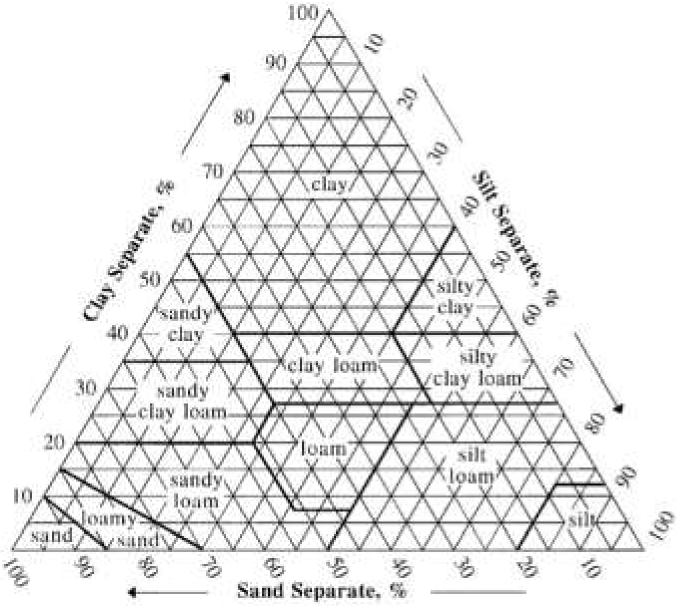

Figure 3.4. Soil texture triangle

CHONS elemental analysis (Intertek) was used to determine the carbon, hydrogen, nitrogen, sulfur, and oxygen elements in the soil. Using a pyrolysis technique, oxygen was analyzed by the Perkin-Elmer 2400 Elemental Analyzer. Sulfur was analyzed by colorimetric titration. Carbon, nitrogen and hydrogen were analyzed using ion chromatography.

3.1.4 Amendments

Fishbone. Down to Earth Organic Fish Bone Meal Fertilizer (Mix 4-12-0, Figure 3.5) was used. Fishbone was added to achieve at minimum the stoichiometric amount of hydroxyapatite [$Ca_5(PO_4)_3(OH)$] needed to bind with metals in the leachate, according to Eq. 2 below.

$$\begin{gathered} Ca_5(PO_4)_3OH + H_2O \rightarrow 5Ca^{2+}(aq) + 3PO_4^{3-}(aq) + 2OH^-(aq) \\ 5M^{2+}(aq) + 3PO_4^{3-}(aq) + 2OH^-(aq) \rightarrow M_5(PO_4)_3OH + H_2O \end{gathered} \tag{2}$$

Eq. 2 applies to all divalent metals, which include all metals in Table 3.2 except As and V. The stochiometric amounts of phosphate and hydroxyapatite needed to bind with each metal in the leachate are shown in the right-hand columns of Table 3.2.

Fertilizer. The fertilizer added with phosphate availability was diammonium phosphate (DAP) 99% with NPK ratio 18-46-0 (LD Carlson) (Figure 3.6).

Fruit peels. The fruit peels were gathered from discarded fruits, placed in the drying oven (name) at 200°F, turning every hour for three hours. Initially, samples from bananas that the research group had saved were pulverized using a food processor and passed through #200 sieve to ensure uniformity of the peel sizes. Due to the large quantity required for the reactor, additional fruit peels were purchased from MB Herbals (Figure 3.7). These did not need to be pulverized; they came in powdered form.

Figure 3.5 Down to Earth Organic Fish Bone Meal Fertilizer

Figure 3.6 LD Carlson DAP fertiliser

Figure 3.7 Banana peel purchased (L) and made (R)

3.1.5 Batch Reactor Set-Up and Operation

Batch tests were conducted in 125 ml bottles at room temperature. Each bottle contained 100 ml of synthetic leachate with amendment and 30 g of soil. The experiment was model from previous reasearch (Mu et al., 2016) using 10g of fly ash to 100ml distilled water. 30g of soil of was chosen to allow for a larger surface area to minimize contact times. Figure 3.1 shows the batch experiment setup (soil only) with the synthetic leachate, and Figure 3.2 shows the final set up with synthetic leachate added.

Figure 3.1. Batch experiment setup (soil only- no amendment)

Figure 3.2. Batch experiment (soil with amendments) with synthetic leachate

3.1.6 Analysis

The chemical composition of the soil and amendments were measured using CHNOS analysis by Intertek Pharmaceutical Services. Concentrations of metals were analyzed Shimadzu Center for Environmental, Forensics, and Material Science using Shimadzu Inductively Coupled Plasma Mass Spectrometer (ICP-MS) (2030) for contact times of 3, 14 and 28 days. The contact times were chosen from their use in prior studies (Table 2.4). The soil amendments that removed the greatest amount of metal from the leachate were used in Objective 2 for the reactor experiment.

3.2 Methods to accomplish Obj. 2

To compare the efficiency of one plant species introduced to Grenada (Vetiver grass) in removing heavy metals, with and without soil amendments.

3.2.1 Bench-scale Reactor Set-Up

Horizontal subsurface bench-scale phytoremediation systems were constructed (Fig. 3.9), using 20 – gallon glass containers (0.9 m L x 0.3 m W x 0.3 m H), divided in half to obtain two completely separated 10-gallon reactor duplicates. As mentioned in Obj. 1, loamy clay soil was obtained from a local nursey. Peat moss (Miracle-Gro Sphagnum) obtained from a local home improvement center was added to increase water retention in soil. The optimum soil amendment application rates from Obj. 1 were used. Table 3.3 shows the quantity of soil mixture, peat moss, and amendment added to each 10-gallon reactor, totaling 25 pounds.

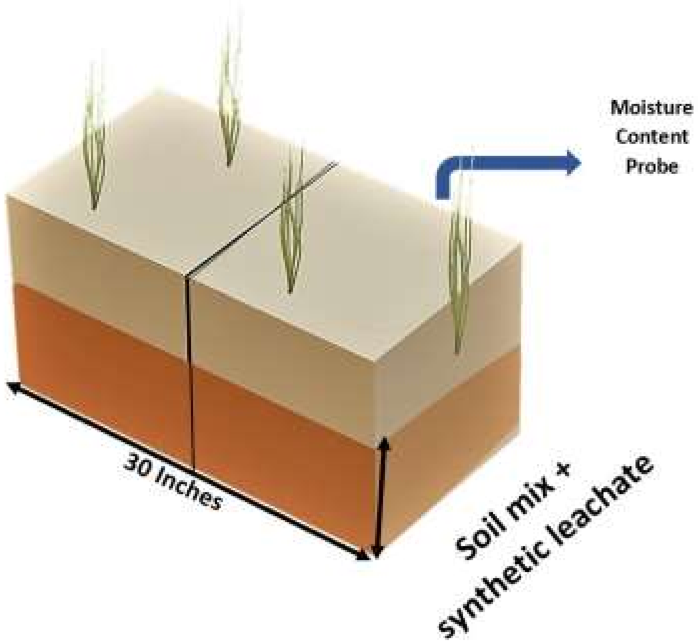

Figure 3.9. Phytoremediation Bench-Scale system

Table 3.3 Quantity of soil & amendments added to 10-gallon reactors.

Reactor Set	**1**	**2**	**3**
Amendment (type)	None (Control)	Fish bone	Banana Peel
Amendment (lbs)	None	0.515	3.87
Soil (lbs)	16.75	16.75	16.75
Peat moss (lbs)	8.25	8.25	8.25

The soil and peat moss were mixed for uniform distribution, as shown in Figure 3.10 (a). Next, the amendment was added in the soil and mixed thoroughly, as shown in Figure 3.10 (b). Finally, synthetic leachate, prepared using the average of concentrations 1 and 2 given in Table 3.2 above, was prepared and added at a rate of 0.5 L of synthetic leachate per 25 lb of soil (Figure 3.10 (c)). The soil/amendment/leachate mixture was transferred to the reactor and the process was repeated for the other reactors. The leachate was not recirculated; the reactors were not flow-through systems.

(a). Mixing soil and peat moss (b) Addition of fishbones to soil/peat mixture

(c) Addition of synthetic leachate to soil/peat moss/amendment mixture

Figure 3.10 Mixing of soil, peat moss, amendment, and leachate

Vetiver grass was purchased from The Herb Cottage online as live grass (Figure 3.11). The plants were bought and shipped bare rooted as individual slips and recommended to be stored in water before replanting to maintain the quality of the plant. The plants were kept at 70-75% moisture content to prevent the plant from stress during the transfer. Each slip was planted at a two-thirds in depth from top and six inches apart from each other.

Figure 3.11. Live Vetiver grass slips

3.2.2 Bench-scale Reactor Operation and Monitoring

A photoperiod of 12 hours representing day-long conditions in Grenada was provided using fluorescent lights (Groome, 1970). The photoperiod was adjusted to 12-hour cycles using a grow lamp for photosynthesis and plant growth. The moisture content was measured prior to adding water. Based on moisture content, 750ml deionized water was added every four days to maintain the moisture content desirable for the vetiver grass and the moisture content was measured as 71± 5%.

Single samples of the soil (top, middle & bottom) were collected on day 14 & day 28 (Figure 3.14). The samples were taken between the plants on day 14 to prevent disturbance of the plant. Day 28 sampling were taken closer to the plant location. The samples were then dried at 105°C using the Fisher Isotemp 500 Series oven for 24 hours then grounded with a mortar and pestle before analyzing.

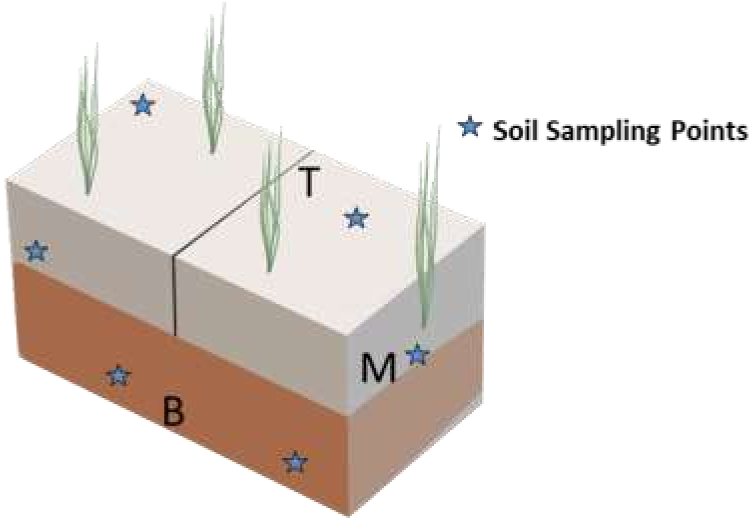

Figure 3.14 Soil Sampling points

Metals in the soil were removed using a modified sequential extraction procedure (Tissier et al., 1979), and the resulting liquid samples were analyzed by Shimadzu ICPMS-2030. Metals in Fraction 3 (reducible fraction) are bound to iron and manganese oxide, metals bound in this phase are considered stable. Metals in Fraction 4 (oxidizable bound) are bound to organic matter and sulfides and is assumed to remain within the solid matrix and are mobilized after a significant period of time, usually by the decomposition of organic matter, or are liberated when exposed to oxidizing conditions (Rodgers et al., 2015). Metals in Fraction 5 (residual) bound to silicate they are strongly bonded with crystalline structures and are the most difficult to extract. The theory is that in nature, the conditions have to be very specific to remove them from the complexes.

Table 3.4: Operating conditions for sequential extraction procedures.*

	Fractions	Time	Agitation/Temp	Quantity	Tessier
0	Water Soluble (Ma et al., 1997)	2 hr		15 ml	Deionized water
1	Exchangeable	1 hr	continuous agitation	8 mL	1 mol $MgCl_2$ pH 7.0
2	Bound to Carbonates	5 hr	continuous agitation-leached at rm temp.	8 mL	1 mol Na OAc pH 5.0 w/acetic acid
3	Bound to Iron and Manganese Oxides	6 hr	or occasional agitation	20 mL	0.04 mol NH_2OH*HCl in 25% (v/v) HOAc
			at 96+/- 3°C		
4	Bound to Organic Matter	2 hr	with occasional agitation at 85+/- 2°C	3 mL	0.02 mol HNO_3
				5 mL	30% H_2O_2 pH 2 with HNO_3
		3 hr	with intermittent agitation at 85+/- 2°C	3 mL	30% H_2O_2 ph 2 with HNO_3
		30 min	continuous agitation	5 mL	3.2 mol NH_4OAc in 20% (v/v) HNO_3-dilute to 20 mL
5	Residual (total or residual trace metal analysis)	2hr	reflux heating	15 mL	Aqua Regia

*After each fraction the samples were centrifuged using the Thermo Sorvall RC 6 Plus centrifuge for 30 minutes at 4200RCF.

The vetiver grass was carefully removed from the reactor, taking precaution not to damage the roots and shoots. The grass was soaked for one hour in deionized water to removed excess soil before being transferred to the sample vial. The grass was analyzed for heavy metals using U.S. EPA Method 200.7 (1994) using Agilent 7800 ICP-MS at Eurofin Labs.

3.2.3 To determine potential bioenergy production from the Vetiver plant.

Heat value of the plants was determined using ASTM D240-87(1991) Standard Test Method for Heat of Combustion of Liquid Hydrocarbon Fuels by Bomb Calorimeter. Heat of combustion is determined in this test method by burning a weighed sample in an oxygen bomb calorimeter under controlled conditions. The heat of combustion is computed from temperature observations before, during and after combustion, with proper allowance for thermochemical and heat transfer corrections. This was analyzed by Parr Oxygen Combustion Vessel (#1108) and Parr Plain Jacket Bomb Calorimeter (#1341) at Eurofin labs, Dallas, Texas.

3.2.4 The statistical method to determine which amendment is the best performing.

Using the t-test analysis will be performed on the batch test to determine which of the fishbone and the fruit peel concentration is the best suited for the bench test. The same analysis will be used to determine with of the two amendments fishbone or fruit peel is the best performing for the overall experiment.

CHAPTER 4

RESULTS & DISCUSSION

This chapter presents the results and discusses the findings from characterization of the soil and amendments, the batch tests and the reactor experiments.

4.1 Soil and amendment characterization

The analysis of the soil using the soil triangle method with duplicate samples, shown in Table 4.1, indicated the soil type to be loam or medium loam. There are various textures that falls within the loamy category. Clay loam is 27-40% clay to 20-45% sand ratio. Loam is almost equal parts sand and silt (40%) and approximately 20% clay. These ratios were the determining factor for selecting the brand "Screen Select Fill" soil for the project. This falls within the loamy category making it suitable for the batch tests and the reactor experiments.

Table 4.1 Soil texture analysis

Soil Texture Analysis				
	Sample 1 (ml)	Sample 2 (ml)	Sample 1 (%)	Sample 2 (%)
Clay	2.5	3	10.9	12.8
Silt	10	9.5	43.5	40.4
Sand	10.5	11	45.7	46.8
Total	23	23.5	100	100
Texture			Medium loam (loam)	Medium loam (loam)

The purity and chemical composition of soil and amendments was determined by elemental analysis (Table 4.2) to determine the carbon, hydrogen, nitrogen, sulfur, and oxygen. The most abundant element for teach amendment was carbon except for the fertilizer (DAP) and the soil. This makes sense since the fruit peels are organic, and the fertilizer and soil are inorganic. These values for the banana peels compare well with previous study carried out (Kabenge et. al 2018). The values for the citrus peels compare well with a previous study done (Pathak et al. 2017). There was negligible sulfur in all samples except fertilizer. The fertilizer is higher in sulfur compared to the other amendments due to the processing method where sulfur is added to dissolve the phosphate rock (Ipni.Net, 2022). Peat moss consists mostly of organic matter similar to the fruit peel and is expected to have similar values.

Table 4.2 CHONS analysis of soil and amendments

	Carbon (%)	**Hydrogen (%)**	**Oxygen (%)**	**Nitrogen (%)**	**Sulfur (%)**
Fishbone	34.12	5.01	7.79	17.84	0.81
Fertilizer (DAP)	0.13	6.61	20.93	14.31	2.44
Banana peel A (Made) Prepared in lab)	38.72	5.63	1.08	34.05	< 0.1
Banana peel B (Store Bought)	41.77	5.69	1.02	31.4	< 0.1
Lime Peel	41.61	6.28	1.17	39.5	0.1
Orange Peel	41.55	6.31	0.43	38.53	< 0.1
Peat Moss	45.11	5.86	1.11	30.26	0.21
Soil	1.08	0.66	< 0.05	2.62	< 0.1
Soil Mix (+ peat moss)	23.37	4.77	0.31	9.18	< 0.1
Banana peel (Kabenge, 2018)	35.65 ± 0.21	6.19 ± 0.07	45.94 ± 0.17	1.94 ± 0.16	
Orange / Lemon Peel (Pathak, 2017)	38.91 / 40.33	6.19 / 5.96	53.64 / 52.25	1.15 / 1.27	0.11 / 0.19

4.2 Results of the batch reactor tests

The results of the batch tests are represented below in Tables 4.3 – 4.6 and Figures 4.1 – 4.8, using arsenic, chromium, zinc and manganese as examples. Appendix C shows the other elements (Cd, Cu, Fe, Ni, Pb, and V) analyzed. Day 3 and 7 are not shown; samples for these days were sent to an external lab and the results were deemed invalid based on the method or analysis performed. Tables 4.3, 4.4, 4.5 & 4.6 show both measured and adjusted concentrations, with the adjusted concentrations calculated by subtracting the concentration measured in solution for the Control from the concentration measured for the other bottle. Hence, the **negative** values in Table 4.3, 4.4, 4.5 & 4.6 show bottles for which the equilibrium metals concentration in solution was lower than that of the Control. This indicates that the amendment was more effective than the Control in lowering metals concentrations in solution. The **positive** values in Table 4.3, 4.4, 4.5 & 4.6 indicate that the metals concentration in solution was higher than that of the Control, which is not desirable. The final determination of the amendments that would be used in the bench scale reactor was based on overall performance and a choice among the fruit peels.

Table 4.3 Arsenic Concentrations with control adjustments

Quantity of Amendment added		Arsenic Concentration 1				Arsenic Concentration 2			
	Quantity of Amendment	Measured Data		Adjusted to remove control		Measured Data		Adjusted to remove control	
		C1 Day 14	C1 Day 28	C1 Day 14	C1 Day 28	C2 Day 14	C2 Day 28	C2 Day 14	C2 Day 28
Fishbone	FB1X stoic	25	30	-93	-19	244	90	40	6
	FB1.5X stoic	287	445	169	396	145	48	-59	-36
	FB2X stoic.	218	406	100	357	87	51	-117	-33
	FB3X stoic.	355	556	237	507	24	35	-180	-49
	FB5X stoic.	306	422	188	373	322	113	119	29
	3% (w/w) of soil	141	73	23	24	236	141	32	57
Fertilizer	DAP	1436	1084	1318	1035	834	701	630	617
Banana Peel	BP	3	273	-115	224	322	383	118	299
Orange Peel	OP	262	183	144	134	174	189	-30	105
Lemon Peel	LP	246	169	128	120	113	228	-91	144
None (control)	Control	118	49	0	0	204	84	0	0

*1.5X stoic means 1.5 times the stoichiometry of fishbones

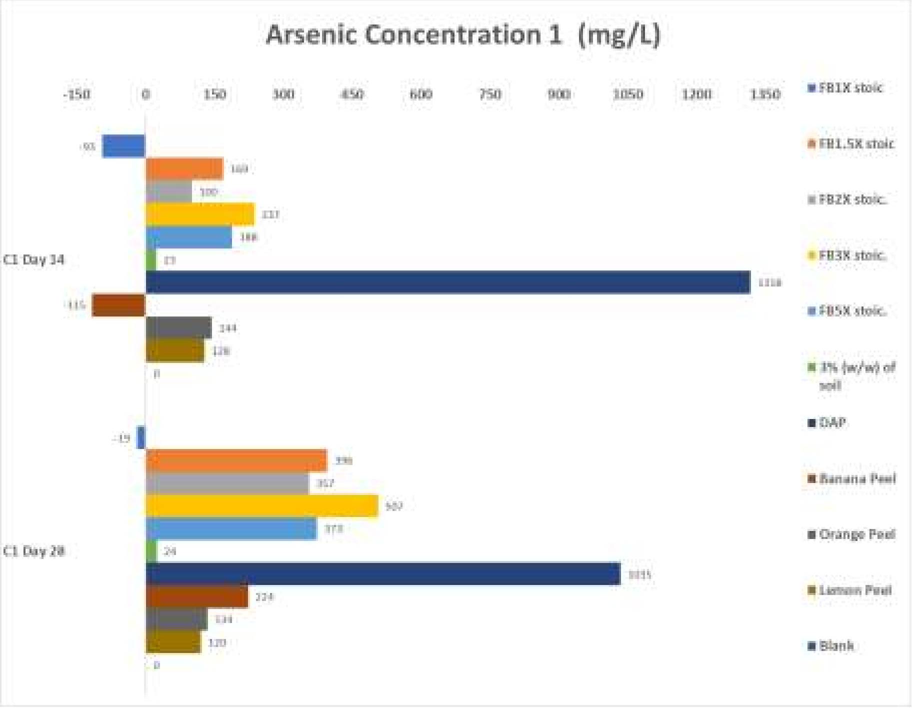

Figure 4.1 Arsenic Concertation 1 with control adjustment

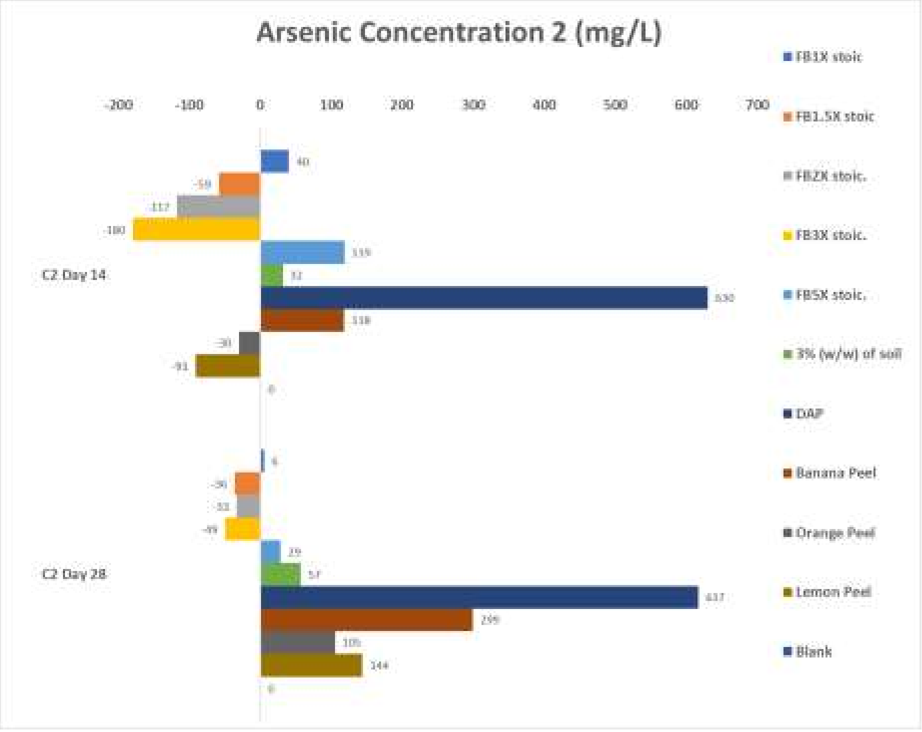

Figure 4.2 Arsenic Concertation 2 (duplicate) with control adjustment

Overall, the metal concentrations of chromium are lower than the arsenic, manganese, and zinc. Figure 4.4 shows for concentration 1 the fishbone with 1X, 1.5X stoichiometry, 3% (w/w) of soil and the banana were the best performers but for concentration 2, the sample including DAP is a good performer also.

Table 4.4 Chromium Concentrations with control adjustments

Quantity of Amendment added		Chromium Concentration 1				Chromium Concentration 2			
		Measured Data		Adjusted to remove control		Measured Data		Adjusted to remove control	
Types of Amendment	Quantity of Amendment	C1 Day 14	C1 Day 28	C1 Day 14	C1 Day 28	C2 Day 14	C2 Day 28	C2 Day 14	C2 Day 28
Fishbone	FB1X stoic	6	6	-2	2	4	5	-1	2
	FB1.5X stoic	6	7	-2	3	4	4	-2	0
	FB2X stoic.	9	9	1	5	6	5	1	2
	FB3X stoic.	9	8	1	4	6	6	1	2
	FB5X stoic.	12	11	4	7	7	6	1	2
	3% (w/w) of soil	5	6	-2	2	4	4	-1	0
Fertilizer	DAP	28	2	20	-2	5	2	0	-1
Banana Peel	BP	7	19	-1	15	11	7	6	4
Orange Peel	OP	30	20	22	16	17	18	12	15
Lemon Peel	LP	42	25	34	21	17	17	12	14
None (control)	Control	8	4	0	0	5	3	0	0

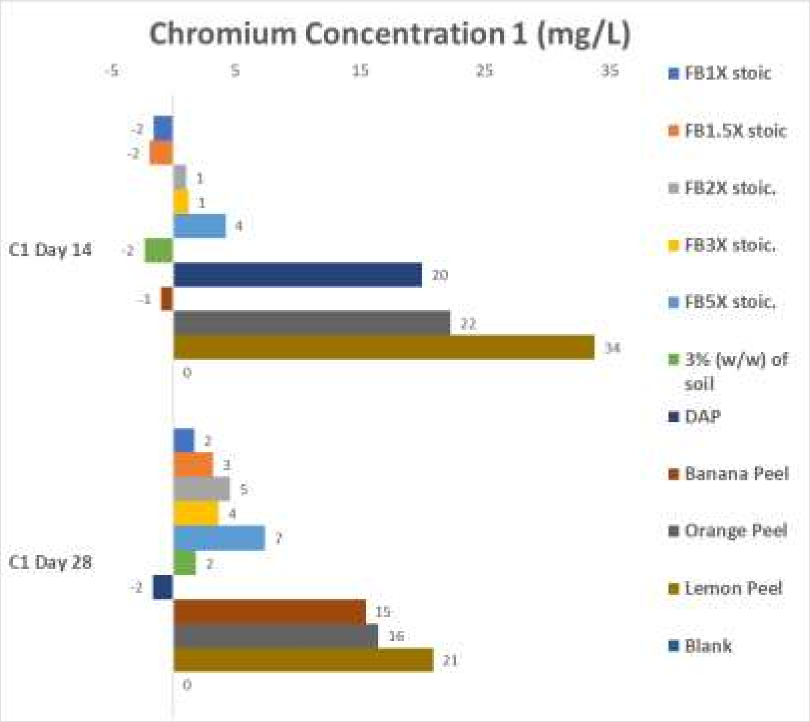

Figure 4.3 Chromium Concertation 1 with control adjustment

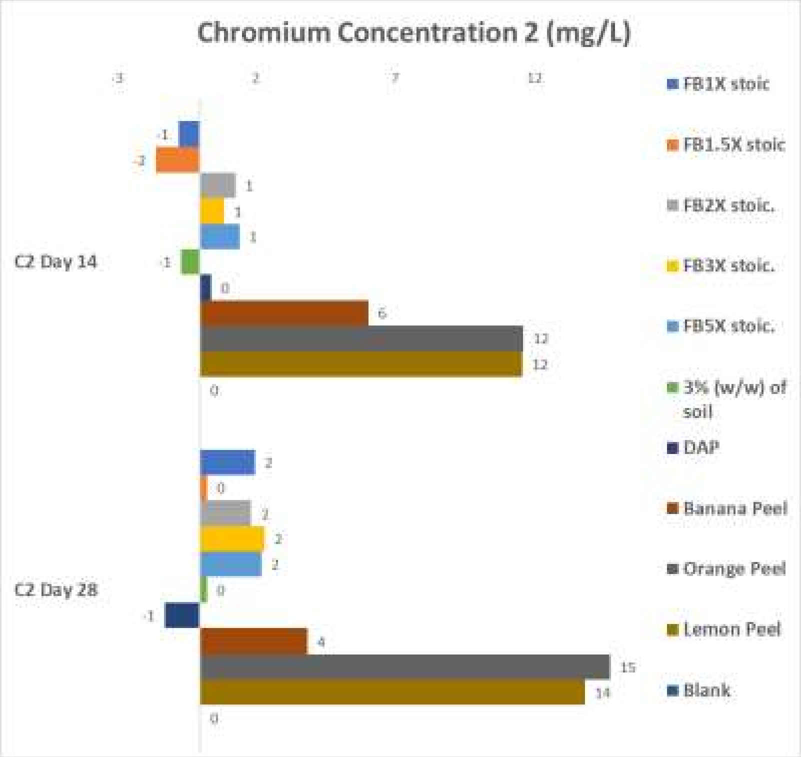

Figure 4.4 Chromium Concertation 2 (duplicate) with control adjustment

Figures 4.5 & 4.6 show the absorption of zinc amendments was more effective than the control. Zinc concentrations 1 & 2 on day 14 were lower than the control except for the fertilizer. On day 28 the number of performing amendments decreased. This was surprising given that the expectation especially for fishbone is to show some stability or more binding. The most of fruit peels performed better than the control except for banana peel.

Table 4.5 Zinc Concentrations with control adjustments

Quantity of Amendment added		Zinc Concentration 1				Zinc Concentration 2			
Types of Amendment	Quantity of Amendment	Measured Data		Adjusted to remove control		Measured Data		Adjusted to remove control	
		C1 Day 14	C1 Day 28	C1 Day 14	C1 Day 28	C2 Day 14	C2 Day 28	C2 Day 14	C2 Day 28
Fishbone	FB1X stoic	40	1012	-17	7	41	1146	-178	14
	FB1.5X stoic	39	1020	-18	15	40	1233	-179	100
	FB2X stoic.	38	1008	-19	3	109	1157	-109	25
	FB3X stoic.	39	997	-18	-9	43	1131	-176	-2
	FB5X stoic.	39	1012	-18	7	41	1036	-178	-97
	3% (w/w) of soil	41	1015	-16	9	42	1004	-176	-129
Fertilizer	DAP	42	1025	-15	19	241	1155	23	23
Banana Peel	BP	50	993	-7	-12	84	1145	-135	12
Orange Peel	OP	48	942	-9	-64	122	1046	-96	-87
Lemon Peel	LP	52	951	-5	-54	134	949	-85	-184
None (control)	Control	57	1005	0	0	218	1133	0	0

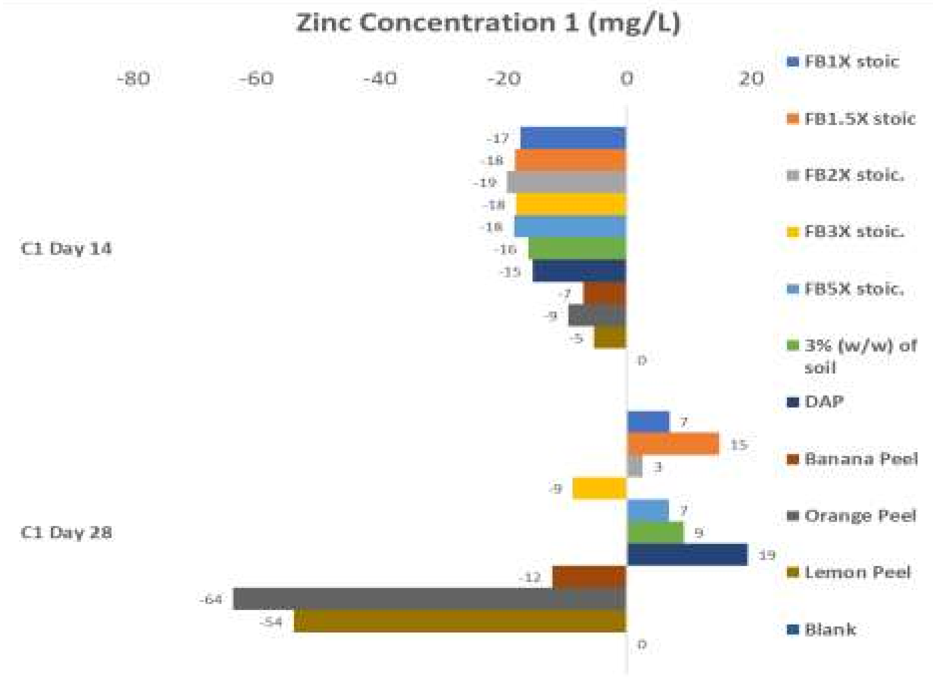

Figure 4.5 Zinc Concertation 1 with control adjustment

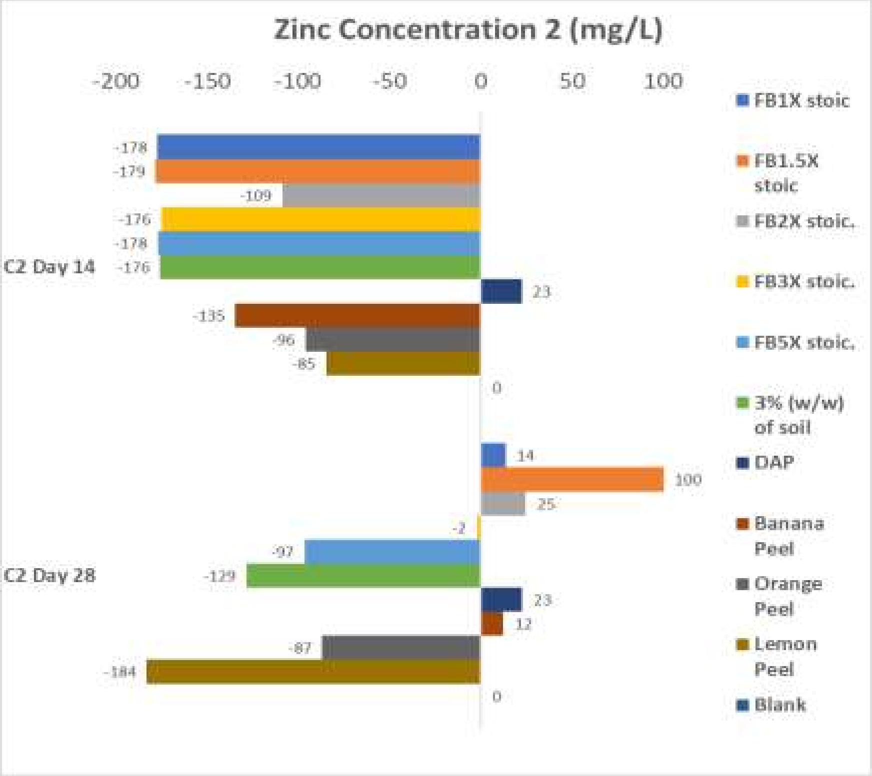

Figure 4.6 Zinc Concertation 2 (duplicate) with control adjustment

Figures 4.7 & 4.8 show that none of the amendments reduced the manganese concentration compared to the control. Orange and lemon peel in particular greatly increased the concentration of manganese in the solution. The amendments did not perform as hoped; there were only 5 cases out of the 28 samples where the fishbone or fertilizer performed better than the control. In this instance the citrus peels were worse performing compared to the banana.

Table 4.6 Manganese Concentrations with control adjustments

Quantity of Amendment added		Manganese Concentration 1				Manganese Concentration 2			
Types of Amendment	Quantity of Amendment	Measured Data		Adjusted to remove control		Measured Data		Adjusted to remove control	
		C1 Day 14	C1 Day 28	C1 Day 14	C1 Day 28	C2 Day 14	C2 Day 28	C2 Day 14	C2 Day 28
Fishbone	FB1X stoic	673	201	652	144	27	69	1	9
	FB1.5X stoic	17	222	-5	166	481	317	456	257
	FB2X stoic.	78	37	56	-19	593	213	568	154
	FB3X stoic.	509	259	487	202	837	124	811	64
	FB5X stoic.	606	254	584	197	559	229	533	169
	3% (w/w) of soil	17	73	-5	16	18	61	-8	1
Fertilizer	DAP	18	58	-3	2	26	75	0	15
Banana Peel	BP	26	999	4	943	1231	310	1206	250
Orange Peel	OP	4891	3644	4869	3587	4869	4366	4843	4306
Lemon Peel	LP	6274	4955	6252	4898	4858	5802	4832	5743
None (control)	Control	22	56	0	0	26	60	0	0

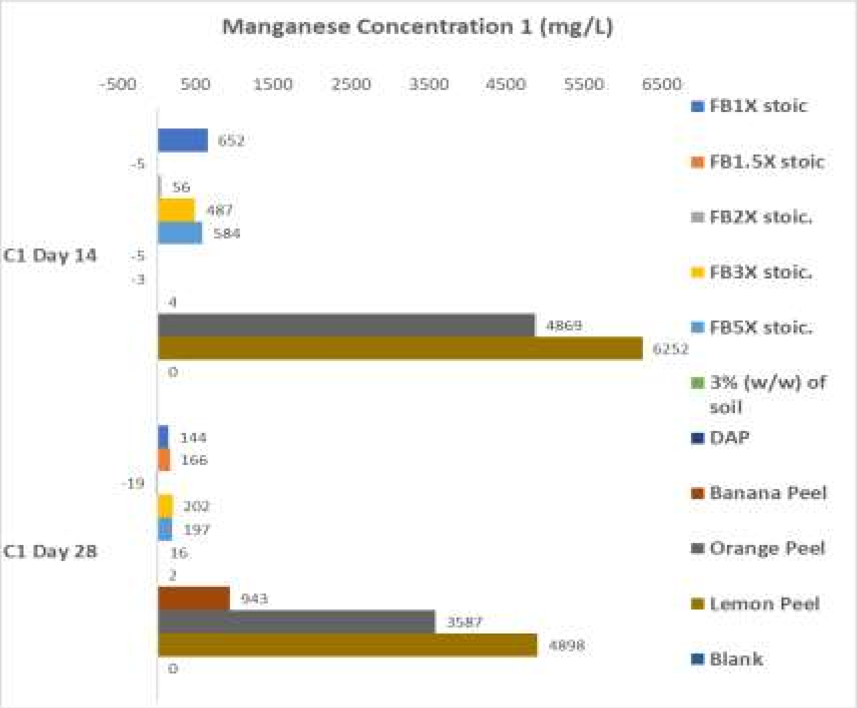

Figure 4.7 Manganese Concertation 1 with control adjustment

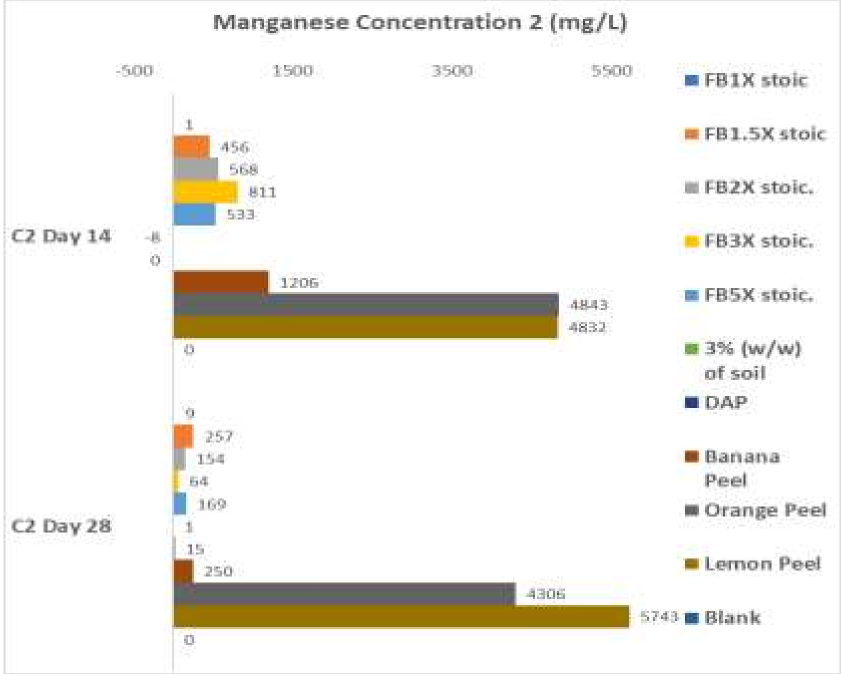

Figure 4.8 Manganese Concertation 2 (duplicate) with control adjustment

Table 4.7 summarizes batch test results for all the metals and amendments. For several of the metal elements (Cd, Cu, Ni, Pb), all of the amendments adsorbed all or most of the metal to the lower detection limit. Hence, data for these metals could not be used in determining the top-performing adsorbent. D3 means at day 3 there was no detectable concentration of the metal and control locations means that there were still residual metal concentrations at day 28.

The fishbone concentration of 3% (w/w) of soil had the best results across all the metals. The fruit peels had the worst performance over all the metals. Although the fertilizer outperformed the fruit peels, the banana peel was chosen to test in the bench-scale reactors to keep with the goal to evaluate a low-cost method of removing heavy metals from leachate.

Table 4.7 Top performing amendments

Elements	**Fishbones**						**Fertilizer**	**Fruit Peels**		
	1 x stoich.	1.5 x stoich.	2 x stoich.	3 x stoich.	5 x stoich.	3% (w/w) of soil	DAP	Banana	Orange	Lemon
As	D28					D28				
Cd	D3	D3	D3	D3	D3	D3	D3	D3	D3	D3
Cr	D28					D28				
Cu	D14	D14	D14	D14	D14	D14				
Fe						D14				
Mn						D28	D28			
Ni	D14	D14	D14	D14	D14	D14	D14	D14		
Pb	D3	D3	D3	D3	D3	D3	D3	D3	D3	D3
Zn						D14				
V		D28	D28							
Count	**6**	**5**	**5**	**4**	**4**	**9**	**4**	**3**	**2**	**2**

*D3 = Day 13

4.3 Results of the bench scale reactors

4.3.1 Metals concentrations in vetiver grass

Table 4.8 shows the metal concentrations measured in the vetiver grass. All the arsenic and four of the six values Cd were below the method detection limit (shown as <detection limit). For all of the other metals, the values indicate that the amendment removed some of the metals available for the plants to

uptake from the soil. The average values measured in the plants (light gray shading) are in the following order: no soil amendment > fishbone > banana peel. The values shown in the columns minus the control (dark gray shading) are thus more negative for (banana peel – control) compared to (fishbone – control). The greatest phytoremediation (uptake of metals by plant) therefore occurs without soil amendment, and the least occurs for the banana peel amendment.

In most cases, the variation in metals concentrations between the two plant duplicates is considerable. Several factors may have cause this. The plants were not all the same mass: for the fishbone and banana peel reactors, plant 1 weighed approximately 8 g and 4 g less than plant 2, respectively. The root system varied from plant to plant (Figure 3.11); this can also be a factor affecting the uptake. Moreover, some of the plants were not as healthy (Figure 4.9); this could have been from shock when transplanting them from a water only system to a highly saturated soil medium. In addition, the distribution of the amendments may not have been not uniform.

Table 4.8 shows that there is a high concentration of iron in particular in the plants. Iron is a naturally occurring metal that is abundant in the earth's crust (20,000 to 550,000 mg/kg) (Bodek et al., 1988); it is not uncommon to see large concentrations in the soil, as will be discussed in the metal's distribution section. Comparing the threshold values to the average values for the three reactors, copper in the control and fishbone reactor exceeded the threshold. The chromium for all reactors and copper concentrations for the banana peel reactor were within the threshold range. Arsenic, cadmium, lead, nickel and zinc were all below the threshold for each reactor.


Figure 4.9 Healthy (left) versus Unhealthy (right) vetiver grass

Table 4.8 Metal Concentrations Measured in Vetiver grass on dry weight basis

Metals	Threshold levels in Vetiver (mg/kg) (Truong et al. 2004)	Vetiver Grass Metal Concentration mg/kg										
		Control			Banana Peel				Fishbone			
		1	2	Avg.	1	2	Avg.	Avg. - Control	1	2	Avg.	Avg. - Control
As	21-72	<4.28	<4.43	<4.43	<2.51	<2.47	<2.51	N/A	<4.26	<3.91	<4.26	N/A
Cd	5-20	0.819	<0.832	N/A	<0.472	<0.464	<0.472	N/A	<0.801	1.05	N/A	N/A
Cr	5-18	12.1	15.5	13.8	10.5	6.6	8.6	-5.3	9.28	14.6	11.9	-1.9
Cu	13-15	77.7	47.7	62.7	16.4	10.4	13.4	-49.3	27.5	55.7	41.6	-21.1
Fe	-	7660	9750	8705	6760	4380	5570	-3135	5460	10,600	8030	-675
Mn	-	260	301	281	108	117	113	-168	87.2	281	184	-96
Ni	347	17.7	15.7	16.7	10	7	8.5	-8.2	7.61	20.3	14.0	-2.7
Pb	>78	8.15	10.2	9.2	7.21	4.75	5.98	-3.20	6.34	12.1	9.2	0.0
Zn	880	239	216	228	83.5	73.4	78.5	-149.1	113	232	173	-55
V	-	18.3	20.1	19.2	13.7	8.13	10.92	-8.29	10.7	19.9	15.3	-3.9

4.3.2 Metal Concentrations in Soil: Result from Sequential Extraction Analysis

The ease of release of metals into the environment, or the effectiveness of the amendments in binding the metals, was determined using the adapted Tessier Method (1979) with 1 g of soil/amendment mixture. Figures 4.9, 4.10 & 4.11 show complete sequential extraction data, with percents in each of the fractions 0-5, for bottom samples for Day 14/far away from the plant for the reactors with no amendment, fishbone, and banana peel, respectively. Similar graphs for the other depths and Day 28/close to the plant are provided in the appendix. Data from fractions 3-5 was compiled for presentation in the tables discussed below. It has been suggested that the mobility and bioavailability of metals decreases approximately in order of the extraction sequence (Tessier et al., 1979). The exchangeable and carbonate fraction are easily leached into the soil and made available for uptake by plants, and metals in the later fractions are expected to be less mobile in the soil.

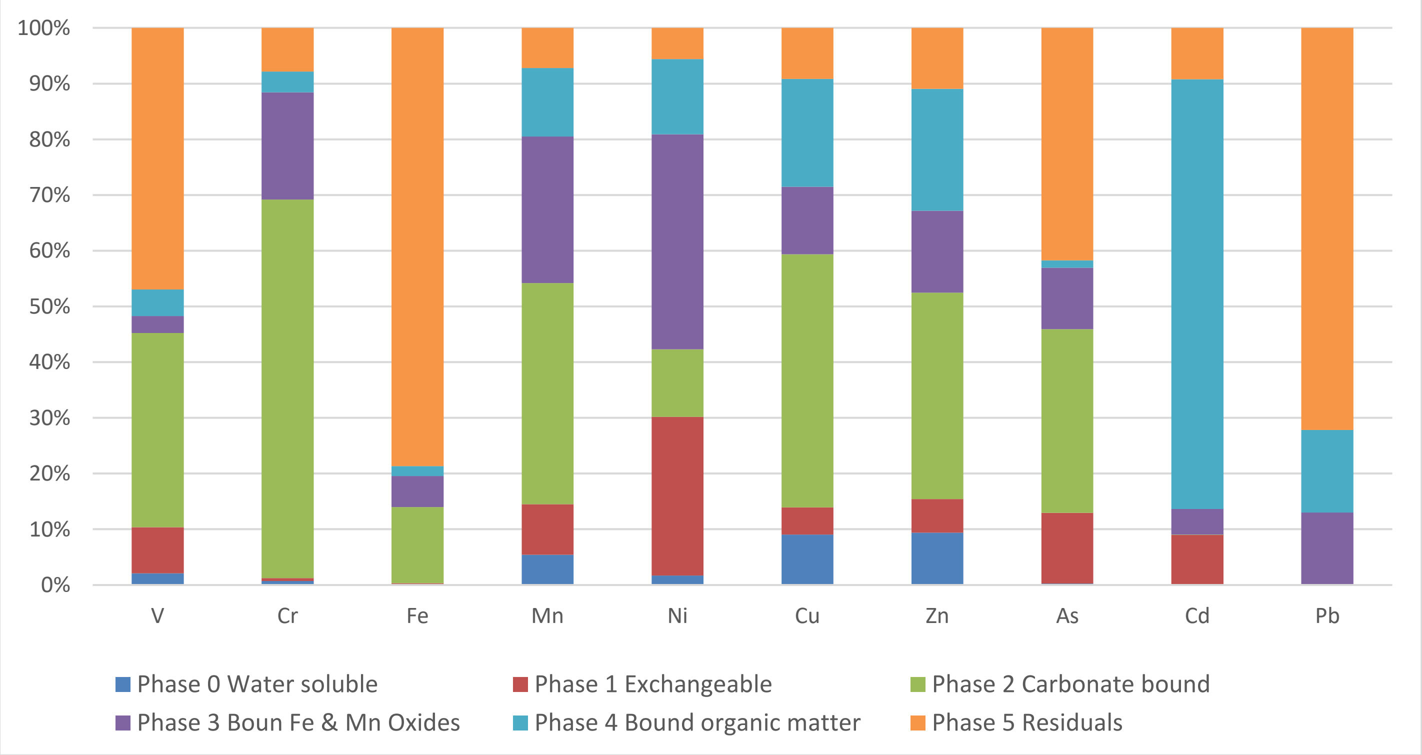

Figure 4.9: Day 14, bottom sampling with no amendment

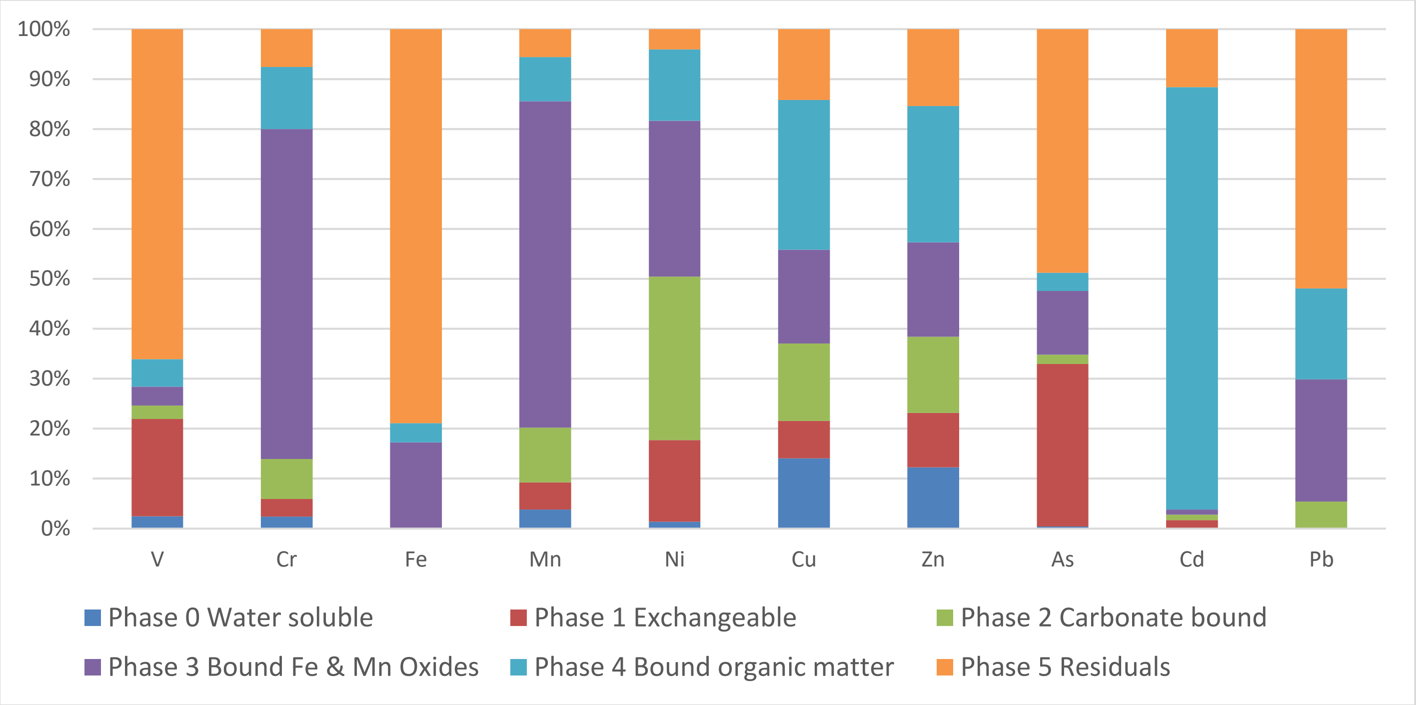

Figure 4.10: Day 14, bottom sampling with fishbone amendment

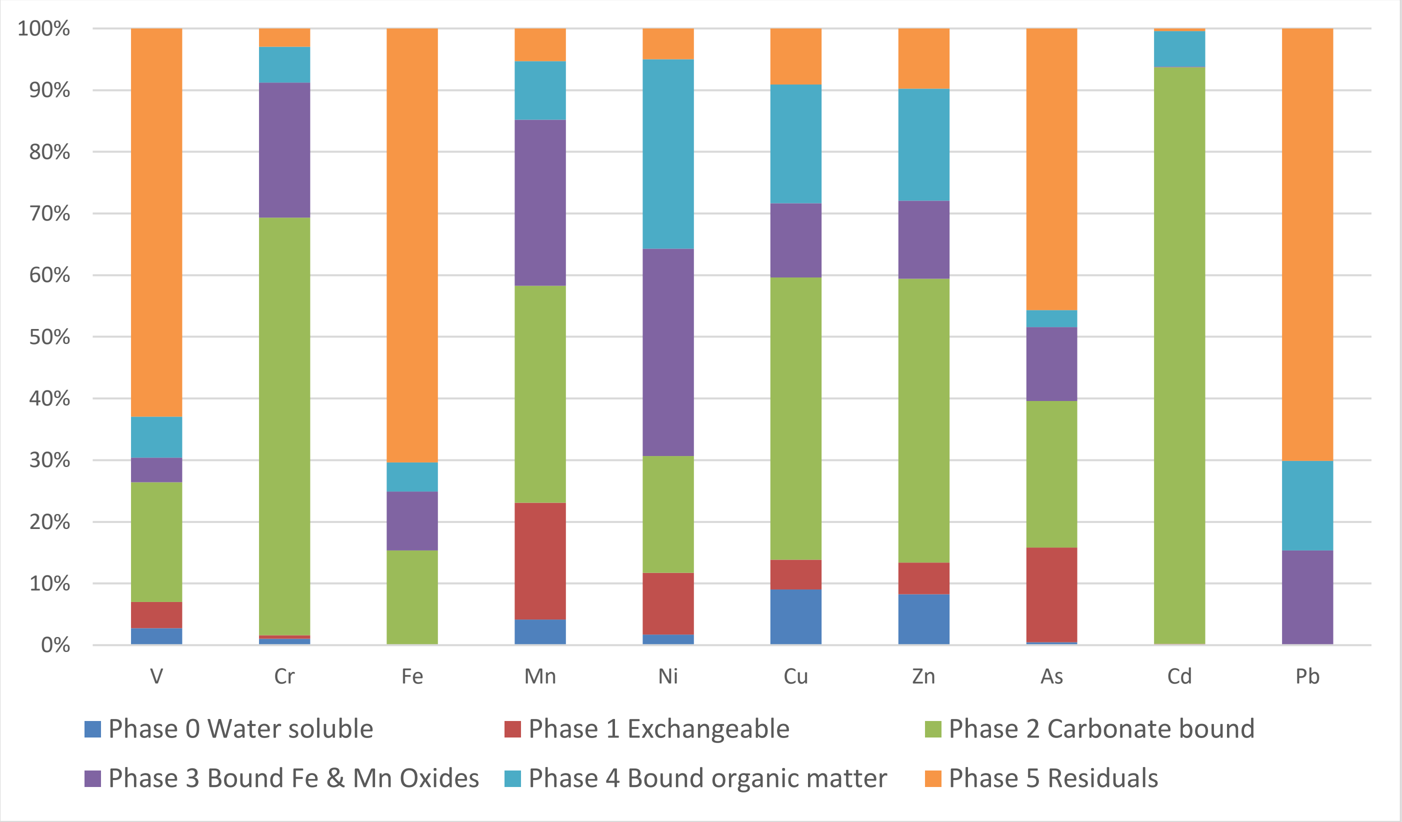

Figure 4.11: Day 14, bottom sampling with banana

Table 4.9 shows the percent of metals in fractions 3-5 near the plant (day 28) versus farther away (day 14), averaged over the top, middle and bottom depths. Fractions 3-5 represent the more stable binding of metals in nature; in these fractions the metals are not easily leached into the environment. The concentration of metals in fractions 3-5 increases closer to the plant compared to far away, as summarized in the average values. Since the samples closer to the plant were collected 2 weeks later, the increase could also represent an increase with time. The increase was not due to the amendments having more time to react (samples near the plant were collected 2 weeks later), because the increase occurred for the reactors with no amendment as well. The increase was thus due to the plants: either the closer proximity to the plants, or the plants having more time to phytoextract the metals. As the plants uptake metals which are mobile/soluble (fractions 0-2), that leaves behind a larger percent of metals in the soil which are in fractions 3-5. This indicates that the plants are playing a role in phytostabilization of the metals, due to vetiver grass being highly tolerant to heavy metals (Table 2.2).

For the no amendment case in Table 4.9, the average difference in metals immobilized in phases 3-5 for near vs. far away was 20.3%; this indicates that **vetiver grass increased metal stabilization by at least 20.3%**. The total immobilization due to the plants may have been greater than 20.3%, because some remediation may have occurred farther away from the plant as well. The difference in percent in fraction 3-5 is largest for Cr (56% increase) and Mn (30% increase), indicating the largest phytoremediation occurred for these metals.

Table 4.9 Binding of metals with proximity to plant

Metal	Percent in fraction 3-5*							
	Fish bone		Banana Peel		No amendment			
	Near plant	Farther away	Near plant	Farther away	Near plant	Farther away	Average	Difference
As	70	65	69	67	82	42	62	40
Cd	87	80	71	67	81	64	72.5	17
Cr	83	77	59	50	82	26	54	56
Cu	43	56	41	42	63	42	52.5	21
Fe	100	99	94	91	100	76	88	24
Mn	70	77	61	47	73	43	58	30

Ni	84	54	80	74	53	68	60.5	-13
Pb	100	97	99	100	87	100	93.5	-13
V	74	69	70	78	74	49	61.5	25
Zn	48	57	42	44	64	46	55	18
Average	**75.9**	**73.1**	**68.6**	**66**	**75.9**	**55.6**	**65.75**	**20.3**

As shown in Table 4.9, iron and lead have the highest percent in fraction 3-5 (see right-hand column with average for no amendment). Copper, zinc, chromium, and manganese have the lowest average percents in fraction 3-5 without amendment; they have substantial percents in fractions 0-2, which indicates a higher potential to be leached back into the environment. See Appendix D for the percentage in each fraction for different reactors. A study by (Jaradat et al, 2005) also investigated the availability of heavy metals within soil using sequential extraction (Tessier et al.,1979). There were some similarities and differences in the prevalence of the heavy metals in each fraction. The similarities are as follows:

(1) In both studies, the highest percent of manganese was found in the Fe-Mn oxide fraction (Fraction 3), which are the scavengers of heavy metals in the soil.

(2) For both studies, the highest percentage of iron was found in the residual fractions (Fraction 5). Metals found in this fraction are expected to be immobile in the soil.

The differences are as follows:

(1) In this research, cadmium was bound to the organic soil fraction (Fraction 4), compared to having the highest percentage in the exchangeable phase (Fraction 2), in the research by (Jaradat et al, 2005).

(2) In this research, zinc and copper were dominant in the carbonate fraction (Fraction 2) compared to the residual & organic soil fractions (Fractions 5 and 4), respectively.

(3) In this research, the highest percentage of lead was in the residuals (Fraction 5) versus the Fe-Mn Oxide fraction (Fraction 3) in the comparing research.

Table 4.10 shows the increased percent of metals bound in fractions 3-5 due to the soil amendments, compared to the reactor with no soil amendment. The main conclusion from the table is that fishbone converted more metals to fractions 3-5 compared to that of banana peel. This is likely because metal binding with fishbone was a chemical bond, whereas banana peel was just physical adsorption. Physical adsorption would likely decrease as the banana peel degrades.

Both amendments worked better farther away from the plant (Day 14). This could be due to the fact that before the amendment was added, soil farther away had a lower in fraction 3-5, so there was more room for improvement. Close to the plant, adding the amendment actually in some cases reduced the amount in fraction 3-5, compared with what the plant by itself had already stabilized. It could also be due to differences in moisture content close to the plant and farther away. It is assumed that the soil moisture content would be lower nearer the plant, due to the plant absorbing water from the soil at the location of the roots. The amendment may work better farther away from the plant, due to water being able to facilitate movement of amendment/metals toward each other to react.

Near the plant (or day 28), fishbone and banana peel worked the best for nickel; it had the highest increased percentage in fractions 3-5. Fishbone increased the percentage in fraction 3-5 the most for chromium and manganese, farther from the plant (Day 14). Since the samples farther away were collected on Day 14 and those nearer the plant were collected on Day 28, it is unclear whether the amendments working better farther away from the plant was due to location or time.

Table 4.10 Increased binding of metals due to soil amendment

Metal	Increased percent in fraction 3-5* compared to no amendment			
	Fish bone		Banana Peel	
	Near plant	Farther away	Near plant	Farther away
As	-12	23	-13	25
Cd	6	16	-10	3
Cr	1	51	-23	24
Cu	-20	14	-22	0
Fe	0	23	-6	15
Mn	-3	34	-12	4
Ni	31	-14	27	6
Pb	13	-3	12	0

V	0	20	-4	29
Zn	-16	11	-22	-2
Average	**0**	**17.5**	**-7.3**	**10.4**

*Remaining percent is in fractions 0-2

Tables 4.11 & 4.12 show the percentage in Fraction 3-5 as a function of sampling depth for points far away from the plant (Day 28) and close to the plant (Day 14), respectively. The average concentrations shown in the last line of the tables show there is no clear trend regarding percent in fractions 3-5 with depth. The highlighted blue areas represent cases where >50% of the metal is in the soluble, exchangeable and carbonate fractions (0-2).

Table 4.11 Top, middle, bottom – far away from plant, day 14

Metal	**Percent in fractions 3-5***								
	Fish bone			**Banana Peel**			**No amendment**		
	Top	**Middle**	**Bottom**	**Top**	**Middle**	**Bottom**	**Top**	**Middle**	**Bottom**
As	67	62	65	73	69	60	29	44	54
Cd	93	49	97	97	97	6	1	100	91
Cr	85	59	86	73	45	31	19	29	31
Cu	63	42	63	43	42	40	29	57	41
Fe	100	98	100	98	90	85	68	74	88
Mn	82	71	80	47	51	42	44	39	48
Ni	44	69	50	73	80	69	80	65	58
Pb	96	100	95	100	99	100	100	100	100
V	66	65	75	83	78	74	41	52	55
Zn	63	46	62	46	45	41	33	58	48

*Remaining percent is in fractions 0-2

Table 4.12 Top, middle, bottom – near plant, day 28

Metal	**Percent in fractions 3-5***								
	Fish bone			**Banana Peel**			**No amendment**		
	Top	**Middle**	**Bottom**	**Top**	**Middle**	**Bottom**	**Top**	**Middle**	**Bottom**
As	66	71	75	49	89	69	82	88	76
Cd	95	97	69	99	100	14	84	77	81
Cr	85	85	78	18	79	81	88	88	71
Cu	43	43	43	38	43	41	63	63	63
Fe	100	100	100	83	100	99	100	99	100

Mn	67	73	70	58	71	56	82	62	76
Ni	91	78	82	79	86	75	53	53	53
Pb	100	100	100	100	100	96	88	87	86
V	75	74	73	43	80	86	77	74	72
Zn	49	48	48	37	47	42	63	64	64

*Remaining percent is in fractions 0-2

4.3.3 Metal distribution between soil and plants

Tables 4.13, 4.14 & 4.15 show the distribution of metals on day 28 between the soil/amendment and the plants, for the reactors with no amendments, fishbone and banana peel respectively. Values with a "<" were below the detection limit. The percentages represent the portion of the total metals found in soil/amendment and plant. The soil samples for day 28 were collected near the plant but were assumed to represent concentrations throughout the reactor.

Cadmium had the highest percentage in the plant, followed by nickel; both of these elements nickel are highly water soluble. Cadmium and nickel are, however, potential toxins for the plants and consumers. They compete with the nutrients in the soil, making the fertility status poor (Rahi et al., 2021). A cause for the high level of metal concentration, especially Ni, might be the source location. The soil was mined from West Texas, which is famous for the large areas of limestone and the Permian Basin. The Permian Basin is an oil and gas producing area; the weathering of the limestone may have leached excess Ni into the soil. All the other metals (As, Cr, Cu, Fe, Mn, Pd, V, Zn) appear to have remained mostly in the soil.

Table 4.13 Distribution of metals between soil/amendment and plants for reactor without amendment

Metal	**Soil/amendment**			**Plant**			**Total**
	Concentration (mg/kg)	**Mass (mg)**	**% of total**	**Concentration (mg/kg)**	**Mass (mg)**	**% of total**	**Mass (mg)**
As	18.9	214.3	100%	10.2	0.4	0%	214.7
Cd	0.1	1.3	14%	216	8.2	86%	9.5
Cr	6.8	76.9	100%	<0.83	0	0%	76.9
Cu	17.9	203.1	95%	301	11.4	5%	214.5
Fe	9953	113,101	100%	15.5	0.6	0%	113,102

Mn	42.2	479.5	100%	47.7	1.8	0%	481.3
Ni	23.6	267.8	42%	9750	370.5	58%	638.3
Pb	10.7	121.1	99%	20.1	0.8	1%	121.9
V	30.7	349	100%	<4.43	0.2	0%	349.1
Zn	14.5	164.7	100%	<2.33	0.1	0%	164.8

Table 4.14 Distribution of metals between soil/amendment and plants for reactor with fishbone

Metals	**Soil/amendment**			**Plant**			**Total**
	Concentration (mg/kg)	**Mass (mg)**	**% of total**	**Concentration (mg/kg)**	**Mass (mg)**	**% of total**	**Mass (mg)**
As	10.9	123.7	100%	12.1	0.4	0%	124.2
Cd	0.1	1.3	13%	232	8.4	87%	9.7
Cr	6.7	76.2	100%	1.05	0	0%	76.3
Cu	26.1	296.7	97%	281	10.1	3%	306.9
Fe	10,351.6	117,632	100%	14.6	0.5	0%	117,632.5
Mn	27	307	99%	55.7	2	1%	309
Ni	19.1	216.5	36%	10,600	381.6	64%	598.1
Pb	7.6	85.9	99%	19.9	0.7	1%	86.6
V	33.3	378	100%	<3.91	0.1	0%	378.1
Zn	19.7	223.9	100%	20.3	0.7	0%	224.6

Table 4.15 Distribution of metals between soil/amendment and plants for reactor with banana peel

Metals	**Soil/amendment**			**Plant**			**Total**
	Concentration (mg/kg)	**Mass (mg)**	**% of total**	**Concentration (mg/kg)**	**Mass (mg)**	**% of total**	**Mass (mg)**
As	21.3	242.3	100%	4.75	0.2	0%	242.5
Cd	0.1	1.1	30%	73.4	2.6	70%	3.7
Cr	12.8	145.8	100%	<0.464	0	0%	145.8
Cu	27	306.9	99%	117	4.2	1%	311.1
Fe	6306	71,659	100%	6.6	0.2	0%	71,659
Mn	29.3	332.6	100%	10.4	0.4	0%	333
Ni	17.6	199.7	56%	4380	157.7	44%	357.4
Pb	6.2	70.3	100%	8.13	0.3	0%	70.6
V	38.2	433.9	100%	<2.47	0.1	0%	434
Zn	21	238.2	100%	7	0.3	0%	238.5

In Section 4.3.2, the largest phytoremediation appeared to have occurred for Cr and Mn, so it would have been expected that these two metals would have shown the highest percentages in the plants. In Section 4.3.2, nickel was shown to not be phytoremediated at all, and Cd to a lesser extent. The plant and soil metal concentrations were measured using different techniques at different labs, which could explain why this data does not match with Section 4.3.2.

4.3.4 Potential bioenergy production from the plant species (Vetiver grass)

Table 4.16 shows the heat of combustion for the vetiver grass. Typical values of the activation energy of vetiver grass ranges 65-70 BTU/lb (Thakur et al, 2017). This is a possible explanation for the lack of data from the samples.

Table 4.16: Heat of Combustion of Vetiver grass

Amendment Type	**BTU/lb**
No Amendment 1	<500
No Amendment 2	<500
Fishbone 1	<500
Fishbone 2	<500
Banana Peel 1	<500
Banana Peel 2	<500

*<500 Indicates the analyte was analyzed for but not detected.

4.4 Metal Concentrations Day 129 in Soil: Result from Sequential Extraction Analysis

In Section 4.4, Table 4.17 shows the result of the bench test after passage of 129 days. Samples were analyzed using the sequential extraction process. Comparing the day 129 to day 28, it was determined that chromium and iron had at least 91% in fraction 3-5 in all three reactors. Chromium showed an increase in percentage over time in the 3-5 fraction, while iron remained unchanged. Copper remained the same, reflecting most of the concentration in 0-2 fraction. The zinc concentration in the 3-5 fraction increased into the 70th percentile for both the banana peel and fishbone. Vanadium concentration decreased, 50/50 percentage in the 0-2/3-5 fraction. Manganese showed some interesting data, the concentration decreased near and far from the plant into the 0-2 fraction. In the banana peel reactor far from plant had a concentration higher than samples near the plant. The opposite occurred in the fishbone reactor near the plant had a concentration higher than samples far from the plant. Lead had a slight decrease in concentration over all three reactors. Table 4.18 show the summary comparison of increased binding of metals due to soil amendment for day 28 and 129. Overall, the banana peel stabilized near and far from plant. The fishbone had a higher binding potential than the fish bone.

Table 4.17 Percentage in Fraction 3-5 for sampling points far away and near to plant (Day 129)

Fishbone Day 129								
Samples taken Near Plant					Samples taken Far From Plant			
Heavy Metal	Reactor 1	Reactor 2	Average		Heavy Metal	Reactor 1	Reactor 2	Average
	% Fraction 3-5	% Fraction 3-5				% Fraction 3-5	% Fraction 3-5	
As	78.6	74.2	76.4		As	76.0	72.7	74.3
Cd	81.2	80.9	81.1		Cd	80.6	80.8	80.7
Cr	99.1	96.2	97.6		Cr	96.0	92.8	94.4
Cu	79.4	16.6	48.0		Cu	81.3	10.4	45.9
Fe	99.5	99.6	99.6		Fe	99.6	96.7	98.2
Mn	84.5	58.2	71.4		Mn	48.8	48.5	48.6
Ni	71.2	47.1	59.2		Ni	81.3	47.9	64.6
Pb	80.8	81.1	80.9		Pb	80.6	74.2	77.4
V	65.4	54.3	59.9		V	63.5	47.3	55.4
Zn	79.4	77.8	78.6		Zn	71.2	79.2	75.2

Banana Peel Day 129								
Near Plant					Far From Plant			
Heavy Metal	Reactor 1	Reactor 2	Average		Heavy Metal	Reactor 1	Reactor 2	Average
	% Fraction 3-5	% Fraction 3-5				% Fraction 3-5	% Fraction 3-5	
As	76.1	72.6	74.3		As	75.5	72.2	73.8
Cd	80.9	80.5	80.7		Cd	80.6	80.5	80.6
Cr	91.9	90.7	91.3		Cr	93.2	97.8	95.5
Cu	67.9	11.2	39.6		Cu	40.4	9.4	24.9
Fe	98.7	97.8	98.2		Fe	99.6	98.5	99.0
Mn	32.6	28.2	30.4		Mn	54.6	91.2	72.9

Ni	76.6	67.2	71.9		Ni	78.1	18.4	48.2
Pb	79.1	79.8	79.5		Pb	80.8	79.5	80.2
V	55.0	51.3	53.1		V	51.6	44.4	48.0
Zn	81.3	74.0	77.7		Zn	91.8	70.0	80.9

Control Day 129								
Near Plant					Far From Plant			
Heavy Metal	Reactor 1	Reactor 2	Average		Heavy Metal	Reactor 1	Reactor 2	Average
	% Fraction 3-5	% Fraction 3-5				% Fraction 3-5	% Fraction 3-5	
As	73.9	72.0	73.0		As	73.3	75.3	74.3
Cd	80.7	80.2	80.5		Cd	80.4	80.6	80.5
Cr	92.9	89.8	91.3		Cr	89.9	89.3	89.6
Cu	23.7	14.3	19.0		Cu	17.2	15.8	16.5
Fe	99.7	99.2	99.4		Fe	99.9	99.4	99.6
Mn	44.2	37.6	40.9		Mn	40.5	26.4	33.5
Ni	48.5	38.7	43.6		Ni	63.6	52.3	57.9
Pb	81.0	80.1	80.6		Pb	80.5	80.2	80.3
V	51.8	49.0	50.4		V	54.3	48.7	51.5
Zn	72.0	74.4	73.2		Zn	70.0	79.4	74.7

Table 4.18 Increased binding of metals due to soil amendment Day 28 versus Day 129

Metal	**Increased percent of metal well-stabilized* by soil/amendment compared to no amendment (Day 28)**			
	Fish bone		**Banana Peel**	
	Near plant	**Farther away**	**Near plant**	**Farther away**
As	-12	23	-13	25
Cd	6	16	-10	3
Cr	1	51	-23	24
Cu	-20	14	-22	0
Fe	0	23	-6	15
Mn	-3	34	-12	4
Ni	31	-14	27	6
Pb	13	-3	12	0
Zn	-16	11	-22	-2
V	0	20	-4	29
Average	0	17.5	-7.3	10.4

Metal	**Increased percent of metal well-stabilized* by soil/amendment compared to no amendment (Day 129)**			
	Fish bone		**Banana Peel**	
	Near plant	**Farther away**	**Near plant**	**Farther away**
As	3	0	1	0
Cd	1	0	0	0
Cr	6	5	0	6
Cu	29	29	21	8
Fe	0	-1	-1	-1
Mn	30	15	-11	39
Ni	16	7	28	-10
Pb	0	-3	-1	0
Zn	9	4	3	-3
V	5	1	5	6
Average	10	6	4	5

*Remaining percent is in phases 0-2

4.5 Statistical Analysis

4.5.1 Statistical Analysis on the Batch Test

To determine the statistical significance of the data, the two concentrations and their duplicates at day 28 were analyzed. A T-test of two samples with unequal variance was used to test the hypothesis that there is a significant difference in metals' concentration in fractions 3-5 for the 3 % w/w fishbone concentration compared to the other concentrations. The same T-test was carried on the fruit peels to test the hypothesis that there is a significant difference in metals' concentration in fractions 3-5 for the banana peel compared to the other peels. The hypotheses tested were as follows:

Fishbone

H_0 = There is no statistical difference between means for 3% w/w and fishbone amendment,

H_1 = 3% w/w fishbone is best performing.

Banana Peel

H_0 = There is no statistical difference between means for banana peel and other fruit peels,

H_1 = Banana peel is best performing.

Table 4.19 shows the results of the T-tests. Values could not be computed for cadmium, copper, nickel and lead due to their standard deviation being zero. It was determined with at least 70% confidence there is a significant difference between the 3% w/w fishbone compared to the other fishbone concentrations for metals concentration 1, except zinc which had a poor performance over all the concentrations. Differences were determined with at least 70% confidence for concentration 2 as well, except zinc and iron, which did not perform well.

Table 4.19 Test of hypothesis that there is a significant difference in metals in fractions 3-5 for the 3 % w/w fishbone concentration compared to the other concentrations

	Comparing metals in fractions 3-5 for 3% (w/w) fishbone to other fishbone concentrations					Comparing metals in fractions 3-5 for banana peel to other fruit peels	
Concentration 1							
	1 X Stoich.	1.5 x stoich.	2 x stoich.	3 x stoich.	5 x stoich.	Orange Peel	Lemon Peel
As							
P(T<=t) one-tail	0.135	0.069	0.058	0.134	0.080	0.326	0.370
Mean	69.93	46.16	56.35	37.59	180.35	136.53	149.44
Cr							
P(T<=t) one-tail	0.166	0.038	0.056	0.116	0.006	0.380	0.093
Mean	6.216	7.502	8.569	8.877	11.78	19.90	23.45
Fe							
P(T<=t) one-tail	0.227	0.262	0.246	0.052	0.052	0.061	0.144
Mean	101.40	113.73	107.72	851.39	1119.54	6885.24	10592.51
Mn							
P(T<=t) one-tail	0.005	0.052	0.303	0.073	0.241	0.027	0.028
Mean	194.02	251.32	119.91	222.54	160.34	3159.42	4868.02
Zn							
P(T<=t) one-tail	0.488	0.497	0.500	0.495	0.499	0.480	0.489
Mean	576.63	561.42	556.08	546.60	557.49	507.13	522.88
V							
P(T<=t) one-tail	0.233	0.191	0.136	0.196	0.257	0.395	0.322
Mean	1.108	0.738	0.674	0.944	1.226	2.346	3.277
Concentration 2							
	1 X Stoich.	1.5 x stoich.	2 x stoich.	3 x stoich.	5 x stoich.	Orange Peel	Lemon Peel
As							
P(T<=t) one-tail	0.133	0.104	0.122	0.093	0.270	0.281	0.396
Mean	69.93	46.16	56.35	37.59	180.35	169.24	226.81
Cr							
P(T<=t) one-tail	0.039	0.418	0.211	0.030	0.040	0.013	0.045
Mean	5.540	4.157	4.657	5.981	6.029	19.35	17.45
Fe							
P(T<=t) one-tail	0.333	0.441	0.469	0.469	0.092	0.004	0.194
Mean	118.46	51.74	65.69	54.48	109.22	11824.91	6805.50
Mn							
P(T<=t) one-tail	0.402	0.262	0.218	0.085	0.031	0.041	0.072
Mean	50.52	175.49	137.99	115.34	235.07	3908.27	4761.53
Zn							
P(T<=t) one-tail	0.476	0.463	0.481	0.485	0.490	0.296	0.324
Mean	633.13	662.33	623.93	615.80	569.19	1012.19	944.80
V							
P(T<=t) one-tail	0.420	0.125	0.145	0.118	0.113	0.050	0.264
Mean	0.965	0.552	0.610	0.524	0.498	4.000	2.197

*Color Representation $p < 0.05$ | >0.05 $p \leq 0.10$ | > 0.10 $P \leq 0.15$ | > 0.15 $P \leq 0.30$

As shown in Table 4.19, it was determined with at least 70% confidence there is a significant difference between banana peel versus the other peels for concentration 1 except for arsenic, zinc and vanadium, and orange peel for chromium, which did not perform well. Differences were determined with at least 70% confidence for concentration 2 as well, except arsenic. Zinc did not perform well with the lemon peel.

Table 4.20 shows the overall performance of the fishbone and the fruit peels amendments. For concentration 3% fishbone performed better than other concentrations of fishbone for 80% of the samples, to at least a 70% level of confidence. 42% of fruit peel samples show that the banana performed better that the other fruit peels to at least a 70% level of confidence. For concentration 2, 75% of the fruit peel samples showed banana peel was the best performing and 57% of the fishbone samples showed that 3% w/w fishbone was the best performing, at 70% level of significance. Since Concentration 1 was higher for all metals except arsenic, it can be concluded that 3% fishbone performed better than the other fishbone concentrations better for higher concentrations of metals, and banana peel performed better than the other peels for lower concentrations of metals.

Table 4.20 Overall performance of the amendments in Batch Test

Total Sample Count	**Fishbone** 30	**Banana Peel** 12

P- Value	**Concentration 1**				**Concentration 2**			
	Fishbone	**Banana Peel**	**Fishbone**	**Banana Peel**	**Fishbone**	**Banana Peel**	**Fishbone**	**Banana Peel**
$p < 0.05$	3	2	10%	17%	4	5	13%	42%
$>0.05\ p \leq 0.10$	8	2	27%	17%	3	1	10%	8%
$> 0.10\ P \leq 0.15$	4	1	13%	8%	7	0	23%	0%
$> 0.15\ P \leq 0.30$	9	0	30%	0%	3	3	10%	25%
Overall Percentage			80%	42%			57%	75%

*30 & 12 represents the total number of samples for the fishbone and banana peel, respectively. The percentages were calculated as a fraction of the total.

4.5.2 Statistical Analysis on the Bench Test

A T-test was carried out on samples taken day 129 to test the hypothesis that there is a statistically significant difference in the metals in fraction 3-5 for fishbone treatment vs. control, and banana peel treatment vs. control. Table 4.21 shows the results of the T-test. Near the plant, there is a greater amount of metals in fraction 3-5 for the fishbone reactor compared to the control reactor, to at least a 70% level of confidence, for all metals except iron. Far away from the plant, there is a greater amount of metals in fraction 3-5 for the fishbone reactor compared to the control reactor, to at least a 70% level of confidence, for 6 of the 10 metals.

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Table 4.21 Test of hypothesis that there is a significant difference in metals in fractions 3-5 for the fishbone treatment vs. control, and banana peel treatment vs. control.

T-Test		Metals									
		As	**Cd**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**V**	**Zn**
Fishbone Near Plant	P(T<=t) one-tail	0.192	0.072	0.050	0.265	0.363	0.133	0.221	0.281	0.173	0.032
	Mean	76.4	81.1	97.6	48.0	99.6	71.4	59.2	80.9	59.9	78.6
		As	**Cd**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**V**	**Zn**
Fishbone Far from Plant	P(T<=t) one-tail	0.494	0.100	0.104	0.280	0.247	0.139	0.385	0.265	0.362	0.464
	Mean	74.3	80.7	94.4	45.9	98.2	48.6	64.6	77.4	55.4	75.2
		As	**Cd**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**V**	**Zn**
Banana Peel Near Plant	P(T<=t) one-tail	0.284	0.273	0.493	0.303	0.072	0.059	0.026	0.101	0.183	0.224
	Mean	74.3	80.7	91.3	39.6	98.2	30.4	71.9	79.5	53.1	77.7
		As	**Cd**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**V**	**Zn**
Banana Peel Far from Plant	P(T<=t) one-tail	0.419	0.242	0.119	0.342	0.247	0.147	0.402	0.442	0.262	0.346
	Mean	73.8	80.6	95.5	24.9	99.0	72.9	48.2	80.2	48.0	80.9

*Color Representation $p < 0.05$ $>0.05\ p \leq 0.10$ $> 0.10\ P \leq 0.15$ $> 0.15\ P \leq 0.30$

As shown in Table 4.21, near the plant, there is a greater amount of metals in fraction 3-5 for the banana peel reactor compared to the control reactor, to at least a 70% level of confidence, for all metals except chromium and copper. Far away from the plant, there is a greater amount of metals in fraction 3-5 for the banana peel reactor compared to the control reactor, to at least a 70% level of confidence, for 5 of the 10 metals. Both the fishbone and banana peel were more effective near the plant.

Table 4.22 show the overall performance of the 3% w/w fishbone and the banana peel based on the vicinity to the vetiver grass. Near the plant, the fishbone and banana peel reactors performed better than the blank for 90% and 80% of samples, respectively, to at least a 70% confidence level. Far away from the plant, the fishbone and banana peel reactors performed better than the blank for 60% and 50% of samples, respectively, to at least a 70% confidence level.

Table 4.22 Overall performance of the 3% w/w fishbone and the banana peel based on the vicinity to the vetiver grass.

Total count	10							
P - Value	**Fishbone**				**Banana Peel**			
	Near Plant	**Far From Plant**	**Near Plant**	**Far From Plant**	**Near Plant**	**Far From Plant**	**Near Plant**	**Far From Plant**
$p < 0.05$	3	0	30%	0%	1	0	10%	0%
$>0.05\ p \leq 0.10$	0	1	0%	10%	2	0	20%	0%
$> 0.10\ P \leq 0.15$	1	2	10%	20%	1	2	10%	20%
$> 0.15\ P \leq 0.30$	5	3	50%	30%	4	3	40%	30%
Overall Percentage			90%	60%			80%	50%

*10 represents the total number of samples for each sample set. The percentages were calculated as a fraction of the total.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Waste management poses one of the major challenges in developing countries. An understanding of phytoremediation and the benefits it has on the soil as a natural heavy metal removal system will have many applications to countries with a low GDP. The goal of this research was to facilitate low-cost removal of heavy metals from soil in developing countries, utilizing a combination of phytoremediation and soil amendments. The plant species Vetiver grass, introduced from Asia to tropical and sub-tropical regions, was tested, in combination with soil amendments (fish bones, fertilizer, and fruit peels), for their ability to remove ten heavy metals common in dumpsite leachate from these regions.

For the batch tests (soil and amendments alone, no plants), the fishbone concentration of 3% (w/w) of soil had the best results across all the metals, compared to the other fishbone concentrations. The difference was statistically significant to at least a 70% level of confidence for all metals except zinc, and iron for the lower concentration. Fruit peels' performance was not as good as fishbone and fertilizer; nevertheless, banana peel (the best-performing fruit-peel) was chosen to test in the bench-scale reactors, in keeping with the goal to evaluate a low-cost method of removing heavy metals from leachate. The performance of banana peel was statistically better than the other 2 fruit peels, to a 70% level of confidence, for 7 of the 10 metals (all except arsenic, zinc, and the higher concentration of vanadium).

Fishbone was the better-performing amendment in the bench-scale tests (amendments and plants). 3% fishbone amendment with plants increased the amount of metals stabilized in fraction 3-5, compared to the control reactor (plants alone). Fractions 3-5 represent the more stable binding of metals in nature; in these fractions the metals are not easily leached into the environment. The increase was statistically significant, to at least a 70% level of confidence, for

9 of 10 metals near the plant, and for 6 of 10 metals farther away from the plant. Banana peel amendment with plants increased the amount of metals stabilized in fraction 3-5, compared to the control reactor (plants alone). The increase was statistically significant, to at least a 70% level of confidence, for 8 of 10 metals near the plant, and for 5 of 10 metals farther away from the plant.

5.2 Recommendations for Future Research

Recommendations related to plants

1. Use wild coffee (*Senna occidentalis*) and nut grass (*Cyperus rotundus*), which are also plants native to Grenada, as phytoremediation options to remove heavy metal in contaminated soil.
2. Use uniform plant sizes and age of the plant to minimize of the variation in uptake concentrations.
3. Use plants that are potted and grown to control the variables such as prior metal uptake, general wellbeing of the plant, transportation and storage.

Recommendations related to soil and amendments

4. Use soil from the location to be researched to ensure the research matches the on-site conditions.
5. Use extracted fruit peel cellulose, to see whether it is more effective than raw peel.
6. Use a method to add fruit that can be removed after a period since peels degrade over time.
7. Instead of fishbones only, use the fish scales to remove heavy metals by biosorption.
8. Use calcium hydroxide (lime) or calcium oxide (quicklime) to precipitate out the metal ions to stabilize or immobilize metals.

Other Recommendations

9. Collect more soil samples at various distances from the plant to better characterize spatial variability. Increase frequency of collecting samples along with spatial variability to better characterize how the binding of metals changes with time.
10. Conduct a field scale study to determine how long the metals are immobilized using the fruit peels or fishbones.

APPENDIX A

SOIL TRIANGLE METHOD

Soil Triangle Method

- Fill the graduated cylinder with 25 mL of your soil sample.
- Add 75 mL of water to the graduated cylinder.
- Cover the graduated cylinder and invert (shake) several times until the soil is thoroughly mixed.
- Place the cylinder on the table and let it settle for approximately 30 minutes.
- Once the soil has settled, there should be 3 distinct layers. Measure the volume of each layer and the total volume of the sample.
- Calculate the percentage of each layer. Repeat three time and take the averages.
- Using the Triangle, triangulate where each layer coincides, this will give the soil texture.

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APPENDIX B

CREATION OF SYNTHETIC LEACHATE

Concentrations of metal ions tested in the synthetic leachate for the batch analysis

Name of the Compound	Formula of Compound	Range of Conc. In Literature (mg/L)	Heavy Metal	MW of compound to add (g/mol)	Mass of whole compound to add in 1L H20 (mg/L) Upper limit conc.	Mass of compound to add (moles*MW) (g/L)	Conc 1 - 500ml Sol'n (g)
Arsenic oxide	As2O3	0.55 - 0.60	As3+	197.84	7.26	0.0073	0.0040
Cadmium Nitrate	$Cd(NO3)_{2.4H2O}$	0.07 -0.29	Cd2+	308.49	7.96	0.0080	0.0040
Chromium Nitrate	$Cr(NO_3)_3.9H2O$	0.36 - 1.20	Cr3+	400.15	92.35	0.0923	0.0462
Copper Chloride	CuCl2	0.54 - 0.70	Cu2+	134.45	14.81	0.0148	0.0074
Iron Chloride	FeCl2· 4H2O	5.45 - 34.07	Fe2+	198.81	1212.90	1.2129	0.6065
Manganese Chloride	$MnCl_2.4H2O$	1.60 - 3.91	Mn2+	197.91	140.85	0.1409	0.0704
Nickel Chloride	$NiCl_2.6H2O$	0.42 - 0.90	Ni2+	237.69	36.45	0.0364	0.0182
Lead Nitrate	$Pb(NO3)_2$	0.49 - 1.00	Pb2+	331.20	15.98	0.0160	0.0080
Zinc Chloride	$ZnCl_2$	1.66 - 4.10	Zn2+	136.29	85.47	0.0855	0.0427
Vanadium Oxide	V2O5	0.039 - 0.139	V5+	181.88	2.48	0.0025	0.0012

*based on upper limit concentration.

Use the following equation breakdown to calculate the mass of each compound to be added to make individual stock solution to be used for the experiment.

$$Mass\ Salt(mg) =$$

$$volume(l)\ X\ Conc.needed\ \left(\frac{mg}{L}\right) X \frac{1}{MM\ cation\ (\frac{mg}{mmol})}\ X\ MM\ of\ salt\ \left(\frac{mg}{mmol}\right) X\ stoichiometry\ )$$

- Molecular Compound As_2O_3 = 197.841 (g/mol)
- Arsenic Molecular Weight 74.922 (g/mol)
- Arsenic Concentration 6.00 (mg/L)

- Equivalent (# of Arsenic molecule) = 2
- Metal Conc. $\frac{(Metal\ conc.\ X\ \frac{1}{1000})}{MW}$ = 8.01E-05 (mol/L)
- Mass of whole compound to add in 1L H2O (mg/L) =

$\frac{Metal\ conc.}{2} X\ MW\ of\ compond\ X\ 1000$ = 7.9219 mg/L

- Mass of compound to add to achieve Metal Conc. (g/L) = 7.9219/1000 = 0.0079 g/L
- 500ml solution = 0.0079 /2 = 0.0040 g/L.

APPENDIX C

RESULTS OF THE BATCH TESTS

Metal: Arsenic

		Fishbone						None (control)	Fertilizer	Banana Peel	Orange Peel	Lemon Peel
		1 X Stoich.	1.5 x stoich.	2 x stoich.	3 x stoich.	5 x stoich.	3% (w/w) of soil	Control	3 g/L	5g	5g	5g
		D3A1	D3A2	D3A3	D3A4	D3A5	D3A6	D3A7	D3A8	D3A9	D3A10	D3A11
Conc 1	Day 3	109.37	3.65	11.71	173.44	306.90	10.25	0.00	260.50	541.89	379.21	367.05
	Day 14	95.80	361.20	234.22	321.90	257.98	208.71	0.00	684.24	138.79	193.16	200.20
	Day 28	36.78	660.59	301.47	250.58	670.11	49.14	0.00	543.94	102.15	89.76	129.67
Conc. 1 Duplicate		D3A1B	D3A2B	D3A3B	D3A4B	D3A5B	D3A6B	D3A7B	D3A8B	D3A9B	D3A10B	D3A11B
	Day 3	271.25	409.07	523.94	422.73	513.49	317.73	0.00	1606.78	1327.38	900.76	762.93
	C1 Day 14	24.63	286.86	218.48	355.26	306.41	140.93	118.09	1436.42	2.80	262.29	245.96
	C1 Day 28	29.75	444.73	405.53	556.07	422.46	73.35	49.01	1083.54	272.72	183.30	169.20
Conc 2		D3B1	D3B2	D3B3	D3B4	D3B5	D3B6	D3B7	D3B8	D3B9	D3B10	D3B11
	Day 3	247.59	366.58	754.11	404.23	413.14	376.21	0.00	793.70	1047.65	802.27	813.41
	Day 14	379.16	339.62	493.48	207.29	237.13	275.19	0.00	888.64	274.50	653.30	639.76
	Day 28	50.04	44.70	61.88	40.12	247.67	93.44	0.00	475.64	149.90	149.06	225.55
Conc. 2 Duplicate		D3B1B	D3B2B	D3B3B	D3B4B	D3B5B	D3B6B	D3B7B	D3A8B	D3B9B	D3B10B	D3B11B
	Day 3	460.06	585.13	402.60	672.65	683.77	606.46	0	1246.00	1271.65	1253.22	1111.42

	C2 Day 14	244.10	145.21	86.61	23.89	322.49	235.68	203.79	834.22	322.12	173.81	112.59
	C2 Day 28	89.82	47.62	50.83	35.05	113.04	141.40	83.94	701.26	382.93	189.42	228.06

Metal: Cadmium

		Fishbone						None (control)	Fertilizer	Banana Peel	Orange Peel	Lemon Peel
		1 X Stoich.	1.5 x stoich.	2 x stoich.	3 x stoich.	5 x stoich.	3% (w/w) of soil	Control	3 g/L	5g	5g	5g
Conc 1		D3A1	D3A2	D3A3	D3A4	D3A5	D3A6		D3A8	D3A9	D3A10	D3A11
	Day 3	0.003	0.003	0.003	0.003	8.096	0.003		0.003	0.003	0.003	0.003
	Day 14	0.003	0.003	0.003	0.003	0.003	0.003		0.003	0.003	0.003	0.003
	Day 28	0.003	0.003	0.003	0.003	0.003	0.003		0.003	0.003	0.003	0.003
Conc. 1 Duplicate		D3A1B	D3A2B	D3A3B	D3A4B	D3A5B	D3A6B	D3A7B	D3A8B	D3A9B	D3A10B	D3A11B
	Day 3	0.003	0.003	0.003	0.003	0.003	0.003	0.000	0.003	0.003	0.003	0.003
	Day 14	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003
	Day 28	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003
Conc 2		D3B1	D3B2	D3B3	D3B4	D3B5	D3B6		D3B8	D3B9	D3B10	D3B11
	Day 3	0.003	0.003	0.003	0.003	0.003	0.003		0.003	0.003	0.003	0.003
	Day 14	0.003	0.003	0.003	0.003	0.003	0.003		0.003	0.003	0.003	0.003
	Day 28	0.003	0.003	0.003	0.003	0.003	0.003		0.003	0.003	0.003	0.003

Conc. 2 Duplicate		D3B1B	D3B2B	D3B3B	D3B4B	D3B5B	D3B6B	D3B7B	D3A8B	D3B9B	D3B10B	D3B11B
	Day 3	0.003	0.003	0.003	0.003	0.003	0.003	0.000	0.003	0.003	0.003	0.003
	Day 14	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003
	Day 28	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003

Metal: Chromium

		Fishbone						None (control)	Fertilizer	Banana Peel	Orange Peel	Lemon Peel
		1 X Stoich.	1.5 x stoich.	2 x stoich.	3 x stoich.	5 x stoich.	3% (w/w) of soil	Control	3 g/L	5g	5g	5g
Conc 1		D3A1	D3A2	D3A3	D3A4	D3A5	D3A6		D3A8	D3A9	D3A10	D3A11
	Day 3	0.22	<1	<1	0.71	1.32	0.63		0.43	18.14	13.63	9.35
	Day 14	10.62	9.50	8.95	10.36	14.98	5.64		7.63	20.78	31.29	27.38
	Day 28	6.80	7.83	8.61	10.16	12.21	4.53		2.35	21.08	19.37	22.07
Conc. 1 Duplicate		D3A1B	D3A2B	D3A3B	D3A4B	D3A5B	D3A6B	D3A7B	D3A8B	D3A9B	D3A10B	D3A11B
	Day 3	6.22	9.81	20.27	2.30	6.10	4.37	0	4.33	22.61	25.00	15.89
	Day 14	6.14	5.83	8.71	8.90	11.90	5.38	7.71	27.68	6.71	29.94	41.50
	Day 28	5.64	7.18	8.53	7.60	11.35	5.76	3.98	2.35	19.40	20.42	24.83
Conc 2		D3B1	D3B2	D3B3	D3B4	D3B5	D3B6		D3B8	D3B9	D3B10	D3B11

	Day 3	2.72	5.74	1.73	2.87	1.35	3.64		1.21	14.58	15.43	11.13
	Day 14	6.42	7.02	6.64	8.52	10.64	4.16		3.27	17.48	29.75	19.01
	Day 28	5.76	4.72	4.13	6.30	6.50	4.39		1.39	9.71	20.60	17.73
Conc. 2 Duplicate		D3B1B	D3B2B	D3B3B	D3B4B	D3B5B	D3B6B	D3B7B	D3A8B	D3B9B	D3B10B	D3B11B
	Day 3	3.93	12.90	3.47	3.43	2.80	4.73	0	3.40	11.16	16.91	14.19
	Day 14	4.34	3.51	6.38	5.96	6.52	4.42	5.10	5.49	11.15	16.72	16.69
	Day 28	5.32	3.60	5.19	5.66	5.56	3.60	3.35	2.07	7.18	18.09	17.16

Metal: Copper

		Fishbone						None (control)	Fertilizer	Banana Peel	Orange Peel	Lemon Peel
		1 X Stoich.	1.5 x stoich.	2 x stoich.	3 x stoich.	5 x stoich.	3% (w/w) of soil	Control	3 g/L	5g	5g	5g
Conc 1		D3A1	D3A2	D3A3	D3A4	D3A5	D3A6		D3A8	D3A9	D3A10	D3A11
	Day 3	0.03	0.03	0.03	0.03	0.03	0.03		0.03	81.85	0.03	0.03
	Day 14	85.04	4.27	50.29	68.30	37.89	31.30		7.18	7.38	34.33	10.21
	Day 28	0.03	0.03	0.03	0.03	0.03	0.03		0.03	0.03	0.03	0.03
Conc. 1 Duplicate		D3A1B	D3A2B	D3A3B	D3A4B	D3A5B	D3A6B	D3A7B	D3A8B	D3A9B	D3A10B	D3A11B
	Day 3	42.59	0.03	15.19	0.03	1420.42	263.19	0	370.86	340.81	150.77	0.03
	Day 14	0.03	0.03	0.03	0.03	0.03	0.03	42.09	27.29	43.34	116.00	8.46
	Day 28	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03

Conc 2		D3B1	D3B2	D3B3	D3B4	D3B5	D3B6		D3B8	D3B9	D3B10	D3B11
	Day 3	0.03	0.03	0.03	0.03	0.03	0.03		0.03	66.62	30.74	28.69
	Day 14	51.68	9.45	2.69	0.03	0.03	0.03		0.03	0.03	46.59	62.67
	Day 28	0.03	0.03	0.03	0.03	0.03	0.03		0.03	0.03	0.03	0.03
Conc. 2 Duplicate		D3B1B	D3B2B	D3B3B	D3B4B	D3B5B	D3B6B	D3B7B	D3A8B	D3B9B	D3B10B	D3B11B
	Day 3	8.13	89.25	124.84	5.70	6.90	39.16	0	15.61	9.03	29.67	46.66
	Day 14	0.03	0.03	38.12	0.03	0.03	1.31	1144.67	1595.56	0.03	0.03	0.03
	Day 28	0.03	0.03	0.03	0.03	0.03	0.03	0.03	21.17	0.03	0.03	0.03

Metal: Iron

		Fishbone						None (control)	Fertilizer	Banana Peel	Orange Peel	Lemon Peel
		1 X Stoich.	1.5 x stoich.	2 x stoich.	3 x stoich.	5 x stoich.	3% (w/w) of soil	Control	3 g/L	5g	5g	5g
Conc 1		D3A1	D3A2	D3A3	D3A4	D3A5	D3A6		D3A8	D3A9	D3A10	D3A11
	Day 3	2.50	2.50	2.50	2.50	2.50	2.50		2.50	1912.56	2031.03	648.41
	Day 14	1456.45	158.81	228.78	4834.62	2482.87	15.27		3620.56	7866.71	18509.61	10755.53
	Day 28	119.47	37.57	49.11	727.27	948.28	2.50		2.50	182.66	5496.40	6499.51
Conc. 1 Duplicate		D3A1B	D3A2B	D3A3B	D3A4B	D3A5B	D3A6B	D3A7B	D3A8B	D3A9B	D3A10B	D3A11B
	Day 3	3688.40	38.33	975.56	2.50	632.04	238.25	0	39.05	783.61	3768.26	1223.84
	Day 14	2.50	282.40	2.50	136.19	831.22	2.50	3055.94	527.43	5295.48	25463.60	11101.35

	Day 28	83.34	189.89	166.34	975.52	1290.79	90.53	152.08	66.00	3115.21	8274.08	14685.52
Conc 2		D3B1	D3B2	D3B3	D3B4	D3B5	D3B6		D3B8	D3B9	D3B10	D3B11
	Day 3	2.50	169.71	2.50	589.56	2.50	119.43		2.50	1398.13	2813.72	1698.58
	Day 14	98.95	443.90	125.92	353.87	859.87	2.50		346.36	6596.29	24333.92	27762.27
	Day 28	21.01	2.38	24.00	-15.81	111.11	47.29		134.19	1868.09	11795.50	3283.60
Conc. 2 Duplicate		D3B1B	D3B2B	D3B3B	D3B4B	D3B5B	D3B6B	D3B7B	D3A8B	D3B9B	D3B10B	D3B11B
	Day 3	45.92	60.69	512.42	73.12	2.50	1374.99	0	626.29	1257.13	4712.30	2651.87
	Day 14	2.50	2.50	1674.08	327.37	1519.75	547.71	463.81	1736.86	2013.06	13118.61	9986.06
	Day 28	215.91	101.10	107.38	124.77	107.33	75.55	100.07	72.27	1651.21	11854.33	10327.40

Metal: Manganese

		Fishbone						None (control)	Fertilizer	Banana Peel	Orange Peel	Lemon Peel
		1 X Stoich.	1.5 x stoich.	2 x stoich.	3 x stoich.	5 x stoich.	3% (w/w) of soil	Control	3 g/L	5g	5g	5g
Conc 1		D3A1	D3A2	D3A3	D3A4	D3A5	D3A6		D3A8	D3A9	D3A10	D3A11
	Day 3	16.42	17.53	17.94	18.06	15.72	18.30		35.60	797.55	352.95	274.07
	Day 14	446.66	595.35	110.15	74.95	70.43	60.55		59.78	2693.46	3498.57	3804.97
	Day 28	187.12	280.59	202.60	186.50	66.78	48.19		29.68	271.04	2675.10	4781.12
Conc. 1 Duplicate		D3A1B	D3A2B	D3A3B	D3A4B	D3A5B	D3A6B	D3A7B	D3A8B	D3A9B	D3A10B	D3A11B
	Day 3	61.72	50.89	59.05	53.04	59.08	60.51	0	56.54	1078.52	642.25	512.70

	Day 14	673.39	17.09	77.53	508.62	605.71	16.69	21.85	18.49	26.17	4890.63	6273.93
	Day 28	200.92	222.05	37.21	258.58	253.90	72.77	56.47	58.43	999.36	3643.75	4954.91
Conc 2		D3B1	D3B2	D3B3	D3B4	D3B5	D3B6		D3B8	D3B9	D3B10	D3B11
	Day 3	27.64	50.46	40.56	42.58	40.39	44.46		41.39	1002.62	350.29	430.46
	Day 14	131.25	90.68	189.93	459.17	526.45	17.21		18.76	2379.92	3065.82	3847.49
	Day 28	32.21	33.93	62.50	106.37	241.15	25.93		43.23	35.64	3450.18	3720.57
Conc. 2 Duplicate		D3B1B	D3B2B	D3B3B	D3B4B	D3B5B	D3B6B	D3B7B	D3A8B	D3B9B	D3B10B	D3B11B
	Day 3	61.32	60.99	62.23	57.58	59.28	61.15	0	57.87	902.40	533.53	665.27
	Day 14	26.55	481.31	593.28	836.74	558.59	18.06	25.62	25.60	1231.40	4868.66	4857.62
	Day 28	68.84	317.06	213.47	124.32	229.00	60.84	59.93	75.25	309.63	4366.35	5802.49

Metal: Nickel

		Fishbone						None (control)	Fertilizer	Banana Peel	Orange Peel	Lemon Peel
		1 X Stoich.	1.5 x stoich.	2 x stoich.	3 x stoich.	5 x stoich.	3% (w/w) of soil	Control	3 g/L	5g	5g	5g
Conc 1		D3A1	D3A2	D3A3	D3A4	D3A5	D3A6		D3A8	D3A9	D3A10	D3A11
	Day 3	1.00	1.00	1.00	1.00	1.00	1.00		1.00	1.00	1.00	101.25
	Day 14	1.00	1.00	1.00	1.00	732.91	1.00		1.00	1.00	89.47	276.79
	Day 28	1.00	1.00	1.00	1.00	1.00	1.00		1.00	1.00	1.00	1.00
		D3A1B	D3A2B	D3A3B	D3A4B	D3A5B	D3A6B	D3A7B	D3A8B	D3A9B	D3A10B	D3A11B

Conc. 1 Duplicate	Day 3	1.00	1.00	1.00	1.00	1.00	1.00	0	1.00	1.00	1.00	1.00
	Day 14	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	89.61	215.40
	Day 28	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Conc 2		D3B1	D3B2	D3B3	D3B4	D3B5	D3B6		D3B8	D3B9	D3B10	D3B11
	Day 3	1.00	1.00	1.00	1.00	1.00	1.00		1.00	1.00	1.00	1.00
	Day 14	1.00	1.00	1.00	1.00	1.00	1.00		1.00	1.00	19.02	70.20
	Day 28	1.00	1.00	1.00	1.00	1.00	1.00		1.00	1.00	1.00	1.00
Conc. 2 Duplicate		D3B1B	D3B2B	D3B3B	D3B4B	D3B5B	D3B6B	D3B7B	D3A8B	D3B9B	D3B10B	D3B11B
	Day 3	1.00	1.00	1.00	1.00	1.00	1.00	0	1.00	1.00	1.00	1.00
	Day 14	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	57.03	91.70
	Day 28	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Metal: Lead

		Fishbone						None (control)	Fertilizer	Banana Peel	Orange Peel	Lemon Peel
		1 X Stoich.	1.5 x stoich.	2 x stoich.	3 x stoich.	5 x stoich.	3% (w/w) of soil	Control	3 g/L	5g	5g	5g
Conc 1		D3A1	D3A2	D3A3	D3A4	D3A5	D3A6		D3A8	D3A9	D3A10	D3A11
	Day 3	0.01	0.01	0.01	0.01	0.01	0.01		0.01	0.01	0.01	0.01
	Day 14	0.01	0.01	0.01	0.01	0.01	0.01		0.01	0.01	0.01	0.01
	Day 28	0.01	0.01	0.01	0.01	0.01	0.01		0.01	0.01	0.01	0.01
Conc. 1 Duplicate		D3A1B	D3A2B	D3A3B	D3A4B	D3A5B	D3A6B	D3A7B	D3A8B	D3A9B	D3A10B	D3A11B
	Day 3	0.01	0.01	0.01	0.01	0.01	0.01	0	0.01	0.01	0.01	0.01
	Day 14	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
	Day 28	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

Conc 2		D3B1	D3B2	D3B3	D3B4	D3B5	D3B6		D3B8	D3B9	D3B10	D3B11
	Day 3	0.01	0.01	0.01	0.01	0.01	0.01		0.01	0.01	0.01	0.01
	Day 14	0.01	0.01	0.01	0.01	0.01	0.01		0.01	0.01	0.01	0.01
	Day 28	0.01	0.01	0.01	0.01	0.01	0.01		0.01	0.01	0.01	0.01
Conc. 2 Duplicate		D3B1B	D3B2B	D3B3B	D3B4B	D3B5B	D3B6B	D3B7B	D3A8B	D3B9B	D3B10B	D3B11B
	Day 3	0.01	0.01	0.01	0.01	0.01	0.01	0	0.01	0.01	0.01	0.01
	Day 14	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
	Day 28	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

Metal: Zinc

		Fishbone						None (control)	Fertilizer	Banana Peel	Orange Peel	Lemon Peel
		1 X Stoich.	1.5 x stoich.	2 x stoich.	3 x stoich.	5 x stoich.	3% (w/w) of soil	Control	3 g/L	5g	5g	5g
Conc 1	**12260.12**	D3A1	D3A2	D3A3	D3A4	D3A5	D3A6		D3A8	D3A9	D3A10	D3A11
	Day 3	59.01	59.51	60.96	58.19	57.73	60.17		431.37	445.59	465.55	828.27
	Day 14	992.18	952.02	1010.80	990.80	976.28	970.19		1003.57	946.75	1017.37	1023.93
	Day 28	140.98	102.40	104.13	96.50	102.81	97.32		101.25	92.88	72.48	94.26
Conc. 1 Duplicate		D3A1B	D3A2B	D3A3B	D3A4B	D3A5B	D3A6B	D3A7B	D3A8B	D3A9B	D3A10B	D3A11B
	Day 3	956.99	755.03	935.35	855.54	1029.82	984.72	0	946.19	1009.84	1033.78	1259.13
	Day 14	39.92	39.01	37.82	39.22	38.89	41.17	57.25	41.88	50.08	47.75	51.91

	Day 28	1012.27	1020.44	1008.04	996.70	1012.16	1014.70	1005.49	1024.98	993.33	941.79	951.50
Conc 2	**13329.19**	D3B1	D3B2	D3B3	D3B4	D3B5	D3B6		D3B8	D3B9	D3B10	D3B11
	Day 3	1623.87	554.98	1920.84	534.92	551.63	1510.54		556.32	562.34	884.46	867.18
	Day 14	40.89	45.69	40.12	41.81	39.25	39.03		40.75	40.22	67.08	12.90
	Day 28	120.01	91.79	90.53	100.95	102.33	170.78		115.56	100.12	978.77	940.60
Conc. 2 Duplicate		D3B1B	D3B2B	D3B3B	D3B4B	D3B5B	D3B6B	D3B7B	D3A8B	D3B9B	D3B10B	D3B11B
	Day 3	952.84	969.39	1040.52	987.13	968.84	981.39	962.11	0	987.02	1036.77	1043.56
	Day 14	40.79	39.73	109.45	42.87	40.91	42.07	218.44	241.08	83.59	122.12	133.53
	Day 28	1146.26	1232.87	1157.33	1130.65	1036.05	1003.96	1132.68	1155.37	1145.00	1045.61	949.01

Metal: Vanadium

		Fishbone						None (control)	Fertilizer	Banana Peel	Orange Peel	Lemon Peel
		1 X Stoich.	1.5 x stoich.	2 x stoich.	3 x stoich.	5 x stoich.	3% (w/w) of soil	Control	3 g/L	5g	5g	5g
Conc 1	**361.43**	D3A1	D3A2	D3A3	D3A4	D3A5	D3A6	D3A7	D3A8	D3A9	D3A10	D3A11
	Day 3	4.04	0.50	0.50	4.33	3.41	0.51	0.00	4.66	10.98	13.33	10.61
	Day 14	2.76	0.94	0.83	3.02	2.58	2.79	0.00	11.50	3.17	7.95	4.21
	Day 28	0.74	0.32	0.21	0.24	0.42	1.28	0.00	6.90	0.08	0.88	0.80
Conc. 1 Duplicate		D3A1B	D3A2B	D3A3B	D3A4B	D3A5B	D3A6B	D3A7B	D3A8B	D3A9B	D3A10B	D3A11B
	Day 3	5.94	7.10	4.49	4.86	6.17	4.00	0	21.34	14.66	20.63	17.74

	Day 14	0.39	0.08	0.50	0.50	0.38	2.50	4.27	16.17	2.58	5.35	5.82
	Day 28	1.47	1.16	1.14	1.64	2.03	3.04	2.94	11.52	3.29	3.82	5.75
Conc 2	**4.93**	D3B1	D3B2	D3B3	D3B4	D3B5	D3B6	D3B7	D3B8	D3B9	D3B10	D3B11
	Day 3	2.46	4.26	1.31	5.59	2.34	3.73	0.00	6.91	6.87	12.85	12.10
	Day 14	0.50	0.41	0.27	0.50	0.50	0.54	0.00	3.67	1.29	5.51	6.41
	Day 28	0.25	0.50	0.50	0.50	0.50	0.91	0.00	2.66	0.70	3.98	1.13
Conc. 2 Duplicate		D3B1B	D3B2B	D3B3B	D3B4B	D3B5B	D3B6B	D3B7B	D3A8B	D3B9B	D3B10B	D3B11B
	Day 3	2.09	1.33	6.45	5.91	2.37	4.59	0	8.28	6.42	10.90	13.11
	Day 14	0.46	0.50	1.57	0.50	0.50	1.36	2.13	5.56	1.20	1.54	1.45
	Day 28	1.68	0.60	0.72	0.55	0.50	1.41	1.57	3.67	1.59	4.02	3.26

APPENDIX D

PERCENTAGE IN EACH FRACTION FOR DIFFERENT REACTORS.

Metals	Reactor with No Amendment, Day 14, Top						
	Fraction 0 Water soluble	Fraction 1 Exchangeable	Fraction 2 Carbonate bound	Fraction 3 Bound Fe & Mn Oxides	Fraction 4 Bound organic matter	Fraction 5 Residuals	% in Fraction 3-5
V	2	16	41	2	4	35	41
Cr	1	1	80	15	3	2	19
Fe	0	0	32	6	2	60	68
Mn	5	7	45	24	10	10	44
Ni	3	17	0	48	23	9	80
Cu	6	28	37	9	14	6	29
Zn	7	25	36	10	15	7	33
As	0	3	68	4	1	25	29
Cd	0	0	99	0	1	0	1
Pb	0	0	0	13	13	74	100

Metals	Reactor with No Amendment, Day 14 Middle						
	Fraction 0 Water soluble	Fraction 1 Exchangeable	Fraction 2 Carbonate bound	Fraction 3 Bound Fe & Mn Oxides	Fraction 4 Bound organic matter	Fraction 5 Residuals	% in Fraction 3-5
V	2	18	29	0	4	47	52
Cr	1	3	67	23	4	3	29
Fe	0	0	26	1	3	70	74
Mn	5	10	46	20	11	7	39
Ni	5	17	13	1	50	14	65
Cu	4	17	21	45	8	4	57
Zn	4	18	20	42	10	5	58
As	0	4	51	4	1	39	44
Cd	0	0	0	0	84	16	100
Pb	0	0	0	0	21	79	100

Metals	Reactor with No Amendment, Day 14 Bottom						
	Fraction 0 Water soluble	Fraction 1 Exchangeable	Fraction 2 Carbonate bound	Fraction 3 Bound Fe & Mn Oxides	Fraction 4 Bound organic matter	Fraction 5 Residuals	% in Fraction 3-5
V	2	8	35	3	5	47	55
Cr	1	0	68	19	4	8	31
Fe	0	0	14	6	2	79	86
Mn	5	9	40	26	12	7	46
Ni	2	28	12	39	13	6	58
Cu	9	5	45	12	19	9	41
Zn	9	6	37	15	22	11	48
As	0	13	33	11	1	42	54
Cd	0	9	0	5	77	9	91
Pb	0	0	0	13	15	72	100

Metals	Reactor with No Amendment, Day 28 Top						
	Fraction 0 Water soluble	Fraction 1 Exchangeable	Fraction 2 Carbonate bound	Fraction 3 Bound Fe & Mn Oxides	Fraction 4 Bound organic matter	Fraction 5 Residuals	% in Fraction 3-5
V	3	17	3	4	6	67	77
Cr	2	2	8	66	12	9	88
Fe	0	0	0	8	39	53	100
Mn	3	6	9	70	7	5	82
Ni	1	15	32	31	17	4	53
Cu	14	7	16	19	30	14	63
Zn	12	8	16	20	29	14	63
As	0	15	2	39	5	38	82
Cd	1	9	6	7	68	10	84
Pb	0	0	12	23	17	48	88

Metals	Reactor with No Amendment, Day 28 Middle						
	Fraction 0 Water soluble	Fraction 1 Exchangeable	Fraction 2 Carbonate bound	Fraction 3 Bound Fe & Mn Oxides	Fraction 4 Bound organic matter	Fraction 5 Residuals	% in Fraction 3-5
V	3	21	3	4	6	64	74
Cr	2	2	7	68	11	9	88
Fe	0	1	0	8	3	88	99
Mn	6	12	19	38	15	9	62
Ni	2	14	31	31	16	6	53
Cu	14	7	16	19	30	14	63
Zn	12	8	16	21	29	15	64
As	0	11	1	62	1	25	88
Cd	0	15	8	16	52	10	77
Pb	0	1	13	20	11	56	87

Metals	Reactor with No Amendment, Day 28 Bottom						
	Fraction 0 Water soluble	Fraction 1 Exchangeable	Fraction 2 Carbonate bound	Fraction 3 Bound Fe & Mn Oxides	Fraction 4 Bound organic matter	Fraction 5 Residuals	% in Fraction 3-5
V	3	22	3	4	7	61	72
Cr	2	3	23	55	10	6	71
Fe	0	0	0	15	4	81	100
Mn	5	9	11	59	10	6	76
Ni	2	16	30	30	18	5	53
Cu	14	7	15	19	30	14	63
Zn	12	8	16	21	29	14	64
As	0	22	2	30	3	44	76
Cd	0	12	7	10	62	9	81
Pb	0	0	14	22	14	50	86

Fishbone Day 14 Top

Metals	Fraction 0 Water soluble	Fraction 1 Exchangeable	Fraction 2 Carbonate bound	Fraction 3 Bound Fe & Mn Oxides	Fraction 4 Bound organic matter	Fraction 5 Residuals	% in Fraction 3-5
V	3	27	4	5	7	53	66
Cr	3	4	8	65	13	7	85
Fe	0	0	0	1	10	88	100
Mn	4	5	8	57	10	15	82
Ni	2	14	41	24	15	4	44
Cu	14	7	16	19	30	14	63
Zn	13	9	16	19	30	13	63
As	0	30	2	13	1	52	67
Cd	0	4	4	1	79	13	93
Pb	0	0	4	21	24	50	96

Fishbone Day 14 Middle

Metals	Fraction 0 Water soluble	Fraction 1 Exchangeable	Fraction 2 Carbonate bound	Fraction 3 Bound Fe & Mn Oxides	Fraction 4 Bound organic matter	Fraction 5 Residuals	% in Fraction 3-5
V	3	21	12	4	6	55	65
Cr	2	2	36	44	10	6	59
Fe	0	0	2	12	2	84	98
Mn	7	8	15	46	15	9	71
Ni	2	21	8	46	17	6	69
Cu	9	5	43	13	20	9	42
Zn	9	6	40	15	20	11	46
As	0	25	13	9	2	50	62
Cd	0	4	47	2	45	2	49
Pb	0	0	0	23	18	59	100

Fishbone Day 14 Bottom

Metals	Fraction 0 Water soluble	Fraction 1 Exchangeable	Fraction 2 Carbonate bound	Fraction 3 Bound Fe & Mn Oxides	Fraction 4 Bound organic matter	Fraction 5 Residuals	% in Fraction 3-5
V	2	20	3	4	6	66	75
Cr	2	4	8	66	12	8	86
Fe	0	0	0	17	4	79	100
Mn	4	5	11	65	9	6	80
Ni	1	16	33	31	14	4	50
Cu	14	7	15	19	30	14	63
Zn	12	11	15	19	27	15	62
As	0	33	2	13	4	49	65
Cd	0	2	1	1	85	12	97
Pb	0	0	5	24	18	52	95

Fishbone Day 28 Top

Metals	Fraction 0 Water soluble	Fraction 1 Exchangeable	Fraction 2 Carbonate bound	Fraction 3 Bound Fe & Mn Oxides	Fraction 4 Bound organic matter	Fraction 5 Residuals	% in Fraction 3-5
V	3	22	0	4	6	65	75
Cr	2	3	10	62	14	9	85
Fe	0	0	0	10	4	86	100
Mn	8	9	16	37	19	11	67
Ni	2	7	0	38	45	8	91
Cu	10	5	42	13	21	10	43
Zn	9	6	36	14	23	12	49
As	0	34	0	11	5	51	66
Cd	0	5	0	3	83	9	95
Pb	0	0	0	11	10	79	100

Fishbone Day 28 Middle

Metals	Fraction 0 Water soluble	Fraction 1 Exchangeable	Fraction 2 Carbonate bound	Fraction 3 Bound Fe & Mn Oxides	Fraction 4 Bound organic matter	Fraction 5 Residuals	% in Fraction 3-5
V	3	23	0	4	6	64	74
Cr	2	3	10	59	14	12	85
Fe	0	0	0	6	1	92	100
Mn	6	6	15	51	14	8	73
Ni	2	15	4	37	35	7	78
Cu	10	5	42	13	21	10	43
Zn	9	6	37	14	22	11	48
As	0	29	0	8	2	60	71
Cd	0	3	0	0	85	12	97
Pb	0	0	0	22	2	76	100

Fishbone Day 28 Bottom

Metals	Fraction 0 Water soluble	Fraction 1 Exchangeable	Fraction 2 Carbonate bound	Fraction 3 Bound Fe & Mn Oxides	Fraction 4 Bound organic matter	Fraction 5 Residuals	% in Fraction 3-5
V	3	22	1	4	6	63	73
Cr	2	8	12	58	13	7	78
Fe	0	0	0	7	3	89	100
Mn	8	8	15	42	17	10	70
Ni	2	14	3	42	33	7	82
Cu	10	5	42	13	21	10	43
Zn	9	6	37	15	21	12	48
As	0	24	1	16	4	54	75
Cd	0	2	29	4	61	4	69
Pb	0	0	0	13	9	78	100

Metals	Banana Peel Day 14 Top						
	Fraction 0 Water soluble	Fraction 1 Exchangeable	Fraction 2 Carbonate bound	Fraction 3 Bound Fe & Mn Oxides	Fraction 4 Bound organic matter	Fraction 5 Residuals	% in Fraction 3-5
V	3	5	9	4	7	71	83
Cr	2	1	24	59	8	6	73
Fe	0	0	2	9	4	85	98
Mn	5	28	20	28	12	7	47
Ni	2	21	4	33	33	7	73
Cu	10	5	43	13	20	10	43
Zn	9	6	40	16	19	11	46
As	1	23	4	14	3	56	73
Cd	1	2	0	72	22	2	97
Pb	0	0	0	17	15	67	100

Metals	Banana Peel Day 14 Middle						
	Fraction 0 Water soluble	Fraction 1 Exchangeable	Fraction 2 Carbonate bound	Fraction 3 Bound Fe & Mn Oxides	Fraction 4 Bound organic matter	Fraction 5 Residuals	% in Fraction 3-5
V	3	5	15	4	7	67	78
Cr	3	4	48	32	9	4	45
Fe	0	0	10	11	6	74	90
Mn	5	23	21	31	10	10	51
Ni	4	16	0	35	39	7	80
Cu	9	5	44	12	20	9	42
Zn	13	5	36	15	19	10	45
As	1	18	12	13	4	52	69
Cd	1	2	0	4	84	9	97
Pb	1	0	0	25	16	59	99

Metals	Banana Peel Day 14 Bottom						
	Fraction 0 Water soluble	Fraction 1 Exchangeable	Fraction 2 Carbonate bound	Fraction 3 Bound Fe & Mn Oxides	Fraction 4 Bound organic matter	Fraction 5 Residuals	% in Fraction 3-5
V	3	4	19	4	7	63	74
Cr	1	1	68	22	6	3	31
Fe	0	0	15	10	5	70	85
Mn	4	19	35	27	10	5	42
Ni	2	10	19	34	31	5	69
Cu	9	5	46	12	19	9	40
Zn	8	5	46	13	18	10	41
As	0	15	24	12	3	46	60
Cd	0	0	94	0	6	0	6
Pb	0	0	0	15	15	70	100

Metals	Banana Peel Day 28 Top						
	Fraction 0 Water soluble	Fraction 1 Exchangeable	Fraction 2 Carbonate bound	Fraction 3 Bound Fe & Mn Oxides	Fraction 4 Bound organic matter	Fraction 5 Residuals	% in Fraction 3-5
V	2	9	46	2	4	37	43
Cr	1	1	80	9	4	5	18
Fe	0	0	17	9	5	69	83
Mn	5	5	31	39	12	7	58
Ni	2	10	9	33	41	6	79
Cu	8	5	49	11	18	9	38
Zn	7	5	51	12	17	8	37
As	1	7	43	34	1	14	49
Cd	1	0	0	9	82	8	99
Pb	0	0	0	32	15	53	100

Metals	Banana Peel Day 28 Middle						
	Fraction 0 Water soluble	Fraction 1 Exchangeable	Fraction 2 Carbonate bound	Fraction 3 Bound Fe & Mn Oxides	Fraction 4 Bound organic matter	Fraction 5 Residuals	% in Fraction 3-5
V	3	16	2	4	7	69	80
Cr	3	2	16	49	20	10	79
Fe	0	0	0	10	7	83	100
Mn	6	8	15	49	14	8	71
Ni	2	8	4	21	56	10	86
Cu	10	5	42	13	20	10	43
Zn	10	6	38	14	21	11	47
As	1	9	1	56	6	27	89
Cd	0	0	0	6	85	10	100
Pb	0	0	0	19	16	66	100

Metals	Banana Peel Day 28 Bottom						
	Fraction 0 Water soluble	Fraction 1 Exchangeable	Fraction 2 Carbonate bound	Fraction 3 Bound Fe & Mn Oxides	Fraction 4 Bound organic matter	Fraction 5 Residuals	% in Fraction 3-5
V	3	7	4	5	0	82	86
Cr	2	2	14	63	12	6	81
Fe	0	0	1	3	3	93	99
Mn	8	12	24	44	2	9	56
Ni	3	21	1	69	1	5	75
Cu	10	5	44	13	17	10	41
Zn	10	7	41	16	16	10	42
As	3	24	4	14	2	53	69
Cd	40	1	46	6	0	8	14
Pb	4	0	0	16	0	80	96

APPENDIX E

PERCENTAGE IN EACH FRACTION FOR DIFFERENT REACTORS DAY 129.

Reactor 1 (Fishbones) Near Plant

Metal	Fraction 0 Water soluble	Fraction 1 Exchangeable	Fraction 2 Carbonate bound	Fraction 3 Bound Fe & Mn Oxides	Fraction 4 Bound organic matter	Fraction 5 Residuals	% in Fraction 3-5
V	1	32	2	3	8	54	65
Cr	0	0	1	6	2	91	99
Fe	0	0	0	3	14	82	99
Mn	0	1	15	6	8	70	84
Ni	1	11	17	7	43	21	71
Cu	1	1	19	17	40	23	79
Zn	9	11	1	7	22	51	79
As	5	11	5	8	9	62	79
Cd	6	6	6	6	11	64	81
Pb	6	6	7	7	7	67	81

Reactor 1 (Banana Peel) Near Plant

Metal	Fraction 0 Water soluble	Fraction 1 Exchangeable	Fraction 2 Carbonate bound	Fraction 3 Bound Fe & Mn Oxides	Fraction 4 Bound organic matter	Fraction 5 Residuals	% in Fraction 3-5
V	2	40	3	3	9	43	55
Cr	2	2	5	53	17	21	92
Fe	1	0	0	8	34	57	99
Mn	0	7	60	21	9	3	33
Ni	0	7	16	12	55	9	77
Cu	1	3	28	16	23	29	68
Zn	6	5	8	12	16	53	81
As	5	13	6	6	6	63	76
Cd	6	6	7	6	11	63	81
Pb	6	8	7	7	7	64	79

Reactor 1 (Control) Near Plant

Metal	Fraction 0 Water soluble	Fraction 1 Exchangeable	Fraction 2 Carbonate bound	Fraction 3 Bound Fe & Mn Oxides	Fraction 4 Bound organic matter	Fraction 5 Residuals	% in Fraction 3-5
V	1	45	2	3	10	39	52
Cr	1	2	4	44	15	34	93
Fe	0	0	0	4	44	51	100
Mn	0	2	54	24	11	9	44
Ni	0	27	24	10	34	5	48

Cu	0	0	76	3	11	9	24
Zn	11	4	14	6	49	17	72
As	5	16	5	6	8	60	74
Cd	6	6	6	7	11	63	81
Pb	6	6	7	7	9	65	81

Reactor 2 (Fishbones) Far from Plant							
Metal	**Fraction 0 Water soluble**	**Fraction 1 Exchangeable**	**Fraction 2 Carbonate bound**	**Fraction 3 Bound Fe & Mn Oxides**	**Fraction 4 Bound organic matter**	**Fraction 5 Residuals**	**% in Fraction 3-5**
V	2	33	2	3	7	54	64
Cr	1	1	2	25	8	62	96
Fe	0	0	0	5	39	56	100
Mn	1	3	47	16	8	25	49
Ni	5	13	10	66	5	1	72
Cu	0	0	19	21	40	21	81
Zn	4	13	11	25	35	10	71
As	6	13	6	7	7	62	76
Cd	7	6	7	6	10	64	81
Pb	6	6	7	7	8	65	81

Reactor 2 (Banana Peel) Far from Plant							
Metal	**Fraction 0 Water soluble**	**Fraction 1 Exchangeable**	**Fraction 2 Carbonate bound**	**Fraction 3 Bound Fe & Mn Oxides**	**Fraction 4 Bound organic matter**	**Fraction 5 Residuals**	**% in Fraction 3-5**
V	1	45	2	3	9	40	52
Cr	1	2	4	41	13	39	93
Fe	0	0	0	4	25	71	100
Mn	0	2	43	18	24	12	55
Ni	0	8	13	9	66	3	78
Cu	0	0	60	13	16	12	40
Zn	4	3	1	7	42	43	92
As	4	15	6	8	9	59	75
Cd	6	6	7	7	11	63	81
Pb	6	6	7	7	7	66	81

Reactor 2 (Control) Far from Plant							
Metal	**Fraction 0 Water soluble**	**Fraction 1 Exchangeable**	**Fraction 2 Carbonate bound**	**Fraction 3 Bound Fe & Mn Oxides**	**Fraction 4 Bound organic matter**	**Fraction 5 Residuals**	**% in Fraction 3-5**
V	1	43	2	3	12	39	54
Cr	3	2	5	56	18	15	90
Fe	0	0	0	6	45	49	100
Mn	0	6	53	24	14	3	41
Ni	1	17	19	9	54	1	64
Cu	0	0	83	3	7	8	17
Zn	9	6	15	9	13	49	70
As	5	17	5	7	7	60	73
Cd	7	7	6	7	10	63	80
Pb	6	7	7	8	9	64	80

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BIOGRAPHICAL INFORMATION

Sasha Jones was born on the Caribbean Island of Grenada. After working 7 years in the brewing industry and 3 years in the microbiology department of St. George's School of medicine, she moved to the United States to pursue a degree in Environmental Science. As an undergraduate, she spent her time as a student/teaching assistant, a tutor in the Chemistry & Mathematics department and during the summer as a teacher for Upward Bound high school students and adults pursuing their GED.

Sasha received a Bachelor of Science degree in Environmental Science from Midwestern State University, and then went on to become a high school science teacher. In her 7 years of teaching, she taught the majority of the science courses, collaborated with colleagues to write the curriculum for Environmental Science, and was a mentor and sponsor for various organization at the James Bowie High School.

As a fitness enthusiast and a means of mental wellness during her studies, she developed a passion for helping anyone she comes across to grow and develop self-confidence. She earned her CrossFit Level 2 and Kids Certificate. She spends some time giving back to the community by coaching.

She interned at Conifer Systems a subsidiary of Archaea Energy in the research and development department. She also had the opportunity to present at Air & Waste Management Association in San Francisco and Intercontinental Landfill Research Symposium in Ashville, North Carolina.

Sasha Jones is a graduate from the department of Earth & Environmental Science at the University of Texas at Arlington. She is passionate about understanding the ways that waste management can be economically sustainable for third world countries. Her research focuses on the removal of heavy metals and low-cost ways to remediate them.