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Bioaccessibility of lead from contaminated soil using phosphate treatment -- Physiologically Based Extraction Test and In Vitro Gastrointestinal Method Test

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BIOACCESSIBILITY OF LEAD FROM CONTAMINATED SOIL USING PHOSPHATE TREATMENT- PHYSIOLOGICALLY BASED EXTRACTION TEST AND IN VITRO GASTROINTESTINAL METHOD TEST

By

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ABSTRACT

Phosphate treatments are used to immobilize lead in soil by forming pyromorphite. Soil from Bonne Terre, Mo was collected to study whether such treatment decreases the bioaccessibility of lead. The soil was treated using 0.5 soil wt% of phosphate. Treatments were: none, phosphoric acid, triple super phosphate, and organic bone meal. Each sample was studied after one, four, sixteen, and twenty weeks; during this time span, water was added approximating the average rainfall rate. Percolated water was collected to test the leached phosphate concentrations. Phosphate was below the detection limit in that leachate. Remediated soil samples were used in Physiologically Based Extraction Tests (PBET) and In Vitro Gastrointestinal Method Tests (IVG). Lead concentrations were determined using Flame Atomic Adsorption (FAA) and Graphite Furnace Atomic Adsorption (GFAA). Titrations of synthetically formed chloropyromorphite were conducted to determine the effect of pH on the dissolution of chloropyromorphite. Results showed that as pH decreased, dissolution between lead and phosphate increased. Ksp's of chloropyromorphite ranged from $10^{-33.3}$ to $10^{-84.4}$ depending on the varying pH and phosphate source. Remediated soil samples were used in a density separation analysis to determine heavy metal composition. Lead compounds such as lead sulfide, lead oxide and lead dioxide were found in trace amounts. The adsorption rate of lead through the stomach lining has been considered but not analyzed throughout this research. A decrease in lead bioaccessibility was observed after a remediation period of 20 weeks.

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NOMENCLATURE

Symbol	Description
Pb	Lead
PbS	Lead Sulfide
PbO	Lead Oxide
PbO_2	Lead Dioxide
$PbCO_3$	Lead Carbonate
$Pb_5(PO_4)_3Cl$	Chloropyromorphite
$Pb_5(PO_4)_3OH$	Hydroxypyromorphite
$Pb_5(PO_4)_3Fl$	Fluoropyromorphite
PBET	Physiologically Based Extraction Test
IVG	In Vitro Gastrointestinal Method
PA	Phosphoric Acid (H3PO4)
TSP	Triple Super Phosphate
BM	Organic Bone Meal
FAA	Flame Atomic Adsorption
GFAA	Graphite Furnace Atomic Adsorption
XRD	X-Ray Diffraction Microscope
SEM-EDS	Scanning Electron Microscope, Energy Dispersive Spectroscopy
BDL	Below Detection Limit
DI	Deionized Water

1. INTRODUCTION

Lead is a naturally occurring heavy metal throughout the earth. Around 600 A.D. lead was used by Romans for plumbing. Today, lead is mined mainly for lead-acid batteries, television screens and used in some forms of gasoline. Approximately six million tons of lead is mined each year, with an estimated 85 million tons still available in mining reserves (EPA (1987); Federal Register 56(1991)). Lead poisoning is very serious for young children. According to the Centers for Disease Control (CDC), approximately four million children are exposed to elevated levels of lead (CDC.gov 2016). The action limit required by the United States Environmental Protection Agency (EPA) for lead exposure is approximately 400mg/ kg of soil (EPA.gov 2000a). High levels of lead exposure are linked to high blood pressure, loss of developmental skills, anemia, and many other adverse health effects (Mayoclinic.org 2014). These symptoms typically develop after individuals have been exposed to high lead levels for extended periods. Young children are most vulnerable to lead poisoning. Lead particles can be ingested when a child places their hands or toys in his or her mouth after exposure to elevated lead concentrations (CDC.gov 1991). Adults also can be exposed to high lead levels. This occurs in a variety of ways such as: consumption of animals exposed to elevated lead levels while grazing, plant uptake of lead, lead paint chips and exposure at shooting ranges. Lead exposure to humans also occurs through ground and surface water contaminated.

Areas with elevated lead levels rarely occur naturally. High lead concentrations are common near old lead deposits where mining practices have occurred. Abandoned smelter sites contain elevated lead levels where lead particles remain near or at the soil surface. This allows lead to easily leach into surface water and contaminate public drinking water (Mosby et al., 2006). Furthermore, prior to 1978, lead was added to paint to decrease the drying time and limit the corrosion of paint (Crow, 2007 and CDC.gov, 2014). Chipping and flaking of lead based paint has caused increased lead levels throughout residential and urban areas. As urban gardening increases, individuals are likely to be exposed to elevated lead levels (Clark et al. 2008). Lead exposure also occurs through inhalation of lead particles blown in the wind. This causes a small coating of lead contaminated particles to be spread throughout plants, clothing, and housing near contaminated sites (New York State Dept. of Health 2013).

Missouri has a significant history of lead exposure to children. Lead mining began in the 1720's when French explorers moved to southeastern Missouri. Lead was a contributor to the development and economic growth of the Missouri economy (MO Dept. of Natural Resources, 2017). However, large smelter sites exposed thousands to elevated lead concentrations. Regions of the state where exposure is the highest include the southeast and southwest portions. In 2012, the Missouri Department of Health reported at least 728 children had blood lead levels exceeding the standard requirement by EPA (MO. Dept. of Health, 2018). It is recommended that children living in elevated lead areas wash their hands frequently;

properly clean all toys that could come into contact with a contaminated area and have limited exposure to bare soil areas. These simple tasks can reduce a child's risks of lead poisoning (Mo. Dept. of Health, 2018).

2. LITERATURE REVIEW

2.1. REMOVING LEAD IN SOIL

There are several different methods used to remediate or treat contaminated sites. Large land disturbances and ecological impacts are probable throughout lead remediation. These methods are labor intensive and involve high capital costs. Remediation methods are determined according to the size of the contaminated area and depth of contaminated soil. EPA recommends using one of the following methods to remediate lead contaminated sites: digging and hauling, capping, or adding clean soil and vegetation to a contaminated site (Task-based et al., 2017). Recently however, phosphate amendments have been researched and tested to potentially develop a more cost effective lead remediation method.

The “dig and haul” method to remediate contaminated soil is the most commonly used. This method has been used where high lead concentrations have been reported. Total costs for this method range from $600 to $1500 per dump truck load. Dig and haul includes both extraction of contaminants and backfill with clean soil (Winter et al., 1999). This is the quickest way to decontaminate and rid an area of high lead concentrations. After extracting contaminated soil, the soil is transported from a site of high risk to human exposure to a site of lower risk (Torik and Dransfield, 2018). The “dig and haul” method is used frequently in urban and residential areas to quickly eliminate exposure to high lead concentrations.

Capping contaminated sites is another remediation strategy. Capping is completed by adding concrete, asphalt, or a geomembrane on top of the contamination. This immobilizes soil particles and traps lead below the cap. Rainwater is then re-routed from the impervious surface of the cap, eliminating the risk for groundwater contaminated. Initial land preparation, water mitigation systems, trenching, grading and cap thickness must be considered when installing a cap. Capital costs generally exceed $750,000 for cap installation, along with annual maintenance costs (EPA, 2010). Capping usually occurs' at large sites that have moderately high concentrations of lead (400-1000 mg/kg soil). After completion, sites are generally developed for industrial, utility and renewable energy facilities (EPA 2017).

Rather than hauling away soil, instead adding several inches of clean soil atop a contaminated site is a popular means of remediation. Clean topsoil produces an area where vegetation can be planted without the risk of uptake of lead. The root systems of the planted vegetation act as a natural erosion control measure. This remediation technique creates an environment for pollinators to inhabit (Task-based et al., 2017). Costs to remediate these sites vary depending on soil depth applied to the area. Sites can be remediated with a three-inch layer of topsoil for $300 per dump truck load, along with costs for vegetation (Journal of Environmental Economics, 2007). Overlaying a site with clean soil is used in areas where low levels of contaminated are present (< 400 mg/kg soil). The main driver for remediation with clean fill is to eliminate the spread of airborne lead particles (World Health Organization 1999).

Phosphate amendments have been considered as a means of remediating lead contaminated soil. Lead and phosphate interact to form the mineral pyromorphite (Mosby, 2017). Adding phosphate to contaminated soil sites has been claimed as an inexpensive and effective way to immobilize lead with minimal land disturbance (Labare et al., 2004). Field studies relating the bioaccessibility of lead to phosphate remediated soils have been conducted. The main areas of these field studies include urban gardens, small arms shooting ranges, and smelter sites with high lead concentrations (Scheckel & Ryan, 2004). Literature has reported a decrease in the bioaccessibility of lead after phosphate remediation. However, the residence time before complete mineral formation was reported to exceed seven years (Beyer et al., 2016; Kientz & Jime, 2003). Phosphate amendments are therefore considered as a potentially simple and cost effective method of lead remediation.

2.2. LEAD IMMOBILIZATION WITH PYROMORPHITE

Adding phosphate to lead contaminated soil sites has been studied to some degree over the past few years. A decreasing bioaccessibility of lead in soil is noted after phosphate addition (Weber et al., 2015). As mentioned above, phosphate amendments transform lead into a stable mineral, pyromorphite ($Pb_5(PO_4)_3Cl$). Pyromorphite, an apatite group mineral, ranges from three endmember compositions. These compounds vary by monovalent anion: chloropyromorphite, hydroxypyromorphite and fluoropyromorphite. Chloropyromorphite is the most common form of the pyromorphite species (mindat.org, 2017). The formation of

pyromorphite has been reported to take place at various temperatures and pH ranges (Zhu et al., 2015). Several factors must occur to initialize pyromorphite formation.

Apatite minerals such as pyromorphite are a group of calcium phosphate minerals containing high concentrations of hydroxide, chloride or fluoride (mindat.org, 2017). The composition of apatite is similar to pyromorphite because both minerals have a hexagonal crystal structure and are bound to oxides, hydroxides and carbonates. Chloropyromorphite and apatite are distinguished by the following molecular formulas: ($Pb_5(PO_4)_3Cl$ and $Ca_5(PO_4)_3Cl$, respectively) (mindat.org, 2017). These two compounds share the exact same molar ratio component of 5:3:1 between lead or calcium, phosphate and chlorine. Phosphate amendment amounts therefore must be calculated to include interaction with Ca^{2+} ions in the soil, as well as with Pb^{2+} ions to form both apatite and pyromorphite. Pyromorphite and calcium apatite have been discovered as combined species in nature because of their molecular make up and the diversity of soil (Mosby et, al. 2016).

In situ remediation shows pyromorphite formation occurs after Ca-apatite has been added to solution containing a detectable lead concentration. Literature reports an exchange of Pb^{2+} ions occupying Ca^{2+} sites when Ca-apatite was added to solution. It was observed in these studies that over time, phosphate in Ca-apatite interacted with lead to create pyromorphite. Dissolution rates vary according to pH values during pyromorphite formation from apatite. Using pH values of 2.00, 5.60, and 9.00, dissolution times ranged from 1h to approximately 5040h. At pH 2.00, the

greatest amount of apatite and pyromorphite dissolution occurs within the first hour. The acidic conditions create an environment with independent lead, calcium and phosphate particles. A steady state concentration of the compounds was not achieved until 5040h after mixing (Xie & Giammar, 2007). Raising the pH to a value greater than 3.50, lead and phosphate particles are likely to interact and form pyromorphite. At pH values of 5.60 and 9.00, significantly less dissolution occurred, creating a steady state environment in a shorter time. Formation of a $(Pb^{2+}/Ca^{2+})_5(PO_4)_3Cl$ also has been found to occur, thus not forming pure pyromorphite (Zhu et al., 2015). Pb^{2+} and Ca^{2+} present in solution precipitate as a solid with detectable lead and calcium concentrations when the molar ratio between the primary element (Pb^{2+} and Ca^{2+}), and secondary elements are ($(PO_4)_3^-$ and Cl^-) 5:3:1.

Soil composition affects the formation of pyromorphite. According to Ruby (1996), Bartlesville type soils have the largest amount of unbound lead. Bartlesville soils are composed of loamy particulates from weathered sandstone (USDA.gov 2016). Lead particles have the greatest opportunity to interact with phosphate amendments and form pyromorphite when they are independent of other compounds. However, lead in soil is comprised of multiple insoluble lead compounds such as galena (PbS), anglesite ($PbSO_4$), and lead phosphate ($Pb_3(PO_4)_2$) (Ruby et al., 1996). It has been estimated that only 30% of lead available in soil is made up of independent lead particles (Ngiaru 1973). Temperature was also reported to have no effect on the solubility of pyromorphite (Topolska et. al., 2016). The Ksp values of

pyromorphite that have been reported range $10^{-18.69}$ to $10^{-84.4}$ (Scheckel & Ryan, 2006; Xie & Giammar, 2007; Zhu et al., 2015; Ngiaru 1973). One issue in describing the Ksp of pyromorphite is the form of phosphate used in the defining equation. The literature reports values using various phosphate compounds, such as hydrogen and dihydrogen phosphate (HPO_4^{2-} and $H_2PO_4^-$ respectively), which increase the value of the reported Ksp. The form of phosphate that reacts to from pyromorphite is PO_4^{3-}, and pH affects the dissociation and relative abundance of protonated species of phosphate (Figure 2.1). Defining Ksp with a form of phosphate other than the trivalent becomes problematic because that Ksp is pH-dependent.

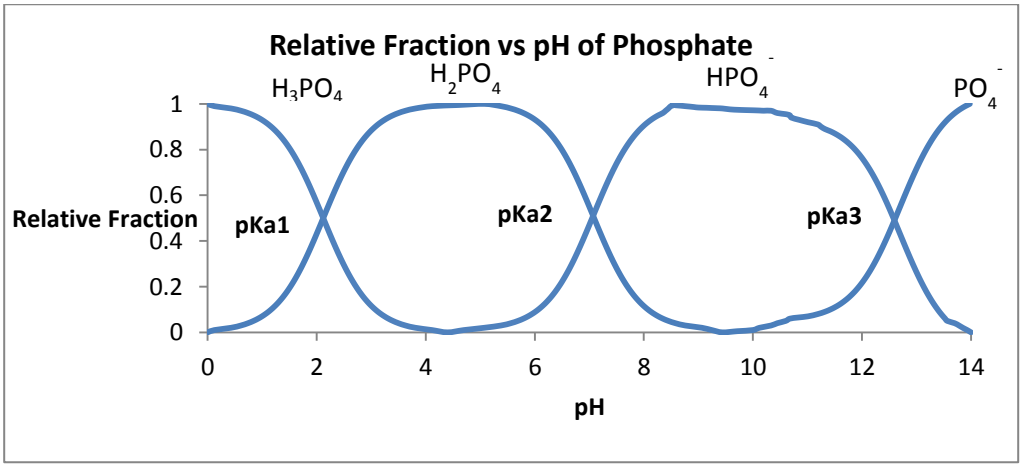


Figure 2.1. pH vs Forms of PO_4^-

2.3. SOLUBILITY OF PYROMORPHITE

Previous literature has reported the formation of pyromorphite occurs almost immediately when lead and phosphate are present in a solution.

Chloropyromorphite precipitates rapidly after the addition of phosphate and chloride in a controlled setting. The molar ratio of lead-phosphate and chloride ratio in precipitated chloropyromorphite is 5:3:1. Once formation occurs, crystallinity of chloropyromorphite samples taken at 1-h and 1-y post precipitation remained unchanged (Scheckel and Ryan Et. al 2002). Many different values for the solubility product (Ksp) of chloropyromorphite have been reported. Values of $10^{-18.79}$ to as low as $10^{-84.4}$ have been reported (Scheckel and Ryan Et. al. 2002). The pH of solution that pyromorphite is precipitated in plays a major role in the stability of the mineral. If the Ksp is defined as including total phosphate ($H_2PO_4^-$, HPO_4^-), rather than only PO_4^{3-}, lower pH values increase the Ksp of pyromorphite when the phosphate source is either $H_2PO_4^-$ or HPO_4^-, creating a more soluble mineral, while higher pH values decrease the Ksp and creates a more stable, insoluble mineral. When pyromorphite is in solution with pH < 2.00, the Ksp (= $[Pb]_5[\Sigma H_1PO_4]_3^-[Cl]$) is highest at $10^{-18.79}$. The soil used in these experiments had an average soil pH of 6.23, leading to a calculated Ksp of $10^{-84.43}$ from PHREEQC and wateq4f.dat. The reported Ksp from Ngiaru and other literature reports a constant Ksp of $10^{-84.43}$. The reported Ksp signifies pyromorphite is very stable and insoluble in naturally occurring soil. However, many considerations must be accounted for when determining the thermodynamics behind the solubility product of pyromorphite. It was reported that particle size could have an impact on the Ksp value of pyromorphite (Giammar and Xie et Al. 2007). Pyromorphite was dissolved in this solution because the surface area of the particles was so small. With such a small surface area and a low pH, according to the Gibbs

free energy of formation equation, the energy emitted was so low that pyromorphite particles were completely dissolved without the bonds being broken to create separate lead and phosphate particles (Xie & Giammar, 2007). The literature reported that the energy emitted from particles less than 1µm was not able to be represented in the solubility of synthetic chloropyromorphite (Xie & Giammar, 2007). pH also has been linked to the stability of pyromorphite. As pH decreases, the stability of pyromorphite decreases. Scheckel reports that chloropyromorphite samples at pH 2.00 released approximately 20% of lead, while samples at pH 6.00 released 0.6% lead (Scheckel and Ryan Et al. 2002). Determining the correct Ksp value can alter the margin of error in dissolution by as much as 50% (Xie & Giammar, 2007). With a low pH being used during half of the *in vitro* experiments (i.e. PBET) in this research, it is important to determine the apparent Ksp of the pyromorphite in a stomach solution (pH 1.70). As described earlier, pyromorphite should be noticeably soluble in a solution with pH <2.00 and very insoluble in solution with pH > 5.50.

Table 2.1. Reported Ksp Values of Chloropyromorphite

Phosphate Source	pH	Literature Ksp	PHREEQC ksp
Ngiaru (1973)	2.21-2.29	$10^{-84.4}$	
Scheckel H_3PO_4	0-2.12	$10^{-18.69}$	$10^{-33.30}$
Scheckel $H_2PO_4^-$	2.12-7.21	$10^{-25.05}$	$10^{-53.63}$
Scheckel HPO_4^{2-}	7.21-12.38	$10^{-46.9}$	$10^{-79.35}$
Scheckel PO_4^{3-}	12.38-14	$10^{-84.4}$	$10^{-84.4}$
Xie & Giammar (2007)	2.00-7.00	$10^{-80.4}$	

Table 2.1 shows a comparison of Ksp values between various literature sources, as well as theoretical equilibrium calculations using the PHREEQC database wateq4f.dat (USGS.gov, 2017). PHREEQC accounted for total equilibrium and formation of pyromorphite, no matter the pH value of the solution. The values differ from values reported by Scheckel because Scheckel accounts for a molar concentration of lead to be present at a given pH, while PHREEQC uses varying molar amounts of lead, phosphate, and chloride to precipitate chloropyromorphite. The molar values used in PHREEQC were converted into lead concentrations and compared to the concentrations recorded from titrations of chloropyromorphite.

2.4. PHOSPHATE AMENDMENTS AS SOIL REMEDIATION

Several forms of phosphate are used as amendments to remediate lead contaminated soil. The three most popular are: triple super phosphate, rock phosphate, and phosphoric acid. Triple super phosphate and rock phosphate do not have a large impact on soil pH. These two are added to remediation sites as a solid or powder. Phosphoric acid has been shown to decrease soil pH and is applied as a liquid. Phosphoric acid, on the other hand is able to move through soil in the shortest amount of time, creating the most efficient amendment method for pyromorphite formation (Scheckel & Ryan, 2004).

Triple super phosphate (TSP) ($Ca(H_2PO_4)_2H_2O$) is a common lawn fertilizer that has been studied as an amendment at lead contaminated sites remediated by EPA and Fish & Wildlife (Mosby et al., 2017). Triple super phosphate is applied by tilling

the contaminated site and applying large amounts of triple super phosphate to exposed soil. According to the literature, as much as 8,000 mg/kg of triple super phosphate have been applied to soil to initiate pyromorphite formation (Mosby, 2017). However, the time period for pyromorphite formation to occur after triple super phosphate remediation has not been reported. Adding high concentrations of triple super phosphate to areas with low vegetative growth in floodplains increases the chances for eutrophication and water pollution.

Rock phosphate ($Ca_3(PO_4)_2$) is commonly used in lead remediation because the mineral is water insoluble. This limits phosphate from being absorbed by plants in remediation areas, while creating an environment suitable for pyromorphite formation. Concentrations ranging from 0.5% phosphate to 1% phosphate per total weight of soil are generally added to sites remediated using rock phosphate. Such sites are prepared by tilling and applying the rock phosphate to exposed soil. Again, no time has been reported for pyromorphite formation after addition of rock phosphate; however, the risk of eutrophication and pollution occurring after the addition of rock phosphate is greatly reduced (Stilwell & Ranciato, 2008).

Phosphoric acid (PA) (H_3PO_4) is considered to be the most effective phosphate amendment to remediate lead contaminated soil (Yang et al., 2001). Phosphoric acid has the ability to leach through soil and come into contact with lead much faster than triple super phosphate and rock phosphate. In addition, phosphoric acid remediation can cover a large volume of soil in a shorter time. However, phosphoric acid application decreases the soil pH at the contaminated site. Additives to raise pH may

be applied after phosphoric acid has been added to increase soil pH and create an environment sustainable for vegetative growth. Lime is not a good choice of raising pH when trying to form pyromorphite because the calcium in lime will likely interact with phosphate before lead and phosphate interact with one another (Scheckel et al., 2006). Health of the general public near the contaminated site must be considered when remediating with phosphoric acid. Young children are at risk of acid burns when coming into contact with phosphoric acid before it has become neutralized in the soil. Although phosphoric acid is the most effective method to remediate lead contaminated sites, there are several safety factors that must be considered before treating a contaminated site with phosphoric acid.

Phosphate amendments including: organic bone meal, fish bones, natural hydroxyapatite (HA) and di-ammonium phosphate (DAP) has also been discussed (Chen et. al., 2006). As previously mentioned, amendments rich in Ca-apatite may not effectively remediate the soil, but create lead calcium phosphates. Furthermore, phosphate amendments have been determined to be effective immobilizers of other heavy metals such as arsenic, cadmium, and zinc (Chen et. al., 2006). Phosphate amendments like organic bone meal, fish bones, natural hydroxyapatite and di-ammonium phosphate potentially could interfere with immobilizing lead in soil by immobilizing other heavy metals. For these considerations, phosphoric acid, triple super phosphate and rock phosphate are the most common phosphate amendments in lead remediation.

2.5. TESTING THE BIOAVAILABILITY OF LEAD

The bioaccessibility of lead is tested either *in vivo*, or *in vitro*. Bioaccessibility tests would be the most accurate if conducted on humans, but there are many ethical issues regarding human testing and lead consumption (Moodie et. al., 2011). Beyer and Ryan conducted experiments using quail, pigs and rats (Mosby et al., 2006). The quail analyzed were fed soil particles remediated with phosphoric acid or triple super phosphate from two contaminated sites. Pigs were fed grasses that had been grown on a separate contaminated site remediated with triple super phosphate. Tests were conducted to determine whether plant uptake of lead had an impact on lead concentrations within the animal's stomach (Beyer et al., 2016). Rather than study a site, rats were used in study on lead adsorption in a fasted or full stomach. Tests on these animals closely resemble human responses because the pH of each animal's stomach is similar to the human stomach (Road & Aberdeen, 1981). Results of these tests show a decrease in the bioaccessibility among the quail, an increase in bioaccessibility among pigs, and no decrease in bioaccessibility among rats fed lead at given meal times versus rats continuously given lead. Testing humans on the bioaccessibility of lead would show the exact decrease in lead bioaccessibility because particles must pass through the GI tract and small intestines before being excreted (Ryan et al., 2004). There has been no definite determination of which animal to study for the most accurate representation on the bioaccessibility of lead in comparison with human beings.

In vitro analysis has been conducted to imitate the digestive system of humans. These methods are performed using Physiologically Based Extraction Tests (PBET) and In Vitro Gastrointestinal Method tests (IVG). PBET is used to simulate the human stomach through digestion. Gastric solution pH used during PBET ranges from 1.7-2.5 depending on the fasted state of the individual. A fasted stomach contains a more acidic gastric solution (pH 1.5-1.7) while a full stomach will have pH values between 2.5 and 4.0 (Road & Aberdeen, 1981). IVG analysis simulates the process in the small intestines. The pH during IVG ranges from 5.5 to 6.5. From the literature, PBET and IVG have been the most accurate *in vitro* analyses performed when testing lead bioaccessibility (Ruby et al., 1996).

PBET is considered one of the most accurate and ethical methods to test the bioaccessibility of lead in humans. However, PBET does not consider lead adsorption through the stomach lining. In 1994, Ruby considered adsorption through the stomach lining as a factor in lead poisoning (Ruby et. al., 1994). In 1996, Ruby analyzed lead contaminated soils using PBET to determine lead bioaccessibility after phosphate remediation (Ruby et. al., 1996). Results of these studies showed dissolution of pyromorphite decreased as solution pH increased from 1.3-2.5 (Ruby et. al., 1996). These results correlate with the Sprague- Dawley model showing a decrease in the bioaccessibility of lead with rats as the pH in the animals' stomach increases (Road & Aberdeen, 1981). The pH during PBET can range between 1.5 and 4.0. An average pH of 1.7 is likely used in solution when performing PBET. Common

pH values of 1.7 to 1.8 are used during PBET analysis because lead is likely ingested while a child is in a fasted state (Ryan et al., 2002).

IVG analysis is conducted following PBET. Gastric solution used in PBET is converted to an intestinal solution using pancreatin and bile salts (Golder Associates 2006). IVG intestinal solution ranges between pH 5.5 and 6.5. From the literature, no dissolution of pyromorphite should take place during IVG. Pyromorphite should precipitate almost immediately at the beginning of IVG and pass through the small intestine as an insoluble mineral (K G Scheckel & Ryan, n.d.; Xie & Giammar, 2007). As reported by various literature sources, pH values above 3.50 showed low concentrations of lead in solution. Thus at pH values expected in the small intestine, there should be a decrease in lead bioaccessibility and formation of pyromorphite (Tang et al., 2009; Yang et al., 2001).

2.6. PHOSPHATE REMEDIATION: DOES IT WORK?

Phosphate remediation has been considered one of the least expensive and most effective ways to remediate lead contaminated soil (Beyer et al., 2016). *In vivo* and *in vitro* experiments have confirmed pyromorphite formation after phosphate addition (Cornish et al., 2004; Ruby et al., 1996). Also, thermodynamic and kinetic analyses show probable pyromorphite formation after phosphate addition (K G Scheckel & Ryan, 2002.; Xie & Giammar, 2007). Soil analyses using X- Ray Diffraction (XRD) and Scanning Electron Microscope Energy-Dispersive X-ray Spectroscopy (SEM-EDS) confirmed pyromorphite formation (Weber et al., 2015; Xie & Giammar, 2007).

Lead phosphate formation has been confirmed, to be an effective way to remediate contaminated soil. However, field results of remediated soils, particularly results of animal testing, have varied; it is likely this variation is due to solubility as pyromorphite travels through the digestive system.

Beyer's (2016) paper testing the lead bioaccessibility on quail shows a decrease in lead bioaccessibility. Quail were tested using phosphate remediated soil from three contaminated sites (two sites in Joplin, Mo and one site from the Big River Floodplain in Missouri) using three phosphate amendments (phosphoric acid , triple super phosphate and composted biosolids). Several in vivo experiments were conducted on the birds to determine lead bioaccessibility in the stomach and small intestines. Experiments analyzed the birds stomach state (fasted or full), the amount of time between feedings, and if the animal was continuously fed or fed at designated meal times. Results of the experiments showed the bioaccessibility of lead in quail decreased by more than 30% in soils remediated with phosphoric acid and triple super phosphate. Soil remediated with composted biosolids did not demonstrate a decrease in bioaccessibility. It must be recognized that soil used during the analyses had been remediated 7-13 years prior to the experiments (Beyer et al., 2016). As reported by Ruby in 1994, a mean residence time of 13 years must be allotted for complete pyromorphite formation (Ruby et al., 1994). Critics argue that this remediation period is too long in urban and residential areas where children are frequently exposed to high lead concentrations. Mosby and Scheckel (Mosby et al., 2006) also conducted tests on lead bioaccessibility using immature pigs.

Immature pigs were analyzed because the stomach of an immature pig is similar to the stomach of a young child. Tests were conducted over a 6.5-year time period at multiple site locations using various phosphate concentrations. After remediating contaminated soil for 10 days using phosphoric acid and potassium chloride (KCl), lime was added to increase soil pH. Soil was then observed using SEM-EDS to determine chloropyromorphite formation, which was confirmed. Next, soil was sent to a separate location where it was fed to pigs in their daily grain. Grass was also grown in the remediated soil. Experiments show that as remediation time increased at each contaminated site, lead bioaccessibility decreased from 21% at three months to 43% at 78 months. However, results after analyzing the blood lead levels in the pigs were not as expected. Higher lead concentrations were reported in the pigs that had ingested treated soil than pigs that had ingested untreated soil. This raises the concern that remediation in areas with low to moderate concentrations of heavy metals create an environment where large amounts of contaminants can be ingested. When dissolution of pyromorphite occurs, lead absorption will occur at a rate faster with lead phosphates than soil ingested containing various other lead compounds. Mosby (et al., 2006) reported plant uptake of lead remained closer to the root nodules of the plant than in the leaf. Given the contrasting results of bioaccessibility studies, more experiments must be conducted to determine if phosphate amendments are an effective remediation technique for lead contaminated soils.

2.7. OTHER RESEARCH RELATED TO PYROMORPHITE FORMATION

While most studies have been conducted using phosphate amendments in contaminated soil, additional experiments have been conducted to determine pyromorphite formation using phosphate amendments. In 2003, Scheckel and Ryan researched pyromorphite formation using phosphoric acid in soft drinks to form pyromorphite; theoretically a child that ingests lead could drink a soft drink and immobilize lead in their stomach through pyromorphite formation. This would reduce the bioaccessibility by allowing the mineral to pass through the GI tract and not be absorbed through the stomach. Experiments showed a 93% lead reduction when phosphoric acid in soda reacted with lead from paint chips (Scheckel & Ryan 2003).

The formation and dissolution of pyromorphite as a function of temperature has also been studied. Topolaska et. al (2016) reported that Ksp values remained constant when analyzed at temperatures ranging from 5-65°C, showing the dissolution rate of pyromorphite is not dependent on temperature, only pH. Initial soil conditions have been studied when determining pyromorphite formation. Ruby et al., (1996) studied different soil types and pyromorphite formation. They reported that Bartlesville soil types are best suited for pyromorphite formation because the soil contains the largest amount of unbound lead particles and allows direct interaction between lead and phosphate (Ruby et al., 1996). Mosby et al., (2006) also considers the heterogeneity of soil on pyromorphite formation. Since soil composition at each remediation site is different, Mosby suggested pyromorphite

formation using the same technique at one contaminated site may not be as effective at different sites.

Phosphorus leaching has also been analyzed. Studies show that leaching values at contaminated sites ranged from 10-20% of the total phosphorus added (Weber et al., 2015). Site preparation and application of phosphate have also been studied heavily. Sites were generally prepared by tilling the soil to a depth of 15 cm prior to the application of a phosphoric acid and rock phosphate mixture. Lime is immediately added to increase soil pH and vegetation is introduced to reduce erosion and runoff risks within the area (Mosby, 2017). Remediating lead contaminated areas with phosphate amendments is cost affective; however it takes months or years for mineral formation to completely immobilize the lead.

3. METHODS/MATERIALS

3.1. FAA/GFAA

Samples were analyzed using either Flame Atomic Absorption (FAA) and/or Graphite Furnace Atomic Absorption (GFAA). Before using the FAA, the instrument had to be properly calibrated. Using a lead standard solution of 1000 mg/L, five samples of known lead concentrations were created in a 1% nitric acid solution. The five samples included the following ppm values: 100, 50, 25, 10, and 5. The adsorption values of these five samples were then graphed versus the concentration of the solution to determine the concentrations of analyzed samples. Standard solution samples were created before every analysis, and reanalyzed after every 12 samples to ensure proper calibration of the machine. Also, before each sample was analyzed, milliQ water was run through the aspirating tube until an adsorption value of “0.000” or “0.001” was recorded by the FAA to ensure that no lead was still in the aspirating tube of the machine.

Before GFAA, samples analyzed by FAA were diluted 100 times. Samples were diluted tenfold twice using a 1% nitric acid solution. The standard curve created from the GFAA was based on the values of 10 and 50 ppb respectively. Since the GFAA was able to be run using the “auto sampler” function, the instrument automatically recalibrated itself every six samples. The possibility of error from samples analyzed using FAA and GFAA is likely because of human error in dilution calculations, as well as proper mixing of analyzed samples.

3.2. PBET/IVG

PBET analysis requires first creating a gastric solution imitating the human stomach. Using a magnetic stir plate and stir bar, 625 mg pepsin, 250 mg malic acid, 250 mg citric acid, 420 µl acetic acid, and 500 µl lactic acid were added to 1 L of distilled water. After mixing, 12 M hydrochloric acid was added to lower the pH in solution to pH 1.70-1.80. After thoroughly mixing the solution, 40 ml of gastric solution was added to four 250 ml separatory funnels (Figure 3.1). 1 g samples of remediated soil were then added to each funnel. Nitrogen gas was pumped into each funnel at a rate of 1 L/min to promote further mixing. After one hour of mixing, the nitrogen was turned off. pH was measured at the initial time and at 5, 10, and 15 minutes. If pH changed, it was adjusted using either DI water for a drop in pH, or a 5% HCL solution to maintain pH values consistent with the human stomach. Subsequently the pH was checked every fifteen minutes. At the end of the two-hour PBET analysis, one 10 ml sample was collected from each separatory funnel. These aqueous samples were analyzed by Flame Atomic Absorption (FAA) and/or Graphite Furnace Atomic Absorption (GFAA).

After PBET, the gastric solution was converted into a solution imitating the human small intestines. A 10% sodium carbonate solution was added to the solution until the pH was increased to 5.5. Next, 2.10 g of bile salts and 0.21 g of porcine pancreatin were added to each funnel. Each separatory funnel was mixed with a stirring rod until the bile salts and pancreatin were dissolved in solution. Following the addition of bile salts and pancreatin, nitrogen gas was pumped into each

separatory funnel at a rate of 1 L/min for one hour to promote further mixing and then is turned off. Throughout the two-hour analysis, pH measurements were recorded every fifteen minutes. After the two-hour period, the IVG solution in each separatory funnel was drained and filtered using No. 42 ash less filter paper. Three 10-mL samples from each separatory funnel were collected after filtering. These samples were analyzed using FAA and/or GFAA.

Figure 3.1. PBET/IVG Testing Set Up

3.3. SOIL CHARACTERISTICS

Soil was collected from the EPA repository in Bonne Terre, Mo. The soil was removed from a residential area in Bonne Terre (Bach, 2017). Removal is used on yards with x-ray fluorescence of samples indicates more than 400 ppm of lead is present. Collection was accomplished by shoveling the contaminated soil into two 5-gallon plastic buckets. Miscellaneous contents in the soil included large rocks, wood

chips, and other debris found in residential neighborhoods. As noted in the results, the soil was recorded to have moderate lead concentrations ranging from 150-250 ppm.

3.4. HOMOGENIZATION AND MIXING

Homogenization began by placing the collected soil (approximately 10 gallons) into a large mound on a tarp. The mound of soil was raked using a steel lawn rake and spread into a large ring. The soil was then shoveled back into the original large mound using a steel shovel. This process was repeated approximately five times to ensure the contaminated soil had been thoroughly mixed. After completing the mixing process, the contaminated soil was sieved through a 600 micron aluminum sieve to remove all large rocks and other debris (Figure 3.2). After sieving, the soil was placed on the tarp again, and the homogenization steps described above were repeated. After the second homogenization cycle, samples were assayed for lead using 12M HCl, and also four one-kilogram samples were collected for phosphate remediation experiments. Samples were placed in separate five gallon plastic buckets. A 5/8" hole was drilled in the bottom of each bucket to allow percolated water to drain out. Each bucket was placed on a wooden stand constructed of 2x4's. Percolated water was collected 1, 3, and 5 days after remediation and analyzed for phosphate concentrations that may have leached through the soil. Each of the one kilogram samples were treated using a different phosphate amendment.

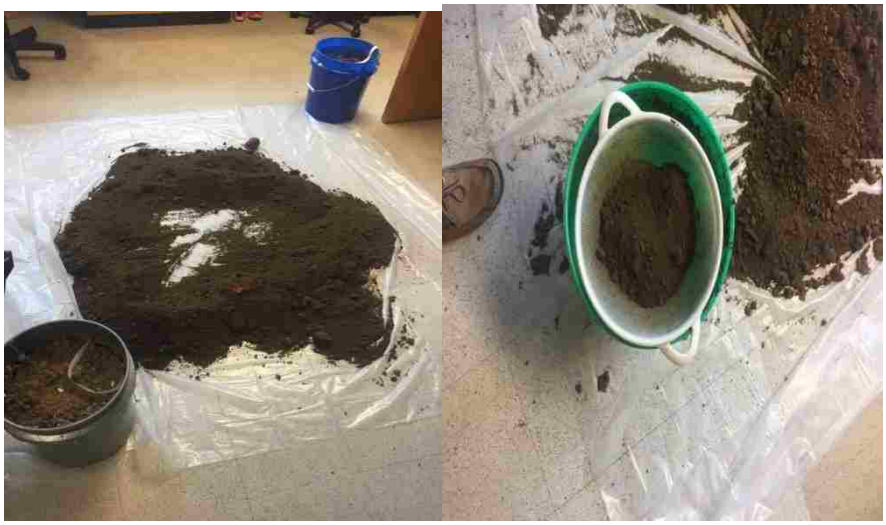


Figure 3.2. Homogenizing and Sieving Contaminated Soil

3.5. INITIAL AND FINAL PH OF THE SOIL

The pH of the soil was determined using two 10-g samples of soil. pH tests were completed using distilled water (DI) and a 5% $CaCl_2$ solution. In the pH analysis, the ratio of DI water volume to soil was 1:1. For the analysis using the $CaCl_2$ solution, the ratio of $CaCl_2$ solution volume to soil was 2:1. Tables 3.1 and 3.2 below show the initial pH readings and final pH readings after 20 weeks of remediation.

Table 3.1. Initial pH of Contaminated Soil

	pH DI water (Ratio 1:1)	pH $CaCl_2$ (Ratio 2:1)	Average pH
Bucket 1	7.70	6.25	6.98
Bucket 2	7.61	6.25	6.93

Table 3.2. pH Values after 20 Week Remediation Period

Remediation Technique	DI Water	5% $CaCl_2$ Solution	
	pH	pH	Average pH Value
None	7.64	6.26	6.95
PA	5.37	4.95	5.16
TSP	6.39	6.09	6.24
BM	7.03	6.07	6.55

3.6. INITIAL LEAD AND CALCIUM CONCENTRATIONS

To determine the initial lead concentration of the soil, 5 g samples of the bulk contaminated soil were collected. Samples were prepared using 50 mL (i.e. 10 mL / per gram) of 12 M concentrated hydrochloric acid. Samples were prepared in triplicate from each bulk soil bucket, resulting in a total of six samples. The soil and HCl mixture was stirred for 5 minutes using a magnetic stir plate and stir bar and then allowed to stand at room temperature overnight. The next day, the solution was drained and filtered through No. 50 ash less filter paper to remove solids. Separate 10-mL samples of the filtrate were analyzed using FAA. Table 3.3 below shows the initial lead concentrations.

Table 3.3. Initial Lead Concentrations

Sample	Adsorption	Soln. Conc. (µmol/L)	Soln. Conc. (mg/L)	Soil Conc. (mg/kg)
B1S1	0.127	250	52	208
B1S2	0.139	277	57	230
B1S3	0.119	233	48	193
B2S1	0.113	219	45	182
B2S2	0.126	248	51	206
B2S3	0.137	273	57	226
Average				208
STD. DEV.				17

To determine the initial calcium concentration, two 10-g samples of soil were collected from the bulk soil. Each sample was added to a large beaker containing 50 ml of 12 M concentrated HCl. The soil and HCl were mixed for 5 minutes using a magnetic stir plate and stir bar. The solution was then allowed to stand at room temperature overnight. After sitting overnight, the solution was filtered through No. 50 ash less filter paper to remove solids. Two 10-ml samples were obtained from each beaker and analyzed using the FAA. As discussed in papers by Scheckel and Mosby, phosphate (specifically PA) will attract calcium to form calcium apatite prior

to phosphate interaction with lead (Scheckel et al. 2004) (Mosby et al. 2001). Knowing the calcium concentration in soil will allow the user to calculate the amount of excess phosphate needed to react with lead and calcium in the soil to ensure a formation of lead phosphates (Table 3.4). Magnesium and strontium ions also interact with phosphates when added to soil. However, these ions were not considered throughout this research.

Table 3.4. Initial Calcium Concentrations

Sample	Adsorption	Sol. Conc. (µmol/L)	Soln. Conc. (mg/L)	Soil Conc. (mg/kg)
1 B1	1.39	6643	266	532
2 B1	1.692	8090	324	648
1 B2	1.459	6973	279	559
2 B2	1.726	8253	331	662

3.7. RAINFALL RATES/ REMEDIATION TECHNIQUES

Four treatment techniques were used to remediate the collected soil. The literature recommends remediating lead contaminated soil with 0.5% phosphate per total soil weight at each remediation site (Stillwell and Ranciato 2008). The four treatment techniques were: none, phosphoric acid, triple super phosphate, and

organic bone meal. For each treatment, 1 kg of soil was placed in a 5-gallon plastic bucket.

3.8. RAINFALL IN BONNE TERRE, MISSOURI

According to U.S Climate Data, the annual rainfall in Bonne Terre, Mo is 44.09 inches (U.S Climate Data). This represents an average rainfall of 0.3cm per day, or for each sample, 222 cm^3 (222 mL).

3.9. NO TREATMENT

One kilogram of contaminated soil was left untreated for one week. 222 mL tap water was added to the bucket and mixed by hand with a hand trowel for 3 minutes. Every 2 days an additional 444 mL of distilled water was added and the soil was re mixed for 3 minutes. The mixing process was intended to approximate extensive tilling of the soil. Water addition and mixing occurred three times over a one week period. Each addition of water was separated by 48 hours. Throughout the treatment process, the sample was left at room temperature (approximately 23˚C).

3.10. PHOSPHORIC ACID (PA)

One kilogram of contaminated soil was treated with phosphoric acid (PA). 5.0 mL of phosphoric acid (85% phosphate) was added to the bucket resulting in 0.5% phosphate by total soil weight (1 kg). 0.5% of phosphoric acid is the recommended quantity to immobilize lead and calcium particles present in the soil. PA was applied

to the soil as a pure HPO_4 solution. The addition of phosphoric acid was expected to result in the formation of pyromorphite and apatite. Equal amounts of distilled water and mixing times occurred for the sample remediated by phosphoric acid as the soil sample with no treatment method.

3.11. TRIPLE SUPER PHOSPHATE (TSP)

One-kilogram of contaminated soil was treated with triple super phosphate. 9.08 g of triple super phosphate was added to the soil resulting in a 0.5% phosphate ratio to total soil weight. 0.5% of triple super phosphate is the recommended quantity to immobilize lead and calcium particles present in the soil. The TSP was applied to the soil as dense rock granules. After adding the triple super phosphate, soil was mixed for 3 minutes by hand using a hand trowel. Equal amounts of tap water and mixing times occurred for the sample remediated by super triple phosphate as the soil sample with no treatment method.

3.12. ORGANIC BONE MEAL (BM)

One kilogram of contaminated soil sample was treated with organic bone meal, from fish bones. 13.62 g of organic bone meal was added to the soil resulting in a ratio of 0.5% phosphate to total soil weight. 0.5% of organic bone meal is the recommended quantity to immobilize lead and calcium particles present in the soil. The bone meal was applied to the soil as a powder. The soil and organic bone meal was mixed by hand for approximately 3 minutes. Equal amounts of tap water and

mixing times occurred for the sample remediated with organic bone meal as the soil sample with no treatment method.

3.13. PHOSPHATE LEACHING ANALYSIS

Percolated simulated samples were collected from each soil-containing bucket on the first, third, and fifth days after phosphate was added to the soil. To test the percolated water for possible phosphate, a phosphate reagent was used. The reagent contained: 50 ml sulfuric acid (H_2SO_4), 5 ml potassium antimonyl tartrate, 15 ml ammonium molybdate, and 30 ml ascorbic acid. These materials were all combined in a 250-mL beaker using a magnetic stir plate and stir bar. Percolated water and the phosphate reagent were combined in a 15-mL conical test vial using 1 mL percolated rainwater and 8 mL reagent. Samples were allowed to mix for 15 minutes before being analyzed using a spectrophotometer.

3.14. IN SILICO ANALYSIS

Before adding phosphate to soil, several PBET/IVG experiments were performed to determine the bioaccessibility of lead using synthetically prepared and precipitated chloropyromorphite.

Synthetic chloropyromorphite was precipitated using 500 mL DI water, 8.00 g lead nitrate ($Pb(NO_3)_2$) , 4.30 g sodium phosphate ($Na_3(PO)_4$), and 1.68 g sodium chloride mixed together using a magnetic stir plate and stir bar. These masses of these compounds have a molar ratio of 5:3:1 (Labare, Butkus, Riegner, Schommer, &

Atkinson, 2004). After adding the compounds to deionized water, a pH value was measured as 1.53. Sodium hydroxide was added to the solution to increase the pH to 7.20. At pH 7.20, pyromorphite precipitated almost instantaneously. The solids were then removed from solution and filtered using No. 50 ash less filter paper and dried at 120°C for 12 hours. Dry solids were then divided into 1 g samples and analyzed using PBET/ IVG.

In another experiment, synthetic chloropyromorphite was precipitated in Missouri River Bottom sand by adding 50 g of silicate based (SiO_3) sterile sand (sterilized with HNO_3), 8.00 g lead nitrate ($Pb(NO_3)_2$), 4.30 g sodium phosphate ($Na_3(PO)_4$), 1.68 g sodium chloride (NaCl) and 500 ml DI water in a 1L beaker. The mixture was stirred for 5 minutes using a magnetic stir plate with stir bar. The initial pH of the mixture was 1.46. Sodium hydroxide was added to the solution to increase the pH to 7.22. The solids were then removed from solution and filtered using No. 50 ash less filter paper and dried at 120°C for 12 hours. The dry solids were then divided into 1 g samples and analyzed using PBET/IVG.

3.15. DENSITY SEPARATION OF HEAVY METALS

Pyromorphite might not observably form in soil for several reasons. One reason is that available lead may be interacting with various compounds in forms such as galena, lead oxide, or lead hydroxide. A density separation technique was used to isolate possible lead compounds in the contaminated soil used by gravity separation. A dense solution (density greater than 2.65 g/ cm^3) was created which

allowed lead dense particles to migrate to the bottom of a test vial. Less dense particles remained at the surface of the solution. The solution was created by mixing 22.5 mL of DI water with 102.5 g of sodium metatungstate using an electric stir plate and stir bar until the sodium metatungstate was completely dissolved. After dissolving the sodium metatungstate, a small piece of quartz (density 2.65 g/ cm^3) was used as a standard calibration for the solution. Observing the floating quartz crystal, the density of the solution was determined to be > 2.65 g/cm^3, and adequate to perform density separation. Two, 1-gram samples of lead contaminated soil with no phosphate treatment were collected and placed into separate vials containing 5 mL of the dense solution. In addition, two samples (1 g each) of each of the three treatments of contaminated soil (phosphoric acid, triple super phosphate, and organic bone meal) after 16 weeks of remediation were collected and placed into separate vials containing 5 mL of the dense solution. Each sample was centrifuged for approximately 30 minutes, forcing the dense particles to the bottom of the vial. The samples were then left undisturbed for 24 hours. After 24 hours, dense particles at the bottom of the sample were extracted using a sludge pipette. The exterior of the pipette was rinsed with DI water after extracting the particles to remove less dense particles present on the exterior of the pipette. The pipette was then drained onto No.42 ash less filter paper. The solids left on each filter paper were collected and placed in separate vials. These samples were analyzed with X-Ray Diffraction (XRD) to determine what compounds were present. XRD did not reveal which lead compounds were in the soil, however, it did show lead particles bound to other

materials in the soil (quartz and dolomite). The peaks recorded during XRD were not high enough to verify that lead compounds were detectable. Scanning Electron Microscopy with Energy Dispersive Spectroscopy (SEM-EDS) was used next to determine the lead compounds in each sample. The SEM showed various lead compounds bound to other elements. Lead oxide was confirmed in a compound also containing calcium oxide. This is consistent with literature reports that lead and calcium interaction is common in a heterogeneous soil sample. One sample from soil remediated using triple super phosphate showed lead phosphate formation under SEM. The species in this sample was a lead phosphate compound also including calcium phosphate. Although large amounts of lead were not detected in these analyses, trace amounts of lead oxide, lead phosphate, and lead sulfide were all confirmed using SEM.

3.16. $PB_5(PO_4)_3CL$ TITRATIONS

Titrations were used to analyze the correlation between detectable aqueous lead concentrations and varying pH values of solution in the presence of pyromorphite. A solution containing, 8.00 g of lead nitrate ($Pb(NO_3)_2$), 4.30 g sodium phosphate ($Na_3(PO)_4$), and 1.60 g sodium chloride (NaCl) was added to 500 mL DI water using a magnetic stir plate and stir bar to precipitate synthetic chloropyromorphite. Sodium hydroxide (NaOH) was added to increase the solution pH to 7.0, creating an almost instantaneous precipitation of synthetic chloropyromorphite. The chloropyromorphite in solution was left on a magnetic stir

plate at 25°C where the pH of solution was measured every 15 minutes. Using 12 M HCl, a titration was performed over a four-hour period as pH was adjusted from 7.0 to 1.5. One 10-mL sample was collected every 15 minutes before reducing the pH of solution by 0.50. When the pH of the solution reached 4.00, the solution was then reduced by 0.25 every 15 minutes until a final pH of 1.50 was reached. Each sample was filtered using individual 0.2-micron nylon syringe filters. After completing the first titration from pH 7.0 to 1.5, a solution of synthetic chloropyromorphite was titrated from pH 1.5 to 7.0. Again, one 10-mL sample was collected before increasing the pH of solution by 0.25 until the pH of solution reached 4.00. At a pH of 4.00, the solution pH was increased 0.50 after each 15-minute time interval to a final pH of 7.00. Samples were filtered using individual use 0.2 micron nylon syringe filters in a 60 mL syringe. Samples were analyzed using FAA to show the impact of varying pH on the solubility of lead when chloropyromorphite is present.

A second set of titrations were performed with a 30-minute equilibrium period. In the first titration using a 15-minute equilibrium period, at pH > 3.50, lead concentrations were measured below detection limit (BDL < 15 mg/kg); it was pondered that lead dissolution might be slow during the titration, so more time was used. During the titration with a 30-minute equilibrium period, chloropyromorphite was titrated from pH 1.50 to 3.75 adjusting the pH by 0.25 during each time interval, (obtaining one 10-mL sample every 30 minutes with varying pH) then continuously mixed at 25°C for 1.50 hours. Two samples, 10 mL each, were measured from the

solution at pH 3.75. The first sample was collected after the initial 30-minute period, while the second sample was collected after a 1.50 hour period. The initial titration began from pH 7.0 to 1.5, and the second titration was conducted from pH 1.5 to 7.0, using the same pH changes as described above. Each sample was filtered using individual 0.2-micron syringe filters. Samples were analyzed using FAA.

4. RESULTS/DISCUSSION

4.1. TITRATIONS

The titration results showed that at pH < 2.50, lead concentrations were detected using FAA. However, when pH > 2.50 lead was BDL. The results showed a direct correlation between formation and dissolution of pyromorphite when pH was greater or less than 3.00. Figure 4.1 below is a comparison between both the 15 minute and 30 minute equilibrium period titration curves.

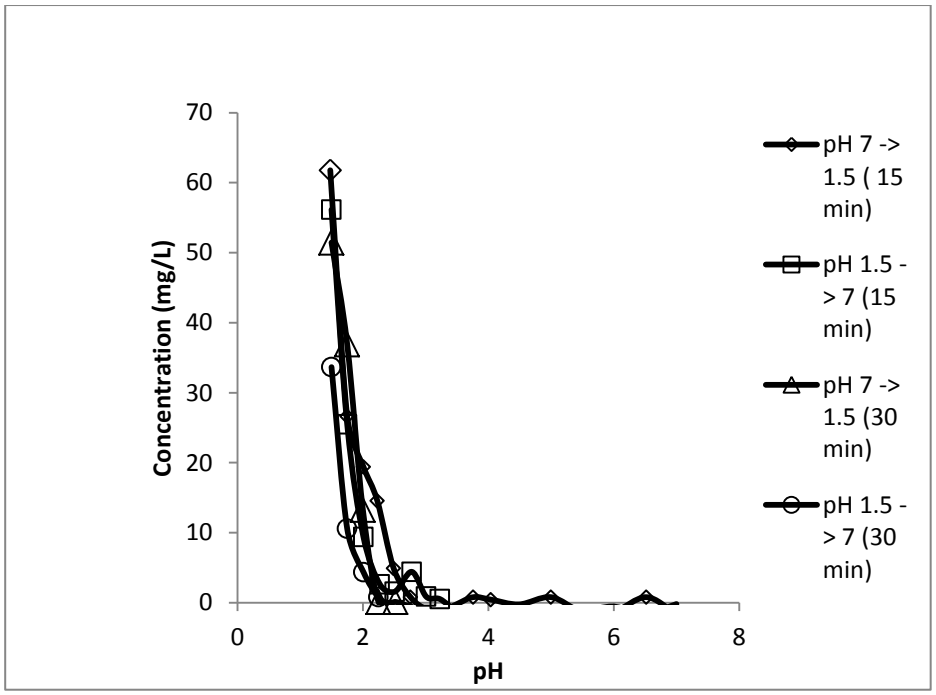


Figure 4.1. pH vs Lead Concentration of Dissolved Chloropyromorphite

4.2. PHREEQC VS TITRATION RESULTS

Results from the titration curve vs the concentrations calculated by PHREEQC show a difference in lead concentrations as pH is increased. PHREEQC calculated that a detectable lead concentration would remain in solution until pH 4.0, while the titration results showed lead concentrations BDL when pH > 3.0. Results here show that a Ksp value of $10^{-84.4}$ is not the exact Ksp of chloropyromorphite. Rather a larger Ksp such as $10^{-84.3}$ represents the data obtained from the titration experiments. These results agree with the results that Xie & Giammar (2007) reported, that the Ksp of chloropyromorphite is higher than the reported value of $10^{-84.4}$. Figure 4.2 as well as Table 4.1 on the next pages show the lead concentrations calculated by PHREEQC vs the lead concentrations reported from the titrations.

4.3. CONTROL TESTS

Control Samples of pyromorphite analyzed by FAA after PBET/IVG showed a large decrease in the bioaccessibility of lead as the pH of solution increased. At a pH < 1.85 during PBET, synthetically precipitated pyromorphite was dissolved to some degree, yielding detectable lead concentrations. As the solution pH was increased to 6.20-6.30 during IVG, pyromorphite re precipitated in solution, greatly decreasing the bioaccessibility of lead. These results correlate with the titration experiments presented earlier. As the pH increased, the detectable lead concentrations decrease, thus signifying a decrease in bioaccessibility of lead after IVG. Figures 4.3 and 4.4 (pgs. 44-45) show the detectable lead concentrations reported after PBET/IVG

analysis from FAA with a 95% confidence interval. The bioaccessibility of lead from these experiments decreased an average of 89.9%.

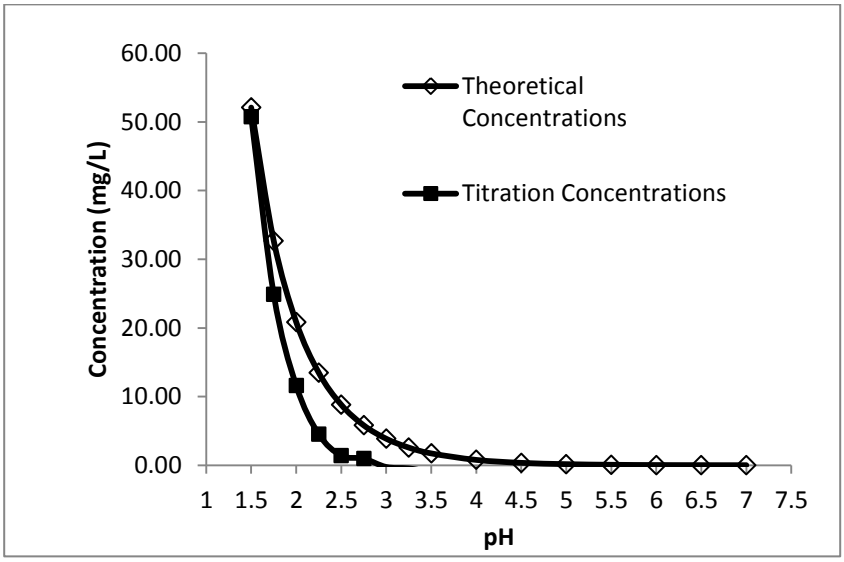


Figure 4.2. Theoretical vs Recorded Concentrations of Lead at Ksp = $10^{-84.43}$

Table 4.1. Reported Ksp Values vs Calculated Ksp Values

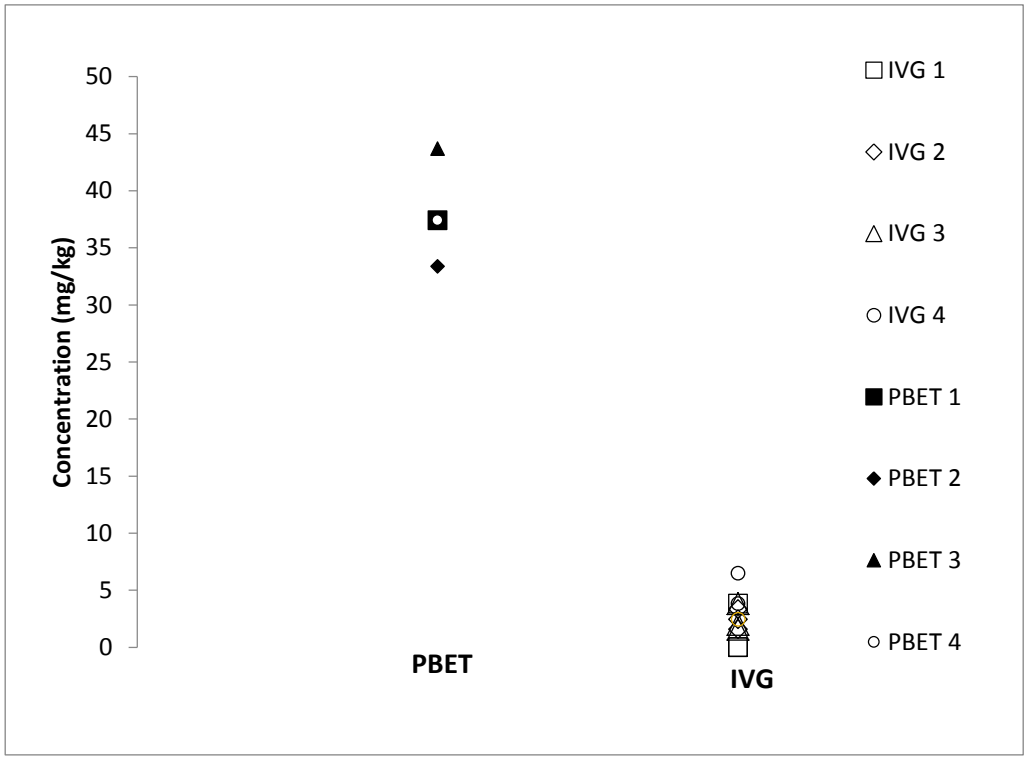
Phosphate Source	pH	Literature Ksp	PHREEQC ksp
Ngiaru (1973)	2.21-2.29	$10^{-84.4}$	
Scheckel H_3PO_4	0-2.12	$10^{-18.69}$	$10^{-33.30}$
Scheckel $H_2PO_4^-$	2.12-7.21	$10^{-25.05}$	$10^{-53.63}$
Scheckel HPO_4^{2-}	7.21-12.38	$10^{-46.9}$	$10^{-79.35}$
Scheckel PO_4^{3-}	12.38-14	$10^{-84.4}$	$10^{-84.4}$
Topolska (2016)	2.00	$10^{-79.6}$	
Xie & Giammar (2007)	2.00-7.00	$10^{-80.4}$	

4.4. 1 WEEK REMEDIATION

Results one week after adding phosphate were inconclusive. As shown in Figure 4.5 and 4.6 (pgs. 46-47) below, the bioaccessibility of lead did not decrease a significant amount during that week. Samples of soil treated with phosphoric acid had the largest average decrease in lead bioaccessibility by 68%, while organic bone meal showed an average decrease of 6% and triple super phosphate did not show a decrease in bioaccessibility. As expected, phosphoric acid resulted in the most rapid formation of pyromorphite over one week. However, it was not expected that phosphate from organic bone meal would reduce bioaccessibility more than phosphate from triple super phosphate. The coarse, dense granules of triple super phosphate may have impacted the effectiveness of breaking down the mineral and allowing phosphate interaction to occur with lead particles. Also, triple super phosphate, because of the large particle size, likely had the smallest amount of surface area exposure in the soil sample, which could have limited the precipitation rate of suspected pyromorphite in the soil. Results from FAA on solution samples with no treatment showed detectable lead through both PBET and IVG, showing no decrease in lead bioaccessibility.

Samples were collected from each soil one week after phosphate addition and analyzed for pyromorphite formation using SEM-EDS. Two additional samples, precipitated synthetic chloropyromorphite and synthetic chloropyromorphite precipitated in clean sand were collected as well. SEM analysis confirmed pyromorphite was present in each sample of synthetic chloropyromorphite (Figure

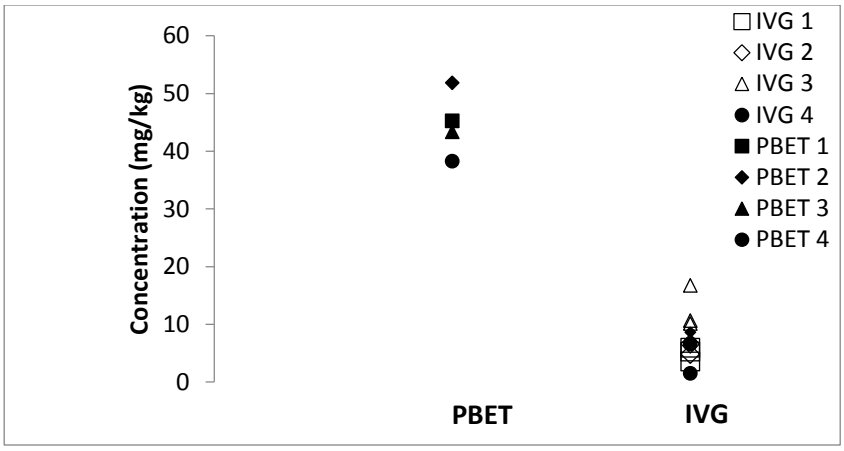
4.7). Synthetic samples had an empirical formula of Pb5(PO4)3Cl. Soil samples showed only a small amount of lead, based on the six samples analyzed. There was no phosphate detected in any of the samples. These samples indicate the interaction between lead and phosphate was minimal during the initial one-week remediation period. Figure 4.8 does show one small piece of lead discovered during analysis of one of the soil samples.



95% Confidence Interval

PBET (mg/kg)	38 +/- 5
IVG(mg/kg)	3 +/- 1

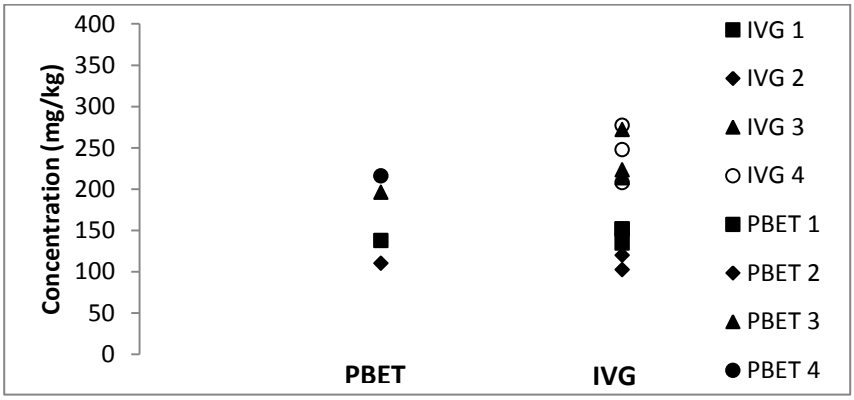
Figure 4.3. Synthetic Pyromorphite PBET/IVG Results



95% Confidence Interval

PBET (mg/kg)	45 +/- 7
IVG	7 +/- 3

Figure 4.4. Pyromorphite in Clean Sand PBET/IVG Results



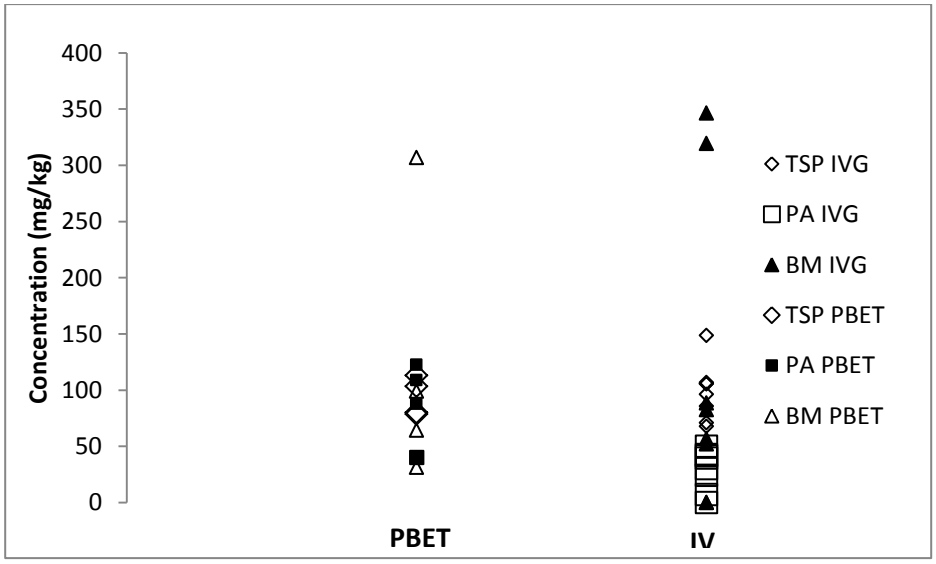
95% Confidence Interval

PBET (mg/kg)	165 +/- 59
IVG (mg/kg)	187 +/- 36

Figure 4.5. PBET/IVG Results, 1 Week, No Treatment

4.5. PHOSPHATE LEACHING ANALYSIS

Results from spectrophotometry of phosphate leachate were inconclusive. It is believed that all samples analyzed contained phosphate concentrations below the detection limit of the spectrophotometer (BDL = 0.3 mg/L). However, due to instrument malfunctions and problems creating standard solution curves, these results cannot be confirmed. Further research and experiments using the spectrophotometer must be conducted to determine a more accurate result of the phosphate concentrations leaching from the soil (Major 2017).



95% Confidence Interval

PBET (mg/kg)	103 +/- 42
IVG (mg/kg)	82 +/- 36

Figure 4.6. PBET/IVG Results 1 Week

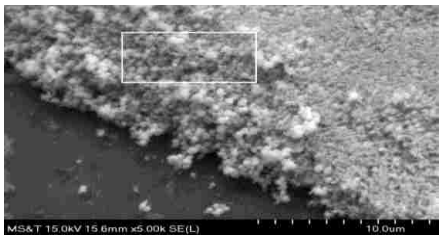


Figure 4.7. Formation of Synthetic Chloropyromorphite from SEM/EDS

Figure 4.8. Lead after 1 Week Remediation No Treatment

4.6. 4 WEEK REMEDIATION

The results from PBET/IVG at four weeks were unexpected. Each sample analyzed using FAA was below the method detection limit. PBET samples were then analyzed using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). ICP-OES showed very low detectable lead concentrations from the analyzed PBET samples. The sample concentrations ranged from 1.05 to 42.8 mg/kg. With such a variation in the data, the sample yielding the highest detectable lead concentration may have had a microscopic lead particle pass through the filter and end up in

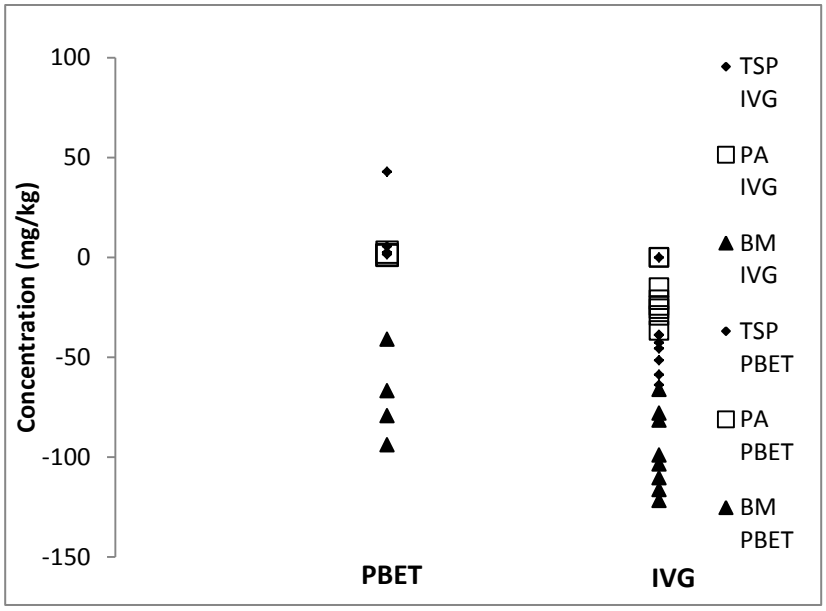
solution before analysis. The results from the four PBET/IVG analysis yielded inconclusive results from FAA and ICP-OES.

Given the inconclusive findings using FAA, GFAA was used. GFAA also showed very low concentrations of lead in each sample. The range of concentrations for the analyzed PBET solutions using GFAA ranged from 0.12 to 12.08 µg/L, while the concentrations of each IVG sample ranged from 0-0.49 µg/L. The largest decrease in bioaccessibility were observed for both phosphoric acid and organic bone meal, 100% decrease, while triple super phosphate displayed a decrease in lead bioaccessibility of 96%. However, these results are not definitive. The samples analyzed using GFAA had been placed in the refrigerator for approximately four months before analysis. Unbeknownst to the author, during this time period, the bile salts in each IVG sample had congealed and created a thick gel in each vial. It is suspected that a large amount of detectable lead was trapped in that gel. The gel could not be dissolved, as that would potentially cause precipitated lead phosphates to dissolve. Even though a decrease in lead bioaccessibility was calculated, the lead concentrations were very low in all cases. Figure 4.9 shows the PBET/IVG lead concentrations reported using FAA, while Figure 4.10 shows the PBET/IVG lead concentrations reported using GFAA.

4.7. 16 WEEK REMEDIATION

At 16 weeks, PBET/ IVG experiments showed an increase in lead bioaccessibility. Lead concentrations recorded from the FAA showed an increase in

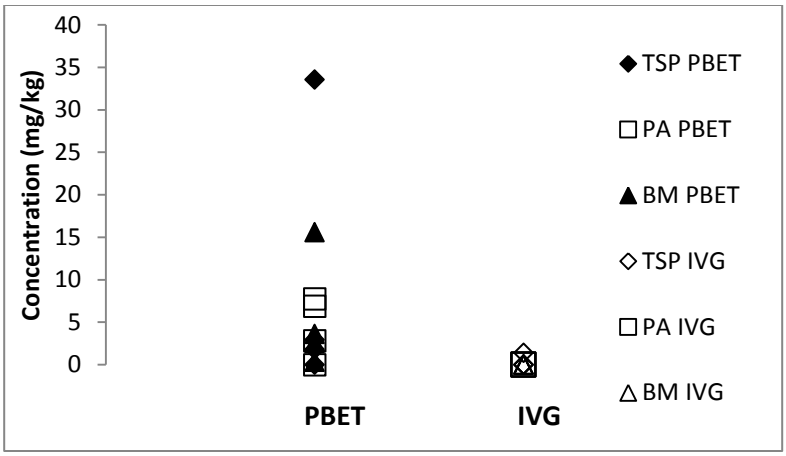
detectable lead during IVG analysis as compared to PBET. These samples were placed in the refrigerator for two weeks while waiting for acetylene gas for the FAA. The samples contained a large amount of congealed material when removed from the refrigerator. To separate the solution and get a large amount of "usable" solution for analysis, samples were heated in a water bath at 37˚C for 30 minutes. After warming the samples, the separated solution was extracted and analyzed. The effects of warming the solution could have impacted these results.



95% Confidence Interval

PBET (mg/kg)	7 +/- 32
IVG (mg/kg)	-53 +/- 15

Figure 4.9. 4 Week PBET/IVG FAA Results



95% Confidence Interval

PBET (mg/kg)	7 +/- 6
IVG (mg/kg)	0.10 +/- 0

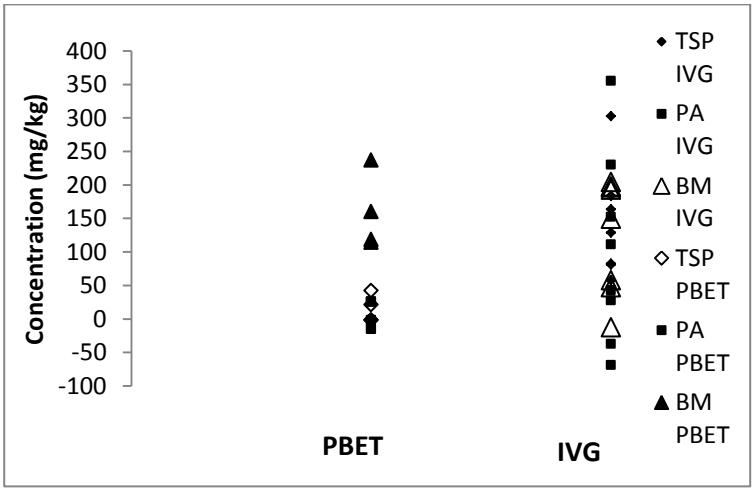
Figure 4.10. Four Week PBET/IVG GFAA Results

Assuming that bile salts were contained in the gel, which did dissolve, the pH of solution could have possibly become acidic after warming the sample. If the IVG sample was below pH 3.0, pyromorphite formed during IVG would not be insoluble and show a detectable lead concentration would be observed when analyzed with the FAA. Because of the additional manipulations to these samples, test results are not representative of the other PBET/IVG results of this thesis. The information presented below in Figure 4.11 (pg. 53) is not considered relevant to the overall results of this research.

4.8. 20 WEEK REMEDIATION

Results from PBET/IVG experiments after 20 weeks of remediation showed a decrease in the bioaccessibility of lead after FAA and GFAA analysis. After PBET, the FAA detected a higher lead concentration from each sample than the detectable lead concentrations recorded after IVG. FAA and GFAA were both used in this analysis because results from the FAA were all near the detection limit of the instrument. GFAA results showed a decrease in the bioaccessibility of lead from phosphoric acid remediation of 88%, while triple super phosphate and organic bone meal remediation showed a decrease in the bioaccessibility of lead by 80 and 70% respectively. These results indicate there was lead phosphate formation in the soil. At low pH (PBET), lead phosphate compounds would be partially dissolved, thus giving a detectable lead concentration. As the pH of the solution was increased (IVG) a large percentage of lead and phosphate should re-precipitate to a lead phosphate compound, reducing the bioaccessibility of lead. It must also be noted lead may have been present in other insoluble lead species during PBET, which could further indicate detectable lead concentrations after PBET when analyzed using GFAA. Detectable lead concentrations may have also been reported using GFAA because of the various lead compounds within the soil. As confirmed with SEM, trace amounts of lead sulfide, lead oxide and lead phosphates were found to be in the soil. At high pH, lead particles previously bound in lead oxides would have the potential to re precipitate with the available oxide or carbonates, as well as precipitate into lead carbonates. If lead was present in excess within a soil sample, it is likely that there

was not enough phosphate in the sample to interact with the lead in solution after PBET.

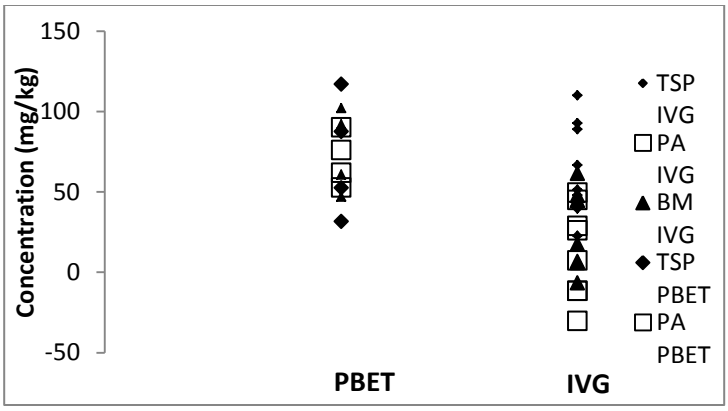


95% Confidence Interval

PBET (mg/kg)	61 +/- 52
IVG (mg/kg)	121 +/- 43

Figure 4.11. 16 Week PBET/IVG Results

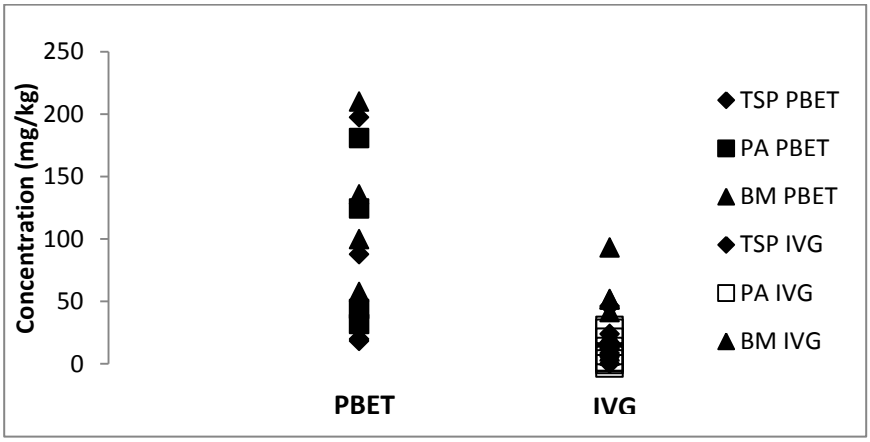
The FAA and GFAA results after PBET/IVG at a 20 week remediation period are presented below in Figures 4.12 and 4.13 respectively, each with a 95% confidence interval. The values from the FAA and GFAA were not identical because before GFAA, each sample had to be diluted approximately 100 times. Human error in dilution calculations, as well as mixing may have skewed the results between FAA and GFAA. However, there was a definitive decrease in the bioaccessibility of lead from PBET and IVG.



95% Confidence Interval

PBET (mg/kg)	73 +/- 15
IVG (mg/kg)	35 +/- 14

Figure 4.12. 20 Week PBET/IVG FAA Results



95% Confidence Interval

PBET (mg/kg)	100 +/- 42
IVG (mg/kg)	21 +/- 9

Figure 4.13. 20 Week PBET/IVG GFAA Results

5. CONCLUSION

Decreasing the bioaccessibility of lead using phosphate amendments is a remediation technique among environmental engineers. This inexpensive technique can immobilize lead below the soil surface, preventing exposure to humans and other wildlife. However, there are many considerations when determining the overall effectiveness of this remediation strategy.

The results of this research showed an average decrease in the bioaccessibility of lead by 79.7% 20 weeks after phosphate addition. These results are consistent with the results that Mosby and Scheckel reported after analyzing the bioaccessibility of lead using quail. Their results showed an average decrease in bioaccessibility of 33 to 63% depending on the soil analyzed (Mosby et al., 2016). Soil collected by Mosby and Scheckel was remediated using either phosphoric acid or triple super phosphate. Overall, the experimental results of Scheckel and Mosby are consistent with those reported here. At low pH values (1.7-2.0) both experiments detected higher lead concentrations, while at pH > 3.0, low concentrations of lead were reported. Considering humans and quail have similar pH values in their stomachs, these results indicate a decrease in lead bioaccessibility. The main difference between the research performed in this thesis and the research of Mosby and Scheckel is that the soil in Mosby and Scheckel's research had been in remediation for approximately 7 years. Soils analyzed in this research had been remediated for 20 weeks. Other research has shown that in some cases that pyromorphite has a mean residence time of approximately 13 years before complete

formation occurs (Ruby et al., 1994). The longer phosphate has to interact with lead particles in the soil, the more likely pyromorphite is to form, thus decreasing the bioaccessibility of lead. The complexity of the soil being remediated must be considered as an additional variable. Scheckel and Mosby reported the presence of various lead species in the soil they studied. With soil being so diverse, many different compounds and elements are bound together, which could limit the interaction between lead and phosphate. As discovered through SEM-EDS and XRD on samples collected in this research, lead oxide, lead sulfide, and lead dioxide compounds in the soil were bound to quartz and dolomite crystals. When lead particles are bound to other minerals, the interaction between phosphate and lead is hindered. Scheckel and Mosby also researched the bioaccessibility of lead by feeding swine food that had been grown in lead contaminated soil remediated with phosphate. The results of this experiment varied greatly and were attributed to heterogeneous soil and the uncertainty that phosphate had been applied evenly to the soil. Again, the composition of soil can lead to multiple concerns when trying to remediate with phosphate. Application of triple super phosphate may not be the most effective strategy of remediation because triple super phosphate is a solid and takes longer than one week to break down and release phosphate. Furthermore, with ample amounts of calcium being present in the soil collected for this research, it is possible that phosphate reacted with calcium and formed calcium apatite. Certainly in one sample of the soil treated in this study, SEM showed a phosphate compound containing both lead and calcium. The in vivo tests performed by Mosby

and Scheckel and in vitro tests performed during this research show a direct correlation between phosphate amendments and a decrease in lead bioaccessibility.

Stomach pH is another factor that must be considered in the effectiveness of phosphate remediation to make lead non-available. Most children play outside in a fasted stomach state (pH 1.5-1.8); from the results reported above, it would be confirmed at this stomach pH that pyromorphite dissolution is almost certain upon ingestion of remediated soil particles. It has been determined that dissolved lead can pass through the stomach lining. At a low stomach pH, lead has a greater chance of passing through the stomach lining because pyromorphite has been dissolved into lead and phosphate ions. This risk is limited when the stomach pH is increased because as the results and titration Tables show, at pH > 3.0, pyromorphite reduces soluble lead to a non-detectable level. This was not the case from the field studies analyzed. This is likely contributed to the diversity of the soil analyzed, and that different elements such as calcium had the opportunity to interact with phosphate before the phosphate reacted with lead. Further research must be conducted to determine the adsorption rate of lead through the stomach lining and how long it would take to become poisoned from pyromorphite dissolving in the stomach.

Phosphate amendments have resulted in decreasing the bioaccessibility of lead throughout this research. As shown in the first week of remediation, phosphoric acid-treated soil had the greatest decrease in bioaccessibility. Soil treated with organic bone meal had a much lower decrease in the bioaccessibility of lead and triple super phosphate did not show a decrease of lead bioaccessibility after one

week. There may have been multiple reasons that triple super phosphate did not show a decrease in bioaccessibility after one week of remediation. With triple super phosphate being in the form of dense granules, the granules may not have broken down and dissolved into the soil during the first week. This would have limited the potential for the release of phosphate into the soil and eliminated the possibility of lead phosphate formation. Also, the triple super phosphate used in this research contacted only a small portion of the soil surface area. This raises the concern that no lead was near the phosphate, which would eliminate the chance for interaction between lead and phosphate at this remediation site (bucket). However, it can be concluded that as triple super phosphate broke down over a 20-week remediation period, lead was able to interact with available phosphate and form lead phosphate compounds, reducing the bioaccessibility of lead at this remediation site by 80.2%. This is likely attributed to the hand mixing that took place periodically throughout the remediation process. Applying organic bone meal as a remediation technique resulted in the lowest decrease in lead bioaccessibility. In the first week of remediation, soil treated with organic bone meal showed a small decrease in lead bioaccessibility as compared to phosphoric acid. The small decrease in bioaccessibility could be attributed to the idea that organic bone meal was not evenly distributed throughout the remediation site. However, since organic bone meal was applied as a powdered substance, the decay rate of dissolution was likely faster than triple super phosphate which created an environment more susceptible to form pyromorphite. Organic bone meal showed the lowest overall decrease in lead

bioaccessibility after twenty weeks, probably because of the high calcium content in the initial composition of organic bone meal; as phosphate is released it is likely to create calcium apatite before lead phosphates because calcium and phosphate ions were in close proximity to one another. Finally, phosphoric acid showed the highest decrease in lead bioaccessibility at 88.1%. Being applied in a liquid form, PA had the greatest ability to cover the entire volume of soil in the least amount of time. Because the phosphoric acid is pure, the phosphate did not have to be released from a compound and thus could interact immediately with lead compounds in soil. There was a concern however that soil pH would be greatly reduced after the application of phosphoric acid. This concern was eliminated by comparing the pH of the initial soil to the final soil pH after 20 weeks of remediation. The final phosphoric acid-treated soil pH was reported to be 5.16. Even though soil pH dropped a significant amount, the risk of dissolution is minimal because dissolution of pyromorphite does not occur at a pH > 3.0. This research has shown a 1% PA solution would likely form lead phosphates on lead contaminated soil sites in the shortest amount of time.

This research shows a decrease in lead bioaccessibility is likely over a remediation period of 20 weeks. Soil diversity, as well as evenly distributed application, must be considered when beginning to remediate lead contaminated soil with phosphate amendments. It is safe to say that this practice is an effective way to immobilize lead in the soil, but further research must be conducted to determine the overall health effects pyromorphite ingestion can have on the human body once the mineral enters the stomach.

APPENDIX A.

POSSIBLE SOURCES OF ERROR

There were many possible sources of error from the experiments conducted. A large source of error could have been that no phosphate or lead interacted throughout PBET and IVG. As mentioned earlier in the report, hand mixing and the "break down" time of different phosphate amendments could potentially limit the interaction between lead and phosphate particles. The soil analyzed was not homogenous and there could have been large lead concentrations in one area of the soil, and minimal lead amounts in other areas. Another possible source of error was the scale of this research. The literature reported remediation sites ranging from several cubic yards, to as large as five acres. Remediating one kilogram samples of soil greatly decreased the chance that a large amount of lead would be present to interact with the phosphate when applied to the soil. As shown in the initial soil conditions, with low to moderate concentrations of lead observed, the opportunity of lead phosphate formation was greatly reduced by the scale of this project.

Another potential source of error could have occurred by using the same separatory funnels repeatedly for PBET/ IVG experiments. This was eliminated by placing each funnel in a 5% HCL solution acid bath after each use. First, the funnels were emptied and rinsed three times using tap water. Next, each bottle was cleaned using AJAX and a wire brush. The funnels were then rinsed an additional three times with tap water. Funnels were then rinsed using DI water and submerged in a 5% HCL solution. Each funnel soaked for 24 hours, then rinsed three times with tap water and three additional times using DI water to eliminate the risk of cross contaminated.

Funnels were allowed to air dry after rinsing with DI water before the next PBET/IVG experiment.

The next possible source of error could have occurred during FAA and GFAA analysis. During FAA analysis, before each sample was analyzed, milliQ water or a 1% HNO_3 solution was used to flush the aspirating tube of the FAA. While these solutions were aspirated through FAA, three readings were measured. The instrument had to read an adsorption value of 0.000 or 0.001 (BDL) before the next sample could be analyzed. This ensured no leftover particulates were still in the aspirating tube from the previous sample. The FAA was also re-calibrated after every 12 samples analyzed to ensure accurate results. During GFAA, the auto sampler feature of the machine ensured that calibration curves were accurate with the varying concentrations reported. The graphite furnace in the GFAA had recently been replaced, thus eliminating the risk of possible error from the furnace being dirty. However, 3 samples analyzed were recorded at a higher concentration than the standard calibration curve of the machine, leading to some error of calculation within the machine.

A major source of error throughout this research was that PBET/IVG samples were placed in the refrigerator after analysis. Previously, samples left at room temperature had green “mold” growing in the test vial after three days of being left at room temperature. As the sample cooled to a temperature around 4˚C in the refrigerator, bile salts present in each sample congealed and created a thick gel at the bottom of the vial. When these samples were filtered and analyzed, lead

concentrations were very low or BDL. The congealed bile salts may have trapped lead and phosphate particles within the mixture, thus showing very low concentrations from GFAA. After the 20 week remediation PBET/IVG analysis, it was decided to leave the samples at room temperature (25˚C) and acidify each sample to minimize the risk of growth within each test vial. Samples were analyzed with FAA and GFAA within three days of PBET/IVG experiments. Finally, other potential sources of error could be contributed to the use of expired chemicals and compounds, human error on mathematical calculations before applying these values to a certain experiment, and instrument malfunctions throughout the research.

APPENDIX B.

EXCESS LEAD CONCENTRATIONS IN SOLUTION

Table AB.1. Lead Concentrations of 30 Minute Equilibrium Titration vs Theoretical Lead Concentrations in PHREEQC

30 Min Equil.				
pH	Moles Pb (PHREEQC)	Conc. PHREEQC (mg/L)	Conc. 30 Min Titration (mg/L)	Soluble Lead in Soln. (mg/L)
1.50	2.51E-04	52.09	51.45	0.64
1.75	1.58E-04	32.70	36.91	-4.21
2.00	1.01E-04	20.84	13.28	7.56
2.25	6.51E-05	13.48	0.11	13.37
2.50	4.25E-05	8.81	0.11	8.71
2.75	2.81E-05	5.81	-0.80	5.81
3.00	1.86E-05	3.86	-0.80	3.86
3.25	1.24E-05	2.58	-1.26	2.58
3.50	8.34E-06	1.73	-1.26	1.73
4.00	3.77E-06	0.78	-1.26	0.78
4.50	1.71E-06	0.35	-1.48	0.35
5.00	7.75E-07	0.16	-1.71	0.16
5.50	3.51E-07	0.07	-1.71	0.07
6.00	1.61E-07	0.03	-1.71	0.03
6.50	7.97E-08	0.02	-1.71	0.02
7.00	4.75E-08	0.01	-1.71	0.01

*Note: At pH = 2.50 method detection limit was reached. All samples analyzed when pH > 2.50 were BDL.

Table AB.2. Lead Concentrations of 30 Minute Equilibrium Titration vs Theoretical Lead Concentrations in PHREEQC

30 Min Equilibrium				
pH	Moles Pb (PHREEQC)	Conc. PHREEQC (mg/L)	Conc. 30 Min Titration (mg/L)	Independent Lead in Solution (mg/L)
1.50	2.51E-04	52.09	33.65	18.44
1.75	1.58E-04	32.70	10.57	22.12
2.00	1.01E-04	20.84	4.36	16.49
2.25	6.51E-05	13.48	0.81	12.67
2.50	4.25E-05	8.81	-0.97	9.78
2.75	2.81E-05	5.81	-0.52	6.34
3.00	1.86E-05	3.86	-1.86	5.72
3.25	1.24E-05	2.58	-1.86	4.43
3.50	8.34E-06	1.73	-1.86	3.58
4.00	3.77E-06	0.78	-1.41	2.19
4.50	1.71E-06	0.35	-1.19	1.54
5.00	7.75E-07	0.16	-0.97	1.13
5.50	3.51E-07	0.07	-1.86	1.93
6.00	1.61E-07	0.03	-1.86	1.89
6.50	7.97E-08	0.02	-1.86	1.87
7.00	4.75E-08	0.01	-1.86	1.86

*Note: when pH = 2.50 method detection limit was reached. All samples analyzed when pH > 2.50 were BDL.

Table AB.3. Lead Concentrations of 15 Minute Equilibrium Titration vs Theoretical Lead Concentrations in PHREEQC

15 Min Equilibrium				
pH	Moles Pb (PHREEQC)	Conc. from PHREEQC (mg/L)	Conc. 15 Min Titration (mg/L)	Independent Lead in Soln. (mg/L)
1.50	2.51E-04	52.09	56.14	-4.05
1.75	1.58E-04	32.7	25.49	7.2
2.00	1.01E-04	20.84	9.37	11.48
2.25	6.51E-05	13.48	2.64	10.84
2.50	4.25E-05	8.81	1.57	7.24
2.75	2.81E-05	5.81	4.41	1.41
3.00	1.86E-05	3.86	0.87	3
3.25	1.24E-05	2.58	0.51	2.07
3.50	8.34E-06	1.73	-1.26	1.73
4.00	3.77E-06	0.78	-1.62	0.78
4.50	1.71E-06	0.35	-1.97	0.35
5.00	7.75E-07	0.16	-1.62	0.16
5.50	3.51E-07	0.07	-1.26	0.07
6.00	1.61E-07	0.03	-1.26	0.03
6.50	7.97E-08	0.02	-0.55	0.02
7.00	4.75E-08	0.01	-1.62	0.01

*Note: At pH = 3.5 method detection limit was reached. All samples analyzed when pH > 3.5 were BDL.

Table AB.4. Lead Concentrations of 15 Minute Equilibrium Titration vs Theoretical Lead Concentrations in PHREEQC

15 Min Equilibrium				
pH	Moles Pb (PHREEQC)	Conc. from PHREEQC (mg/L)	Conc. 15 Min Titration (mg/L)	Independent Lead in Soln. (mg/L)
1.50	2.51E-04	52.09	61.77	-9.68
1.75	1.58E-04	32.70	26.63	6.07
2.00	1.01E-04	20.84	19.39	1.45
2.25	6.51E-05	13.48	14.57	-1.09
2.50	4.25E-05	8.81	4.92	3.89
2.75	2.81E-05	5.81	0.79	5.03
3.00	1.86E-05	3.86	0.79	3.08
3.25	1.24E-05	2.58	0.79	1.79
3.50	8.34E-06	1.73	0.79	0.94
4.00	3.77E-06	0.78	0.44	0.34
4.50	1.71E-06	0.35	-0.25	0.35
5.00	7.75E-07	0.16	-0.25	0.16
5.50	3.51E-07	0.07	-0.94	0.07
6.00	1.61E-07	0.03	-1.28	0.03
6.50	7.97E-08	0.02	-1.28	0.02
7.00	4.75E-08	0.01	-1.28	0.01

*Note: At pH 4.5 method detection limit was reached. All samples analyzed when pH > 4.5 were BDL.

APPENDIX C.

RAINFALL RATES AND PHOSPHATE CALCULATIONS

Table AC.1. Average Rainfall Rate in Bonne Terre, Mo over a 7 day Period

Annual rainfall (in.)	Volume of 5 Gallon Bucket (in^3)
44.1	1594.9
Bucket Diameter (cm)	Volume (cm^3)
30.3	26133.5
Estimated Soil Depth (cm)	Volume of rain/yr./ bucket (cm^3)
5	80858.8
Estimated soil volume in bucket (cm^3)	Rain Volume (L)/ yr./ bucket
3610.1	80.9
Soil surface Area (cm^2)	Daily Rainfall Volume (L)
722.0	0.222
1 Liter = 1000 cm^3	Daily Rainfall (mL)
	221.5
	Watering Every 2 Days (mL)
	443
	Total rainfall after 7 days (mL)
	1551

Table AC.2. Amount of Phosphoric Acid Needed for Soil Amendment

Phosphoric Acid	85% Phosphorus Content
1 L	.85 L Phosphorus Content
1000 ml	850 ml Phosphorus Content
1 kg	.85 kg Phosphorus Content
1000 g	850 g Phosphorus Content
Amendment Amount	**TOTAL AMOUNT OF PA NEEDED**
.5% weight	**mL**
4.25 g	**5.0**

Table AC.3. Amount of TSP Needed for Soil Amendment

Triple Super Phosphate	45% Phosphorus Content/ Bag
4 lb. bag	1.8 lb. Phosphorus/ Bag
1.818 kg bag	.818 kg Phosphorus / Bag
1818 g bag	818 g Phosphorus / Bag
Amendment Amount	**TOTAL AMOUNT OF TSP NEEDED**
.5% weight (g of P/ bag)	**Weight in Grams**
4.09	**9.09**

Table AC.4. Amount of BM Needed for Soil Amendment

Bone Meal	16% Phosphorus Content/ Bag
6 lb. bag	.96 lb. Phosphorus/bag
2.724 kg bag	.436 kg Phosphorus/ bag
2724 g bag	435.84 g Phosphorus/ bag
Amendment Amount	**TOTAL AMOUNT OF BONE MEAL NEEDED**
.5% weight (g of P/Bag)	**Weight in Grams**
2.18	**13.6**

APPENDIX D.

TITRATION INFORMATION

Table AD.1. Titration pH 1.50-> 7.0 With 15 Minute Equilibrium Period

Time	pH	12 M HCL added (mL)	12.5% NaOH Added (mL)	Adsorption	Concentration (µmol/L)	Concentration (mg/L)
0 min	1.50	0	0.00	0.165	271.0	56.1
15 min	1.75	0	10.50	0.079	123.0	25.5
30 min	2.01	0	7.50	0.033	45.2	9.4
45 min	2.27	0.01	4.25	0.014	12.7	2.6
1:00 Hr.	2.51	0	5.50	0.011	7.6	1.6
1:15 Hr.	2.78	0	3.00	0.019	21.3	4.4
1:30 Hr.	3.01	0.1	4.50	0.009	4.2	0.9
1:45 Hr.	3.23	0.05	3.25	0.008	2.5	0.5
2:00 Hr.	3.51	0.1	3.00	0.003	-6.1	-1.3
2:15 Hr.	3.78	0	0.25	0.002	-7.8	-1.6
2:30 Hr.	4.05	0.015	1.25	0.001	-9.5	-2.0
2:45 Hr.	4.52	0.1	2.50	0.002	-7.8	-1.6
3:00 Hr.	5.00	0.05	1.20	0.003	-6.1	-1.3
3:15 Hr.	5.51	0.01	1.25	0.003	-6.1	-1.3
3:30 Hr.	5.99	0	1.50	0.005	-2.7	-0.6
3:45 Hr.	6.50	0	1.25	0.002	-7.8	-1.6
4:00 Hr.	7.00	0	1.33	0.006	-1.0	-0.2

Table AD.2. Standard Solution Calibration Curve Values

PPM	Adsorption	Concentration (µmol/L)
5	0.012	24.1
10	0.026	48.3
25	0.063	120.7
50	0.119	241.3
100	0.222	482.6

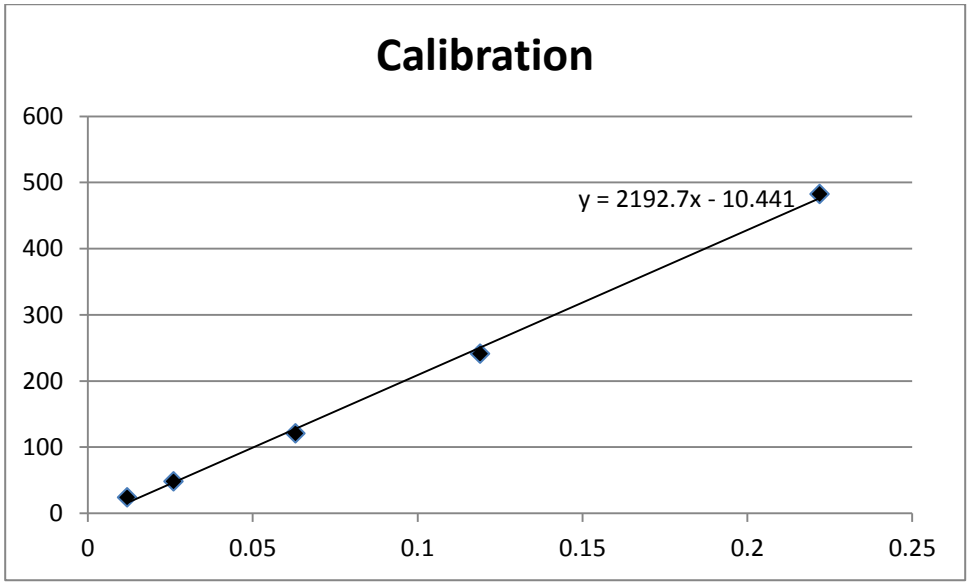


Figure AD.1. Standard Solution Calibration Curve of Titrations with 15 Minute Equilibrium Period

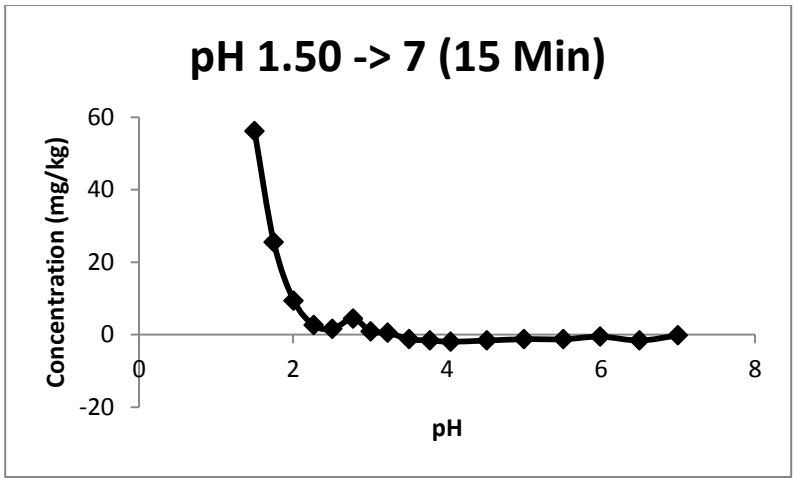


Figure AD.2. Titration Curve from pH 1.5 -> 7.0 with 15 Minute Equilibrium Period

Table AD.3. Titration pH 7.0-> 1.50 With 15 Minute Equilibrium Period

Time	pH	Adsorption	Concentration (µmol/L)	Concentration (mg/L)
0 min	7.00	0.001	-7.8	-1.6
15 min	6.52	0.008	3.8	0.8
30 min	6.00	0.002	-6.2	-1.3
45 min	5.49	0.002	-6.2	-1.3
1:00 Hr.	5.00	0.008	3.8	0.8
1:15 Hr.	4.49	0.005	-1.2	-0.2
1:30 Hr.	4.04	0.007	2.1	0.4
1:45 Hr.	3.76	0.008	3.8	0.8
2:00 Hr.	3.49	0.005	-1.2	-0.2
2:15 Hr.	3.24	0.002	-6.2	-1.3
2:30 Hr.	3.01	0.003	-4.5	-0.9
2:45 Hr.	2.76	0.008	3.8	0.8
3:00 Hr.	2.49	0.02	23.8	4.9
3:15 Hr.	2.23	0.048	70.3	14.6
3:30 Hr.	2.01	0.062	93.6	19.4
3:45 Hr.	1.75	0.083	128.5	26.6
4:00 Hr.	1.48	0.185	298.1	61.8

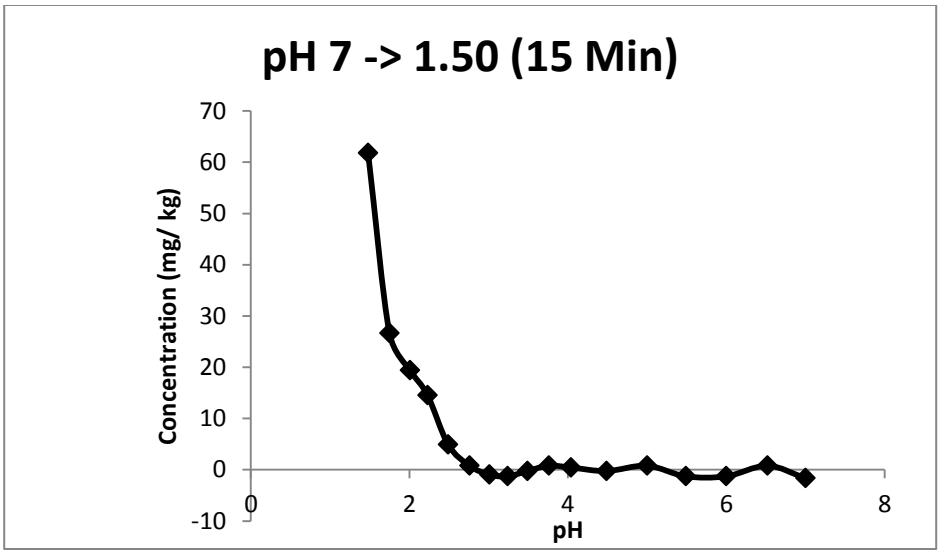


Figure AD.3. Titration Curve from pH 7.0 -> 1.50 with 15 Minute Equilibrium Period

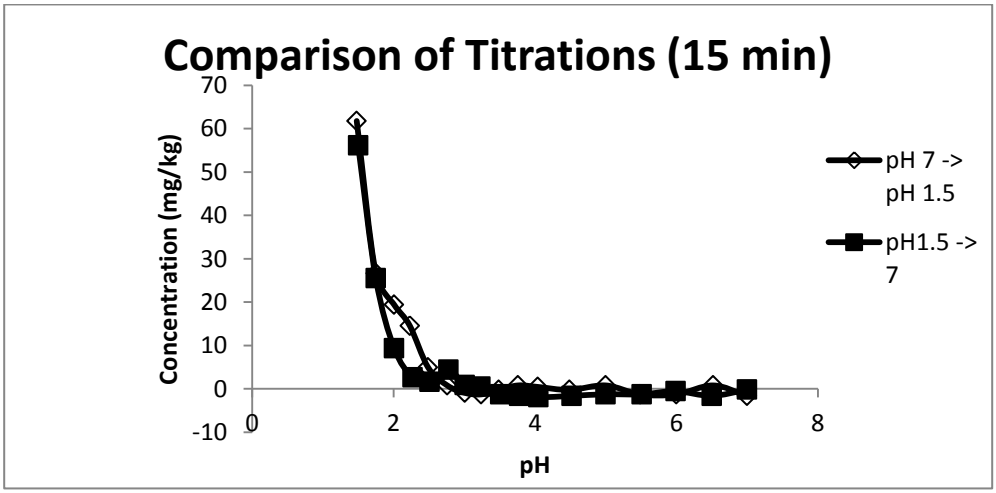


Figure AD.4. Comparison of 15 Minute Equilibrium Period Titration Curves

Table AD.4. Titration pH 7.0-> 1.50 With 30 Minute Equilibrium Period

Time	pH	Adsorption	Concentration (µmol/L)	Concentration (mg/L)
0 Hr.	7.00	0.002	-6.1	-1.3
0.5 Hr.	3.78	0.002	-6.1	-1.3
1.0 Hr.	3.78	0.0015	-7.2	-1.5
1.5 Hr.	3.78	0.001	-8.2	-1.7
2.0 Hr.	3.55	0.002	-6.1	-1.3
2.5 Hr.	3.28	0.003	-3.9	-0.8
3.0 Hr.	3.01	0.001	-8.2	-1.7
3.5 Hr.	2.81	0.003	-3.9	-0.8
4.0hr	2.52	0.005	0.5	0.1
4.5 Hr.	2.25	0.005	0.5	0.1
5.0 Hr.	2.00	0.034	64.1	13.3
5.5 Hr.	1.75	0.086	178.1	36.9
6.0 Hr.	1.50	0.118	248.3	51.4

Table AD.5. Standard Solution Calibration Curve Values

PPM	Adsorption	Concentration (µmol/L)
5	0.012	24.1
10	0.026	48.3
25	0.062	120.7
50	0.123	241.3
100	0.226	482.6

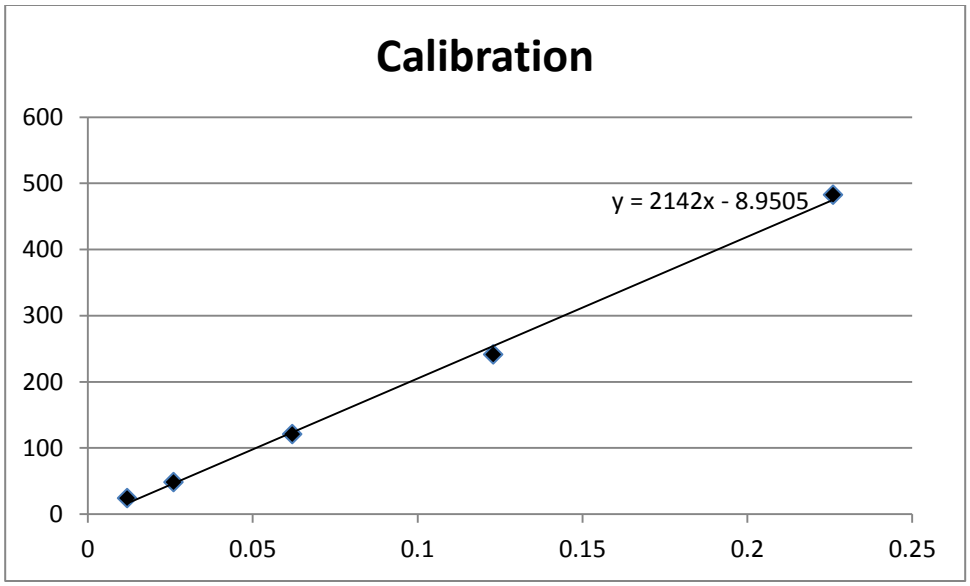


Figure AD.5. Standard Solution Calibration Curve of Titrations with 30 Minute Equilibrium Period

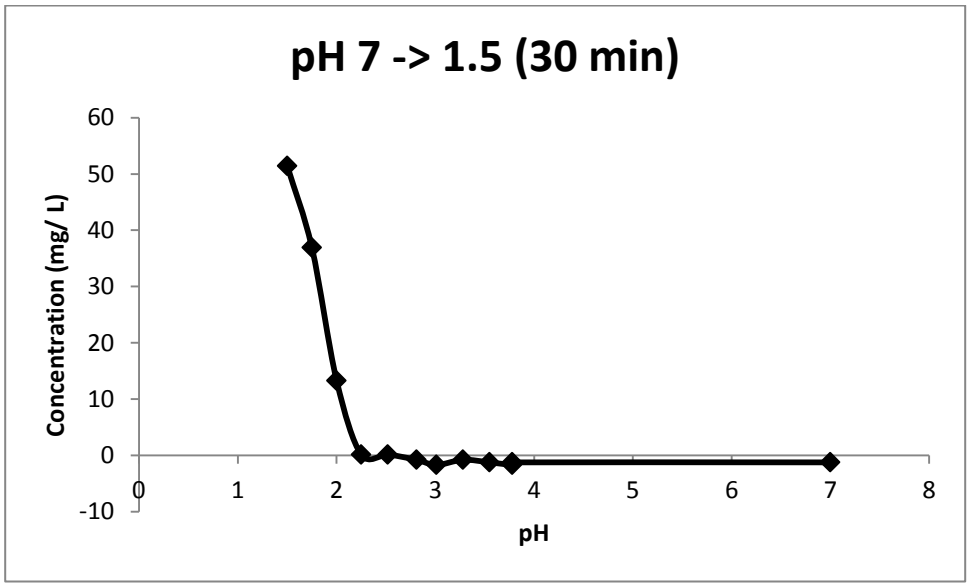


Figure AD.6. Titration Curve from pH 7.0 -> 1.50 with 30 Minute Equilibrium Period

Table AD.6. Titration pH 1.50-> 7.0 With 30 Minute Equilibrium Period

Time	pH	Adsorption	Concentration (µmol/L)	Concentration (mg/L)
0 Hr.	1.50	0.08	162.4	33.7
0.5 Hr.	1.75	0.028	51.0	10.6
1.0 Hr.	2.01	0.014	21.0	4.4
1.5 Hr.	2.25	0.006	3.9	0.8
2.0 Hr.	2.52	0.002	-4.7	-1.0
2.5 Hr.	2.75	0.003	-2.5	-0.5
3.0 Hr.	3.00	0	-9.0	-1.9
3.5 Hr.	3.25	0	-9.0	-1.9
4.0hr	3.50	0	-9.0	-1.9
4.5 Hr.	3.76	0.001	-6.8	-1.4
5.0 Hr.	3.76	0.0015	-5.7	-1.2
5.5 Hr.	3.76	0.002	-4.7	-1.0
6.0 Hr.	7.00	0	-9.0	-1.9

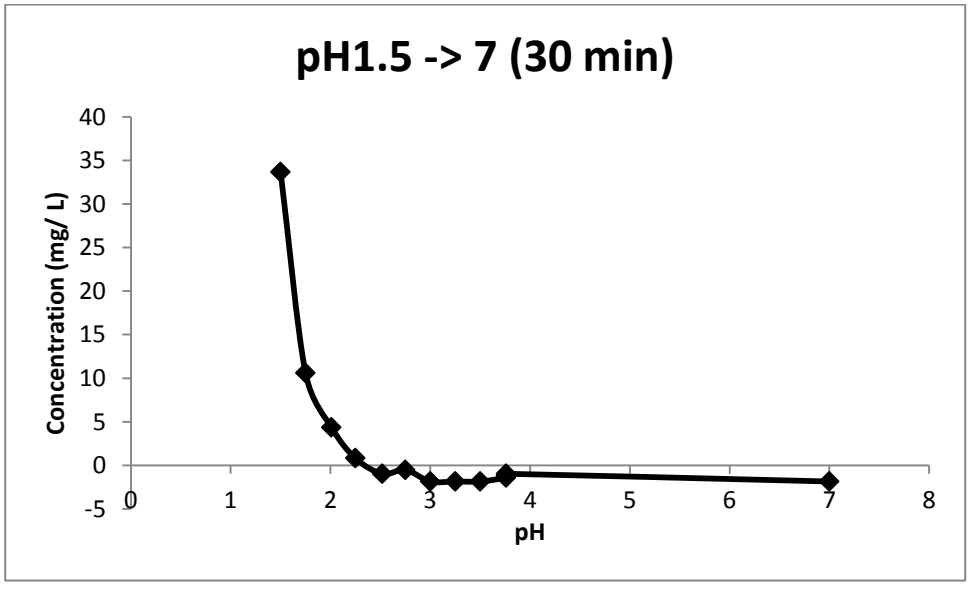


Figure AD.7. Titration Curve from pH 1.50 -> 7.0 with 30 Minute Equilibrium Period

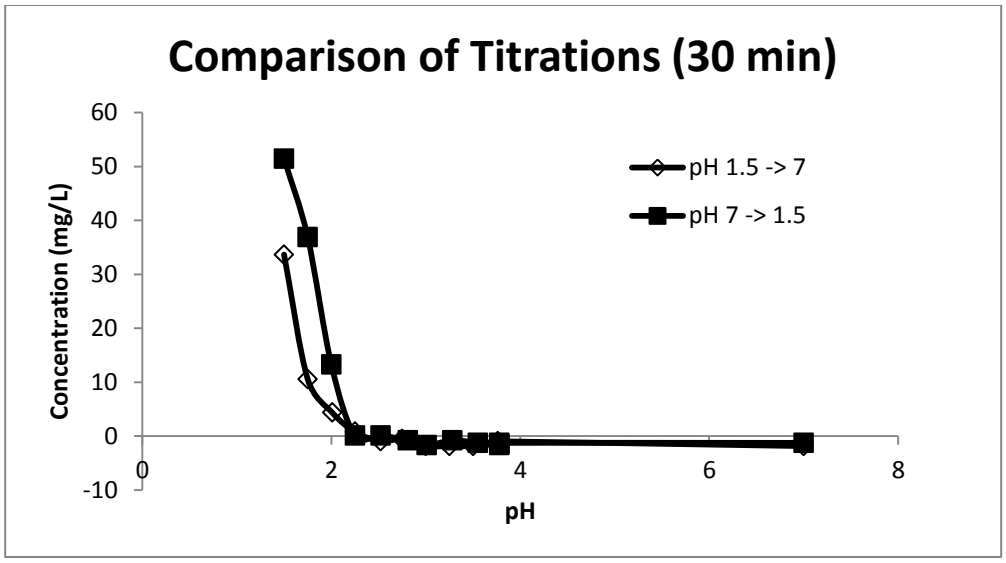


Figure AD.8. Comparison of Titration Curves with 30 Minute Equilibrium Period

APPENDIX E.

CONTROL TESTS IN SILICA

Table AE.1. pH from PBET/ IVG of Synthetic Pyromorphite

Time	Bottle 1	Addition 1	Bottle 2	Addition 2	Bottle 3	Addition 3	Bottle 4	Addition 4
	pH		pH		pH		pH	
0	1.83	Na	1.83	NA	1.83	NA	1.83	NA
5	1.79	1mL DI	1.79	1mL DI	1.74	3 mL DI	1.72	5 mL DI
10	1.76	3 mL DI	1.76	3 mL DI	1.73	5 mL DI	1.7	5 mL DI
15	1.78	3 mL DI	1.74	5 ml DI	1.71	5 mL DI	1.68	7 ml DI
30	1.77	3 mL DI	1.75	3 mL DI	1.7	5 mL DI	1.76	2 mL DI
45	1.77	2 mL DI	1.78	3 mL DI	1.72	5 mL DI	1.81	NA
1:00	1.82	NA	1.81	NA	1.74	5 mL DI	1.76	5 mL DI
1:15	1.79	2 mL DI	1.81	NA	1.79	3 mL DI	1.83	NA
1:30	1.81	NA	1.79	3 mL DI	1.85	NA	1.82	NA
1:45	1.78	3 mL DI	1.81	NA	1.85	NA	1.83	NA
2:00	1.82	NA	1.83	NA	1.86	NA	1.83	NA
2:15	6.05	NA	6.11	NA	6.04	NA	6.09	NA
2:30	6.03	NA	6.07	NA	6.01	NA	6.1	NA
2:45	6.07	NA	6.09	NA	6.04	NA	6.09	NA
3:00	6.06	NA	6.09	NA	6.02	NA	6.08	NA
3:15	6.1	NA	6.1	NA	6.02	NA	6.08	NA
3:30	6.12	NA	6.09	NA	6.02	NA	6.08	NA
3:45	6.09	NA	6.1	NA	6.02	NA	6.08	NA
4:00	6.08	NA	6.09	NA	6.02	NA	6.09	NA
Total Additions (mL)		17 mL DI		18 mL DI		31 mL DI		24 mL DI

Table AE.2. Standard Solution Calibration Curve Values of Synthetic Chloropyromorphite

PPM	Adsorption	Concentration (µmol/L)
5	0.023	24.1
10	0.039	48.3
25	0.064	120.7
50	0.124	241.3
100	0.236	482.6

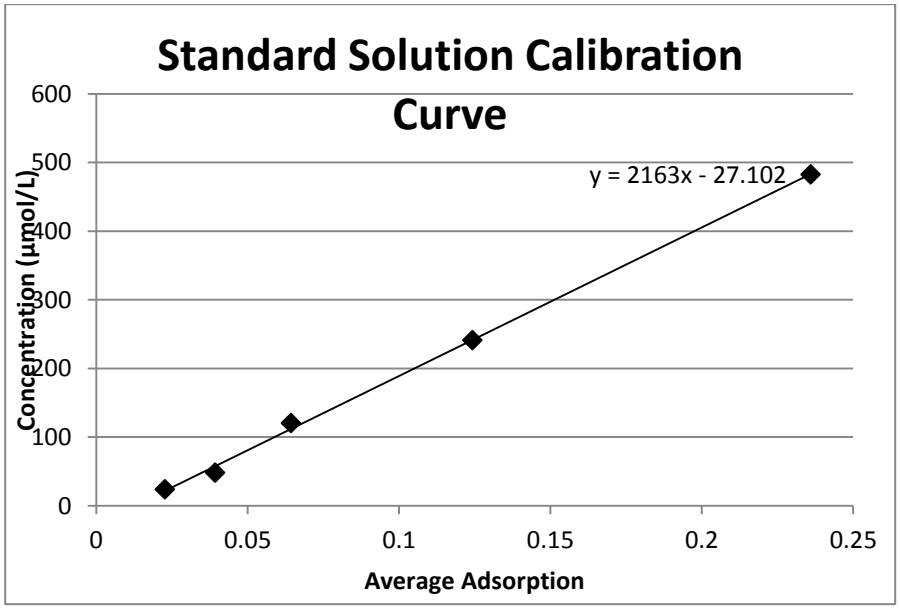


Figure AE.1. Standard Solution Calibration Curve of Synthetic Chloropyromorphite

Table AE.3. PBET/IVG Concentrations of Synthetic Chloropyromorphite

Bottle	Concentration (µmol/L)	Concentration (mg/kg)
PBET 1	180.6	37.4
IVG 1	0.0	0.0
IVG 1	0.0	0.0
IVG 1	18.3	3.8
PBET 2	161.1	33.4
IVG 2	11.8	2.5
IVG 2	16.2	3.4
IVG 2	7.5	1.6
PBET 3	210.8	43.7
IVG 3	7.5	1.6
IVG 3	9.7	2.0
IVG 3	18.3	3.8
PBET	180.6	37.4
IVG 4	11.8	2.5
IVG 4	18.3	3.8
IVG 4	31.3	6.5

Table AE.4. pH from PBET/IVG Analysis of Clean Sand with Synthetic Chloropyromorphite

TIME	Bot 1	Add 1	Bot 2	Add 2	Bot 3	Add 3	Bot 4	Add 4
	pH		pH		pH		pH	
0 min	1.82	NA	1.82	NA	1.82	NA	1.82	NA
5 min	1.73	5 mL DI	1.66	10 mL DI	1.68	10 mL DI	1.67	10 mL DI
10 min	1.80	1mL DI	1.86	NA	1.80	1 mL DI	1.80	1 mL DI
0.25	1.81	NA	1.83	NA	1.76	4 mL DI	1.79	3 mL DI
0.50	1.79	2 mL DI	1.82	NA	1.79	2 mL DI	1.78	2 mL DI
0.75	1.79	3 mL DI	1.81	NA	1.78	5 mL DI	1.81	NA
1.00	1.82	NA	1.82	NA	1.88	NA	1.83	NA
1.25	1.83	NA	1.83	NA	1.89	NA	1.83	NA
1.50	1.80	2 mL DI	1.82	NA	1.84	NA	1.82	NA
1.75	1.83	NA	1.82	NA	1.85	NA	1.83	NA
2.00	1.85	NA	1.83	NA	1.86	NA	1.82	NA
2.25	6.23	NA	6.26	NA	6.25	NA	6.26	NA
2.50	6.25	NA	6.26	NA	6.25	NA	6.25	NA
2.75	6.27	NA	6.21	NA	6.20	NA	6.20	NA
3.00	6.27	NA	6.22	NA	6.20	NA	6.23	NA
3.25	6.28	NA	6.26	NA	6.25	NA	6.25	NA
3.50	6.29	NA	6.26	NA	6.22	NA	6.20	NA
3.75	6.28	NA	6.25	NA	6.24	NA	6.21	NA
4.00	6.27	NA	6.29	NA	6.25	NA	6.24	NA
Total Additions (mL)		13 mL DI		10 mL DI		22 mL DI		16 mL DI

Table AE.5. Standard Solution Calibration Curve Values of Clean Sand with Synthetic Chloropyromorphite

PPM	Adsorption	Concentration (µmol/L)
5	0.014	24.1
10	0.027	48.3
25	0.051	120.7
50	0.106	241.3
100	0.201	482.6

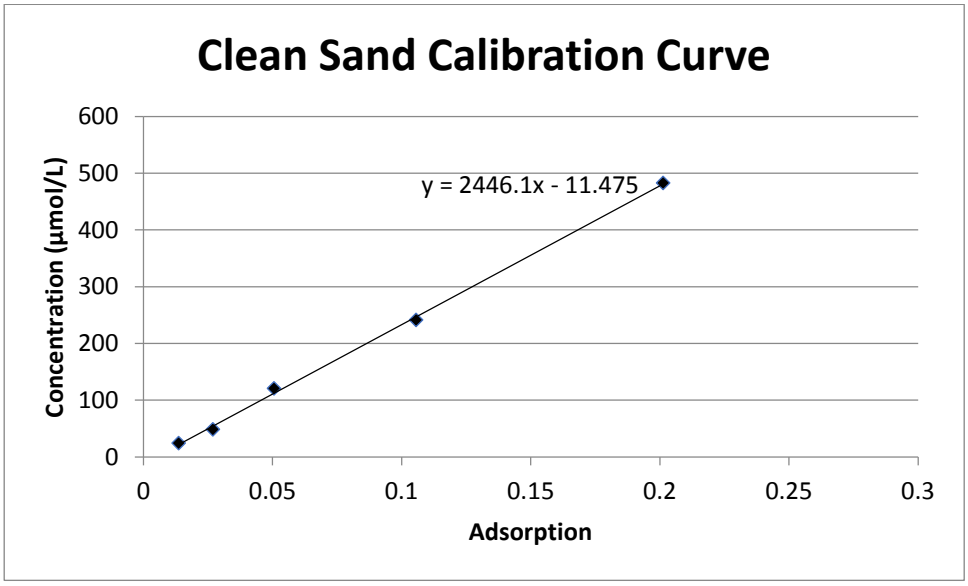


Figure AE.2. Standard Solution Calibration Curve of Clean Sand with Synthetic Chloropyromorphite

Table AE.6. PBET/IVG Concentrations of Clean Sand with Synthetic Chloropyromorphite

Bottle	Concentration (µmol/L)	Concentration (mg/kg)
PBET 1	218.2	45.2
IVG 1	25.3	5.2
IVG 1	28.6	5.9
IVG 1	17.1	3.5
PBET 2	250.1	51.8
IVG 2	23.2	4.8
IVG 2	41.6	8.6
IVG 2	31.8	6.6
PBET 3	209.2	43.4
IVG 3	51.4	10.7
IVG 3	80.9	16.8
IVG 3	49.0	10.2
PBET	184.7	38.3
IVG 4	7.3	1.5
IVG 4	31.8	6.6
IVG 4	-7.4	-1.5

APPENDIX F.

1 WEEK RESULTS

Table AF.1. pH from PBET/IVG Analysis with No Treatment

	Bottle 1	Addition 1	Bottle 2	Addition 2	Bottle 3	Addition 3	Bottle 4	Addition 4
	pH		pH		pH		pH	
	1.79	NA	1.79	NA	1.79	NA	1.79	NA
5 min	1.82	NA	1.8	NA	1.88	NA	1.87	NA
10 min	1.74	NA	1.77	NA	1.76	NA	1.85	NA
15 min	1.72	4 mL DI	1.73	3 mL DI	1.8	NA	1.82	NA
0.50	1.73	7 mL DI	1.69	10 mL DI	1.72	8 mL DI	1.63	15 mL DI
0.75	1.82	NA	1.78	NA	1.7	8 mL DI	1.79	NA
1.00	1.81	NA	1.73	4 mL DI	1.75	2 mL DI	1.76	NA
1.25	1.78	NA	1.79	NA	1.77	NA	1.76	2 mL DI
1.50	1.81	NA	1.78	NA	1.77	NA	1.77	NA
1.75	1.78	NA	1.78	NA	1.76	2 mL DI	1.77	NA
2.00	6.33	NA	6.31	NA	6.28	NA	6.26	NA
2.25	6.21	10 mL DI	6.22	5 mL DI	6.24	NA	6.26	NA
2.50	6.33	NA	6.29	NA	6.26	NA	6.26	NA
2.75	6.23	10 mL DI	6.22	8 mL DI	6.22	3 mL DI	6.22	5 mL DI
3.00	6.26	NA	6.22	NA	6.23	NA	6.23	NA
3.25	6.31	NA	6.24	NA	6.24	NA	6.27	NA
3.50	6.34	NA	6.28	NA	6.25	NA	6.27	NA
3.75	6.28	NA	6.22	NA	6.24	NA	6.27	NA
4.00	6.31	NA	6.24	NA	6.26	NA	6.27	NA
Solution ADDED (mL)		31		30		23		22

Table AF.2. Standard Solution Calibration Values No Treatment

PPM	Adsorption	Concentration (µmol/L)
5	0.014	24.1
10	0.035	48.3
25	0.066	120.7
50	0.117	241.3
100	0.201	482.6

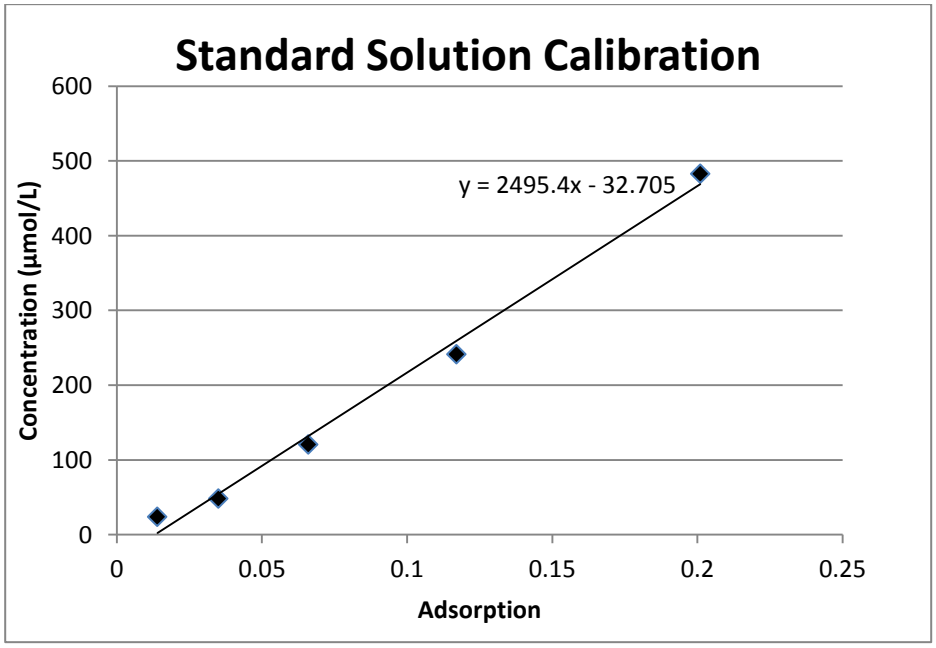


Figure AF.1. Standard Solution Calibration Curve No Treatment

Table AF.3. PBET/IVG Concentrations No Treatment

Sample	Adsorption	Concentration (µmol/L)	Concentration (mg/L)	Concentration (mg/kg)
PBET 1	0.032	47.1	9.8	137.6
IVG 1	0.031	44.7	9.3	151.7
IVG 1	0.031	44.7	9.3	151.7
IVG 1	0.029	39.7	8.2	134.7
PBET 2	0.028	37.2	7.7	110.0
IVG 2	0.027	34.7	7.2	119.7
IVG 2	0.025	29.7	6.2	102.5
IVG 2	0.03	42.2	8.7	145.6
PBET 3	0.037	59.6	12.4	196.1
IVG 3	0.035	54.6	11.3	213.6
IVG 3	0.036	57.1	11.8	223.3
IVG 3	0.041	69.6	14.4	272.1
PBET 4	0.039	64.6	13.4	215.9
IVG 4	0.041	69.6	14.4	277.3
IVG 4	0.034	52.1	10.8	207.8
IVG 4	0.038	62.1	12.9	247.5

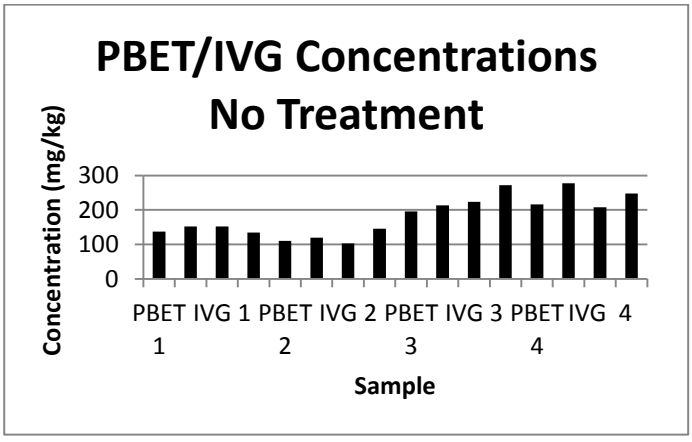


Figure AF.2. PBET/IVG Concentrations No Treatment

Table AF.4. pH from PBET/IVG Analysis PA 1 Week

	Bottle 1	Addition 1	Bottle 2	Addition 2	Bottle 3	Addition 3	Bottle 4	Addition 4
	pH		pH		pH		pH	
Time								
0	1.74	NA	1.74	NA	1.74	NA	1.74	NA
5	1.73	NA	1.73	NA	1.73	NA	1.73	NA
10	1.72	NA	1.72	NA	1.71	NA	1.71	NA
0.25	1.70	NA	1.70	NA	1.69	2 mL DI	1.69	2 mL DI
0.50	1.63	7 mL DI	1.63	7 mL DI	1.66	4 mL DI	1.65	5 mL DI
0.75	1.70	NA	1.71	NA	1.62	10 mL DI	1.71	NA
1.00	1.69	2 mL DI	1.70	NA	1.77	NA	1.69	2 mL DI
1.25	1.71	NA	1.72	NA	1.77	NA	1.71	NA
1.50	1.74	NA	1.74	NA	1.78	NA	1.75	NA
1.75	1.73	NA	1.75	NA	1.78	NA	1.76	NA
2.00	6.64	NA	6.60	NA	6.26	NA	6.37	NA
2.25	6.18	NA	6.20	NA	6.16	NA	6.34	NA
2.50	6.18	NA	6.21	NA	6.19	NA	6.29	NA
2.75	6.18	NA	6.19	NA	6.19	NA	6.23	NA
3.00	6.19	NA	6.18	NA	6.18	NA	6.26	NA
3.25	6.20	NA	6.19	NA	6.21	NA	6.24	NA
3.50	6.21	NA	6.20	NA	6.19	NA	6.25	NA
3.75	6.20	NA	6.19	NA	6.18	NA	6.23	NA
4.00	6.20	NA	6.18	NA	6.20	NA	6.22	NA
	Total added (mL)	9 mL DI		7 mL DI		16 mL DI		9 mL DI

Table AF.5. Weight of Bile Salts and Pancreatin Addition PA 1 Week

	Bile Salt Weight (g)	Pancreatin Weight (g)	Actual Bile Salt Weight (g)	Actual Pancreatin Weight (g)
Bot. 1	2.10	0.21	2.01	0.18
Bot. 2	2.10	0.21	2.04	0.19
Bot. 3	2.10	0.21	1.99	0.18
Bot. 4	2.10	0.21	2.03	0.17

Table AF.6. Standard Solution Calibration Values PA 1 Week

ppm	Adsorption	Concentration (µmol/L)
5	0.008	24.1
10	0.025	48.3
25	0.061	120.7
50	0.124	241.3
100	0.235	482.6

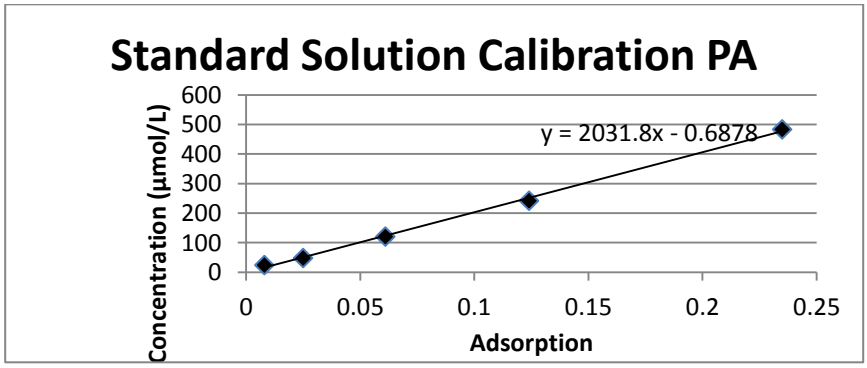


Figure AF.3. Standard Solution Calibration Curve PA 1 Week

Table AF.7. PBET/IVG Concentrations PA 1 Week

PA RESULTS				
	Adsorption	Concentration (µmol/L)	Concentration (mg/L)	Concentration (mg/kg)
PBET 1	0.005	9.5	2.0	40.1
IVG 1	0	0.0	0.0	0.0
IVG 1	0.001	1.3	0.3	7.1
PBET 2	0.014	27.8	5.8	122.4
IVG2	0.004	7.4	1.5	41.7
IVG 2	0.003	5.4	1.1	30.3
PBET 3	0.012	23.7	4.9	87.7
IVG 3	0.005	9.5	2.0	42.7
IVG 3	0.003	5.4	1.1	24.4
PBET 4	0.013	25.7	5.3	108.8
IVG 4	0.005	9.5	2.0	50.3
IVG 4	0.004	7.4	1.5	39.5

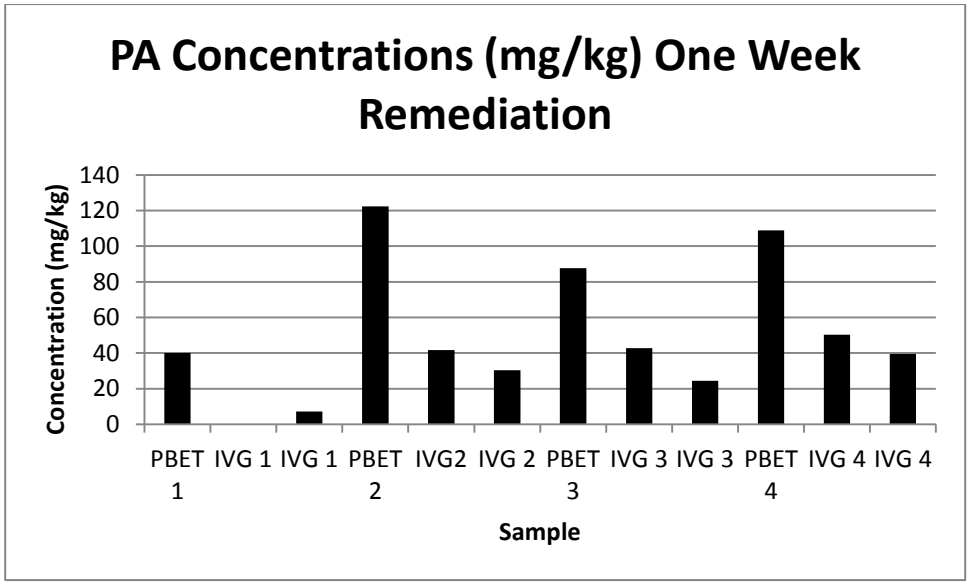


Figure AF.4. PBET/IVG Concentrations PA 1 Week

Table AF.8. pH from PBET/IVG Analysis TSP 1 Week

	Bottle 1	Addition 1	Bottle 2	Addition 2	Bottle 3	Addition 3	Bottle 4	Addition 4
Time								
0	1.7	NA	1.7	NA	1.7	NA	1.7	NA
5	1.63	5 mL DI	1.62	7 mL DI	1.6	9 mL DI	1.61	8 mL DI
10	1.7	NA	1.7	NA	1.7	NA	1.71	NA
0.25	1.69	1 mL DI	1.67	3 mL DI	1.71	NA	1.68	3 mL DI
0.50	1.67	5 mL DI	1.67	5 mL DI	1.69	3 mL DI	1.68	4 mL DI
0.75	1.73	NA	1.66	5 mL DI	1.69	3 mL DI	1.7	NA
1.00	1.74	NA	1.76	NA	1.74	NA	1.75	NA
1.25	1.76	NA	1.77	NA	1.77	NA	1.76	NA
1.50	1.76	NA	1.76	NA	1.76	NA	1.77	NA
1.75	1.76	NA	1.76	NA	1.76	NA	1.76	NA
2.00	6.22	NA	6.48	NA	6.15	NA	6.27	NA
2.25	6.14	NA	6.12	NA	6.12	NA	6.23	NA
2.50	6.16	NA	6.11	NA	6.08	NA	6.21	NA
2.75	6.15	NA	6.11	NA	6.08	NA	6.17	NA
3.00	6.15	NA	6.1	NA	6.09	NA	6.17	NA
3.25	6.15	NA	6.11	NA	6.09	NA	6.15	NA
3.50	6.15	NA	6.11	NA	6.1	NA	6.16	NA
3.75	6.15	NA	6.11	NA	6.1	NA	6.16	NA
4.00	6.15	NA	6.12	NA	6.1	NA	6.15	NA
	Total liquid added (mL)	11 mL DI		20 mL DI		15 mL DI		15 mL DI

.

Table AF.9. Weight of Bile Salts and Pancreatin Addition TSP 1 Week

	Bile Salt Weight (g)	Pancreatin Weight (g)	Actual Bile Salt Weight (g)	Actual Pancreatin Weight (g)
Bot. 1	2.10	0.21	2.03	0.17
Bot. 2	2.10	0.21	2.06	0.15
Bot. 3	2.10	0.21	2.04	0.17
Bot. 4	2.10	0.21	2.08	0.18

Table AF.10. Standard Solution Calibration Values TSP 1 Week

ppm	Adsorption	Concentration (µmol/L)
5	0.008	24.1
10	0.016	48.3
25	0.055	120.7
50	0.118	241.3
100	0.204	482.6

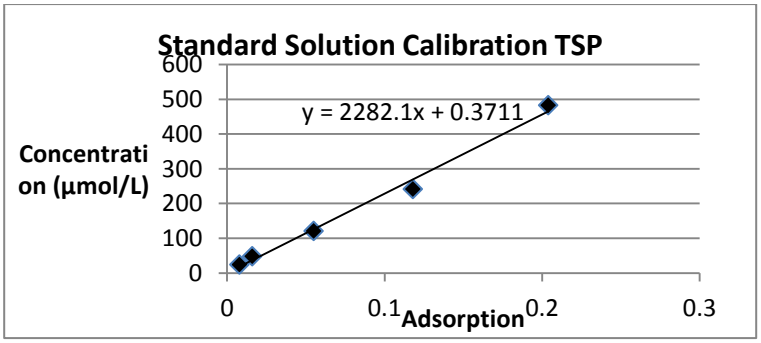


Figure AF.5. Standard Solution Calibration Curve TSP 1 Week

Table AF.11. PBET/IVG Concentrations TSP 1 Week

TSP RESULTS				
	Adsorption	Concentration (µmol/L)	Concentration (mg/L)	Concentration (mg/kg)
PBET 1	0.011	25.5	5.3	103.5
IVG 1	0.006	14.1	2.9	71.1
IVG 1	0.009	20.9	4.3	105.7
PBET 2	0.01	23.2	4.8	80.1
IVG2	0.011	25.5	5.3	105.6
IVG 2	0.007	16.3	3.4	67.7
PBET 3	0.013	30.0	6.2	113.2
IVG 3	0.014	32.3	6.7	148.8
IVG 3	0.01	23.2	4.8	106.8
PBET 4	0.009	20.9	4.3	78.8
IVG 4	0.008	18.6	3.9	85.8
IVG 4	0.009	20.9	4.3	96.3

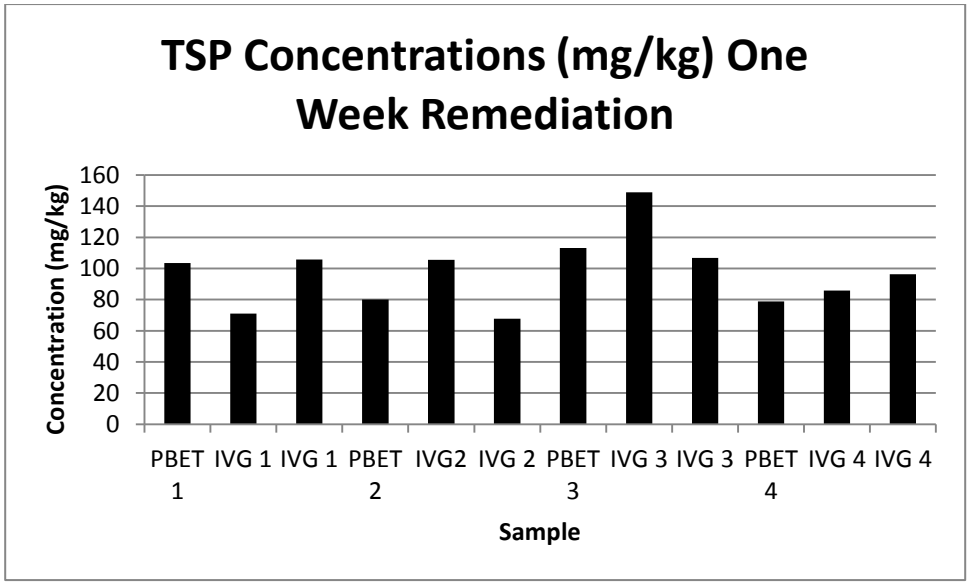


Figure AF.6. PBET/IVG Concentrations TSP 1 Week

Table AF.12. pH from PBET/IVG Analysis BM 1 Week

	Bottle 1	Addition 1	Bottle 2	Addition 2	Bottle 3	Addition 3	Bottle 4	Addition 4
Time								
0	1.71	NA	1.71	NA	1.71	NA	1.71	NA
5	1.49	15 mL DI	1.49	15 mL DI	3.50	NA	1.59	7 mL DI
10	1.80	NA	1.76	NA	3.92	0.7 mL 12 M HCL	1.67	3 mL DI
0.25	1.47	15 mL DI	1.45	20 mL DI	0.48	2.3 mL NaOH & 0.3 mL HCL	1.35	20 mL DI
0.50	1.51	10 mL DI	1.56	7 mL DI	1.84	NA	1.62	7 mL DI
0.75	1.59	7 mL DI	1.61	7 mL DI	1.86	NA	1.67	4 mL DI
1.00	1.63	7 mL DI	1.64	7 mL DI	1.87	NA	1.68	3 mL DI
1.25	1.68	2 mL DI	1.67	4 mL DI	1.86	NA	1.70	NA
1.50	1.70	NA	1.69	2 mL DI	1.85	NA	1.70	NA
1.75	1.69	NA	1.70	NA	1.81	NA	1.70	NA
2.00	5.75	NA	5.96	NA	6.21	NA	6.14	NA
2.25	6.08	NA	6.04	NA	6.19	NA	6.12	NA
2.50	6.06	NA	6.05	NA	6.16	NA	6.11	NA
2.75	6.10	NA	6.07	NA	6.16	NA	6.06	NA
3.00	6.10	NA	6.10	NA	6.13	NA	6.10	NA
3.25	6.08	NA	6.10	NA	6.15	NA	6.11	NA
3.50	6.09	NA	6.11	NA	6.13	NA	6.13	NA
3.75	6.05	NA	6.06	NA	6.11	NA	6.07	NA
4.00	6.04	NA	6.04	NA	6.12	NA	6.07	NA
	Total Liquid added (mL)	56		62		NA		44

Table AF.13. Weight of Bile Salts and Pancreatin Addition BM 1 Week

	Bile Salt Weight (g)	Pancreatin Weight (g)	Actual Weight of Bile Salt (g)	Actual Weight of Pancreatin (g)
Bottle 1	2.10	0.21	2.19	0.19
Bottle 2	2.10	0.21	2.18	0.18
Bottle 3	2.10	0.21	2.13	0.19
Bottle 4	2.10	0.21	2.16	0.16

Table AF.14. Standard Solution Calibration Values BM 1 Week

ppm	Adsorption	Concentration (µmol/L)
5	0.015	24.1
10	0.03	48.3
25	0.07	120.7
50	0.134	241.3
100	0.23	482.6

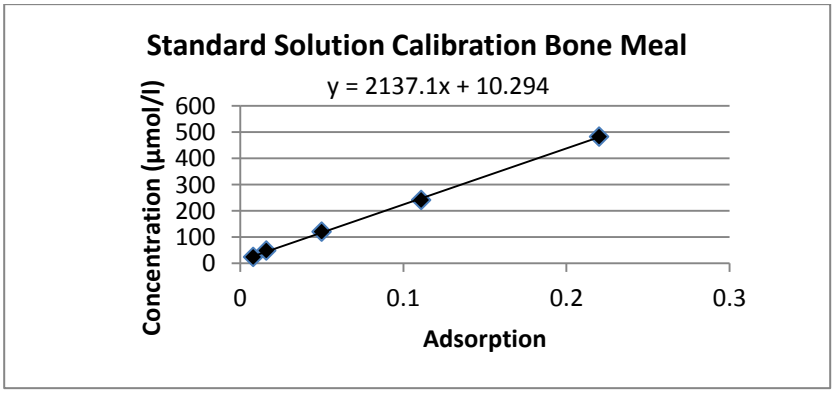


Figure AF.7. Standard Solution Calibration Curve BM 1 Week

Table AF.15. PBET/IVG Concentrations BM 1 Week

BM RESULTS				
	Adsorption	Concentration µmol/L	Concentration (mg/L)	Concentration (mg/kg)
PBET 1	0.002	14.6	3.0	31.4
IVG 1	0	0.0	0.0	0.0
IVG 1	0	0.0	0.0	0.0
PBET 2	0.01	31.7	6.6	64.3
IVG 2	0.006	23.1	4.8	52.1
IVG 2	0.007	25.3	5.2	56.9
PBET 3	0.025	63.7	13.2	307.0
IVG 3	0.021	55.2	11.4	346.4
IVG 3	0.019	50.9	10.5	319.6
PBET 4	0.014	40.2	8.3	99.2
IVG 4	0.01	31.7	6.6	88.7
IVG 4	0.009	29.5	6.1	82.7

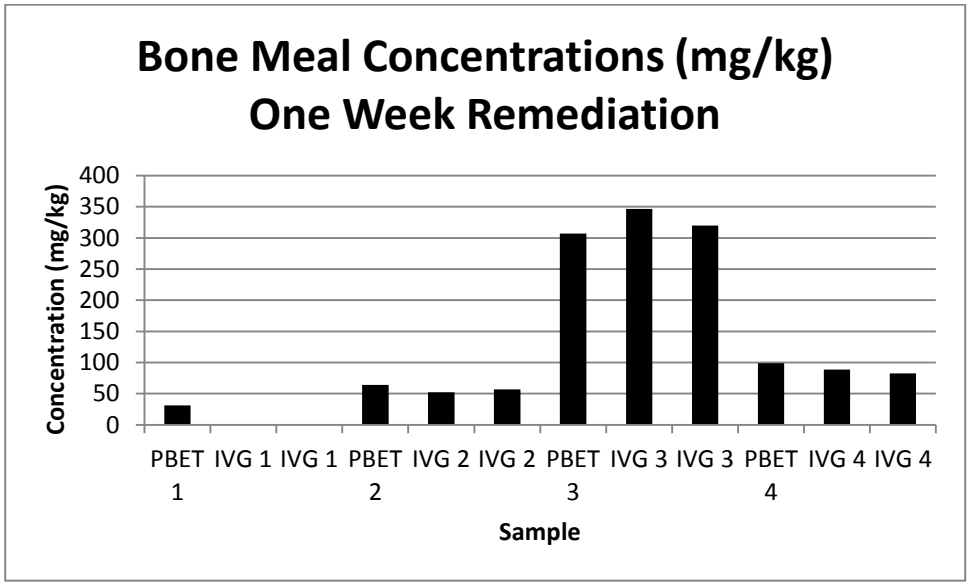


Figure AF.8. PBET/IVG Concentrations BM 1 Week

APPENDIX G.

4 WEEK RESULTS

Table AG.1. pH from PBET/IVG Analysis PA 4 Weeks

	Bottle 1	Addition 1	Bottle 2	Addition 2	Bottle 3	Addition 3	Bottle 4	Addition 4
Time								
0 min	1.72		1.72		1.72		1.72	
5 min	1.71	NA	1.69	1 mL DI	1.69	1 mL DI	1.66	5 mL DI
10 min	1.69	1 mL Di	1.69	1 mL DI	1.69	1 mL DI	1.69	1 mL DI
0.25	1.7	NA	1.69	1 mL DI	1.69	1 mL DI	1.69	1 mL DI
0.50	1.62	8 mL DI	1.63	7 mL DI	1.63	7 mL DI	1.63	7 mL DI
0.75	1.7	NA	1.68	2 mL DI	1.69	1 mL DI	1.66	4 mL DI
1.00	1.7	NA	1.7	NA	1.7	NA	1.7	NA
1.25	1.71	NA	1.71	NA	1.71	NA	1.71	NA
1.50	1.71	NA	1.71	NA	1.71	NA	1.72	NA
1.75	1.7	NA	1.72	NA	1.71	NA	1.72	NA
2.00	6.38	NA	6.46	NA	6.6	NA	6.35	NA
2.25	6.19	NA	6.29	NA	6.3	NA	6.23	NA
2.50	6.25	NA	6.26	NA	6.28	NA	6.13	NA
2.75	6.28	NA	6.27	NA	6.24	NA	6.19	NA
3.00	6.27	NA	6.26	NA	6.25	NA	6.21	NA
3.25	6.28	NA	6.25	NA	6.25	NA	6.24	NA
3.50	6.31	NA	6.26	NA	6.25	NA	6.21	NA
3.75	6.32	NA	6.25	NA	6.25	NA	6.23	NA
4.00	6.33	NA	6.24	NA	6.24	NA	6.25	NA
	Total liquid added (mL)	9 mL DI		12 mL DI		11 mL DI		18 mL DI

Table AG.2. Weight of Bile Salts and Pancreatin Addition PA 4 Weeks

	Bile Salt Weight (g)	Bile Salt Weight in Bottle (g)	Pancreatin Weight (g)	Pancreatin Weight in Bottle (g)
Bottle 1	2.1	2.08	0.21	0.18
Bottle 2	2.1	2.05	0.21	0.19
Bottle 3	2.1	2.09	0.21	0.17
Bottle 4	2.1	2.07	0.21	0.19

Table AG.3. Standard Solution Calibration Values PA 4 Weeks

PPM	Adsorption	Concentration (µmol/L)
5	0.026	24.1
10	0.051	48.3
25	0.144	120.7
50	0.265	241.3
100	0.463	482.6

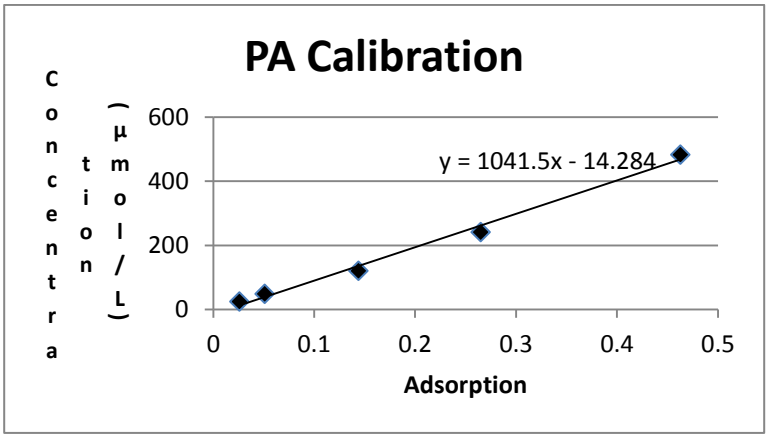


Figure AG.1. Standard Solution Calibration Curve PA 4 Weeks

Table AG.4. PBET/IVG Concentrations PA 4 Weeks

PA				
	Adsorption	Concentration (µmol/L)	Concentration (mg/L)	Concentration (mg/kg)
PBET 1				
IVG 1	0.01	-2.9	-0.6	-15.2
IVG 1	0.01	-6.9	-1.4	-36.6
PBET 2				
IVG2	0.01	-5.9	-1.2	-29.0
IVG 2	0.01	-4.9	-1.0	-24.1
PBET 3				
IVG 3				
IVG 3				
PBET 4				
IVG 4	0.01	1.2	0.2	0.0
IVG 4	0	-7.9	-1.6	-0.1

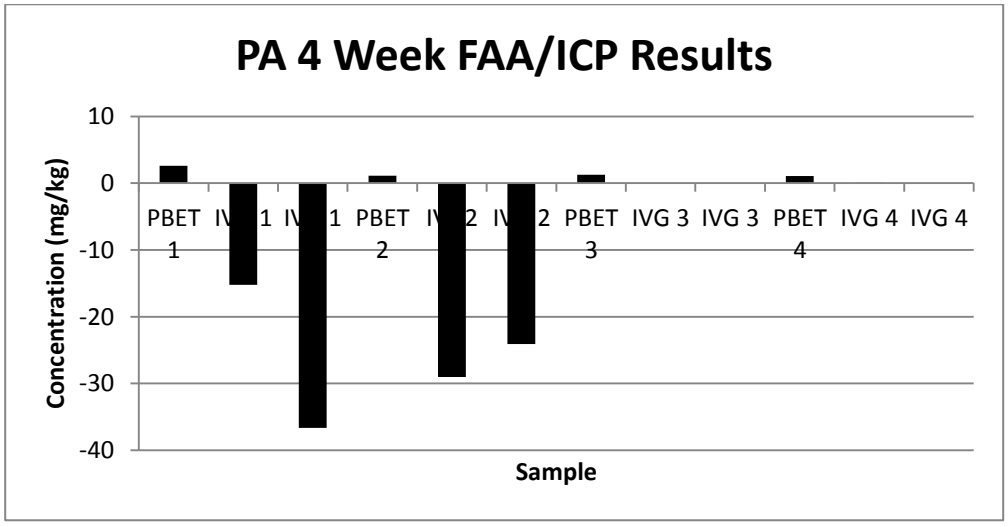


Figure AG.2. PBET/IVG Concentrations PA 4 Weeks

Table AG.5. GFAA PBET/IVG Concentrations PA 4 Weeks

SAMPLE	Concentration (µg/L)	Concentration (µg/L)	Concentration (mg/L)	Concentration (mg/kg)	Std. Dev
PA PBET 1 4 WKS	2.47	2.47	0.25	6.86	0.13
PA IVG 1 4 WKS	BDL	0.00	0.00	0.00	0.02
PA PBET 2 4 WKS	1.01	1.01	0.10	2.81	0.06
PA IVG 2 4 WKS	BDL	0.00	0.00	0.00	0.05
PA PBET 3 4 WKS	BDL	0.00	0.00	0.00	0.11
PA IVG 3 4 WKS	BDL	0.00	0.00	0.00	0.04
PA PBET 4 4 WKS	2.79	2.79	0.28	7.75	0.03
PA IVG 4 4 WKS	BDL	0.00	0.00	0.00	0.33

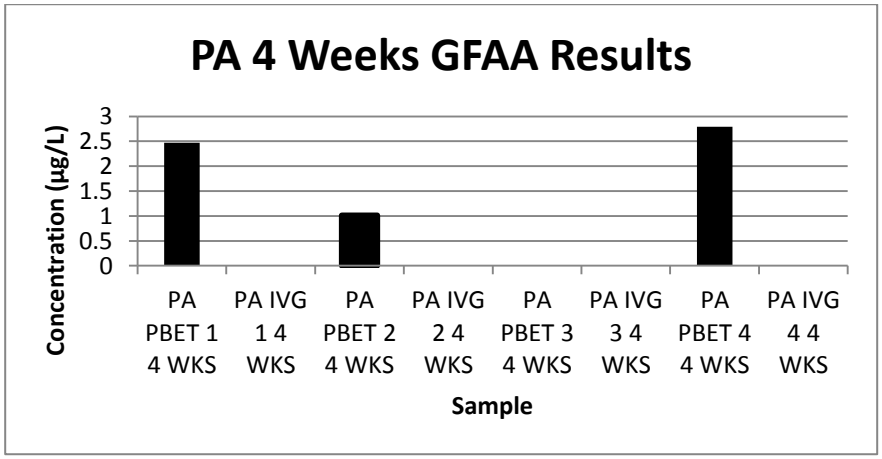


Figure AG.3. GFAA PBET/IVG Concentrations PA 4 Weeks

Table AG.6. pH from PBET/IVG Analysis TSP 4 Weeks

	Bottle 1	Addition 1	Bottle 2	Addition 2	Bottle 3	Addition 3	Bottle 4	Addition 4
Time								
0 min	1.7		1.7		1.7		1.7	
5 min	1.56	10 mL DI	1.52	15 mL DI	1.55	13 mL DI	1.56	10 mL DI
10 min	1.7	NA	1.69	2 mL DI	1.72	NA	1.71	NA
0.25	1.68	3 mL DI	1.69	2 mL DI	1.69	2 mL DI	1.69	2 mL DI
0.50	1.68	3 mL DI	1.66	5 mL DI	1.68	4 mL DI	1.69	1 mL DI
0.75	1.7	NA	1.7	NA	1.73	NA	1.72	NA
1.00	1.71	NA	1.7	NA	1.72	NA	1.73	NA
1.25	1.72	NA	1.71	NA	1.74	NA	1.73	NA
1.50	1.73	NA	1.72	NA	1.74	NA	1.74	NA
1.75	1.74	NA	1.72	NA	1.74	NA	1.73	NA
2.00	6.38	NA	6.44	NA	6.28	NA	6.24	NA
2.25	6.16	NA	6.13	NA	6.1	NA	6.18	NA
2.50	6.17	NA	6.13	NA	6.17	Na	6.19	NA
2.75	6.15	NA	6.1	NA	6.15	NA	6.23	NA
3.00	6.17	NA	6.16	NA	6.12	NA	6.21	NA
3.25	6.15	NA	6.11	NA	6.13	NA	6.23	NA
3.50	6.16	NA	6.13	NA	6.17	NA	6.24	NA
3.75	6.15	NA	6.15	NA	6.23	NA	6.25	NA
4.00	6.15	NA	6.14	NA	6.24	NA	6.25	NA
	Total liquid added (mL)	16 mL DI		24 mL DI		19 mL DI		13 mL DI

Table AG.7. Weight of Bile Salts and Pancreatin Addition TSP 4 Weeks

	Actual Bile Salts Weight (g)	Bile Salts Weight in Bottle (g)	Actual Pancreatin Weight (g)	Pancreatin Weight in Bottle (g)
Bottle 1	2.1	2.06	0.21	0.18
Bottle 2	2.1	2.08	0.21	0.17
Bottle 3	2.1	2.1	0.21	0.19
Bottle 4	2.1	2.04	0.21	0.20

Table AG.8. Standard Solution Calibration Values TSP 4 Weeks

PPM	Adsorption	Concentration (µmol/L)
5	0.027	24.1
10	0.056	48.3
25	0.146	120.7
50	0.255	241.3
100	0.486	482.6

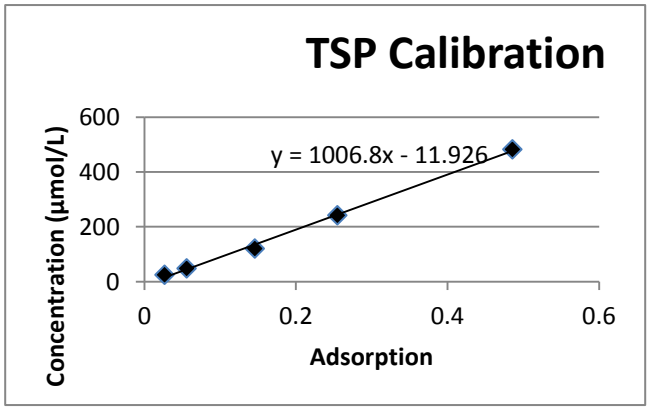


Figure AG.4. Standard Solution Calibration Curve TSP 4 Weeks

Table AG.9. PBET/IVG Concentrations TSP 4 Weeks

TSP				
	Adsorption	Concentration (µmol/L)	Concentration (mg/L)	Concentration (mg/kg)
PBET 1				
IVG 1	0.004	-10.1	-2.1	-45.6
IVG 1	0.005	-9.1	-1.9	-0.1
PBET 2				
IVG2	0.004	-10.1	-2.1	-38.8
IVG 2	0.004	-10.1	-2.1	-38.8
PBET 3				
IVG 3	0.002	-12.2	-2.5	-51.6
IVG 3	0.004	-10.1	-2.1	-42.8
PBET 4				
IVG 4	0.002	-12.2	-2.5	-58.8
IVG 4	0.001	-13.2	-2.7	-63.8

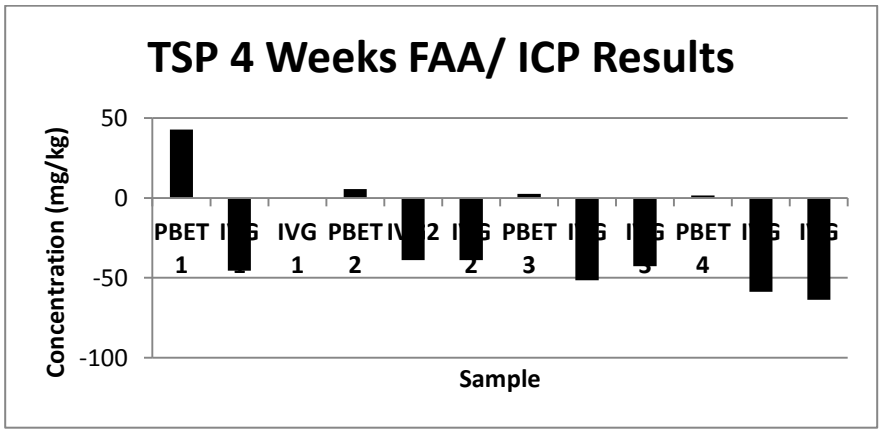


Figure AG.5. PBET/IVG Concentrations TSP 4 Weeks

Table AG.10. GFAA PBET/IVG Concentrations TSP 4 Weeks

SAMPLE	Concentration (µg/L)	Concentration (µg/L)	Concentration (mg/L)	Concentration (mg/kg)	Std. Dev
TSP PBET 1	12.08	12.08	1.21	33.56	0.03
TSP IVG 1	BDL	0	0.00	0.00	0.02
TSP PBET 2	1.05	1.05	0.11	2.92	0.01
TSP IVG 2	BDL	0	0.00	0.00	0.15
TSP PBET 3	0.64	0.64	0.06	1.78	1.92
TSP IVG 3	BDL	0	0.00	0.00	0.02
TSP PBET 4	BDL	0	0.00	0.00	0.08
TSP IVG 4	0.49	0.49	0.05	1.36	0.42

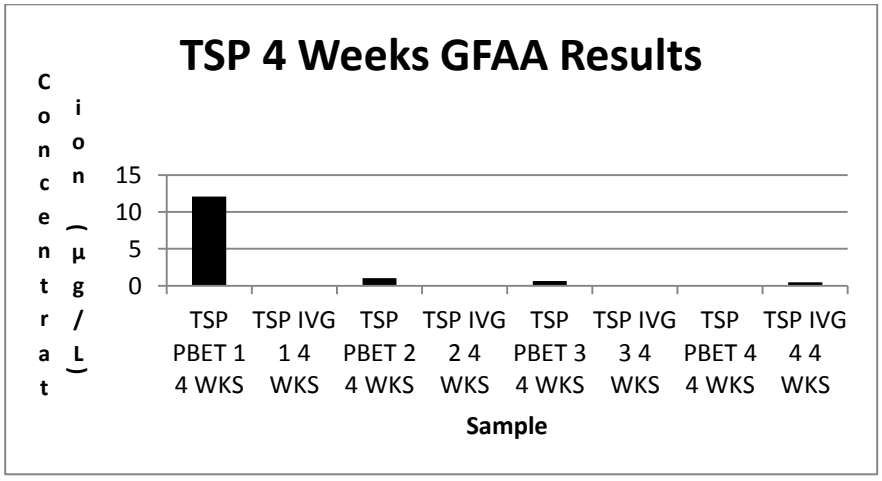


Figure AG.6. GFAA PBET/IVG Concentrations TSP 4 Weeks

Table AG.11. pH from PBET/IVG Analysis BM 4 Weeks

	Bottle 1	Addition 1	Bottle 2	Addition 2	Bottle 3	Addition 3	Bottle 4	Addition 4
Time								
0 min	1.7		1.7		1.7		1.7	
5 min	1.68	2 mL DI	1.67	3 mL DI	1.67	3 mL DI	1.69	1 mL DI
10 min	1.69	1 mL DI	1.65	5 mL DI	1.68	2 mL DI	1.7	NA
0.25	1.69	1 mL DI	1.71	NA	1.64	5 mL DI	1.69	1 mL DI
0.50	1.65	5 mL DI	1.62	10 mL DI	1.65	5 mL DI	1.66	4 mL DI
0.75	1.7	NA	1.71	NA	1.72	NA	1.72	NA
1.00	1.7	NA	1.71	NA	1.71	NA	1.72	NA
1.25	1.71	NA	1.71	NA	1.72	NA	1.72	NA
1.50	1.71	NA	1.71	NA	1.72	NA	1.72	NA
1.75	1.71	NA	1.71	NA	1.73	NA	1.73	NA
2.00	6.21	NA	6.18	NA	6.08	NA	6.38	NA
2.25	6.23	NA	6.12	NA	6.13	NA	6.23	NA
2.50	6.18	NA	6.11	NA	6.11	NA	6.23	NA
2.75	6.15	NA	6.1	NA	6.11	NA	6.2	NA
3.00	6.16	NA	6.1	NA	6.09	NA	6.23	NA
3.25	6.14	NA	6.13	NA	6.11	NA	6.19	NA
3.50	6.15	NA	6.14	NA	6.13	NA	6.18	NA
3.75	6.15	NA	6.12	NA	6.11	NA	6.17	NA
4.00	6.13	NA	6.12	NA	6.11	NA	6.19	NA
	Total liquid added (mL)	9 mL DI		18 mL DI		15 mL DI		6 mL DI

Table AG.12. Weight of Bile Salts and Pancreatin Addition BM 4 Weeks

	Actual Bile Salts Weight (g)	Bile Salts Weight in Bottle (g)	Actual Pancreatin Weight (g)	Pancreatin Weight in Bottle (g)
Bottle 1	2.1	2.06	0.21	0.16
Bottle 2	2.1	2.05	0.21	0.17
Bottle 3	2.1	2.07	0.21	0.16
Bottle 4	2.1	2.05	0.21	0.15

Table AG.13. Standard Solution Calibration Values BM 4 Weeks

PPM	Adsorption	Concentration (µmol/L)
5	0.039	24.1
10	0.085	48.3
25	0.153	120.7
50	0.324	241.3
100	0.604	482.6

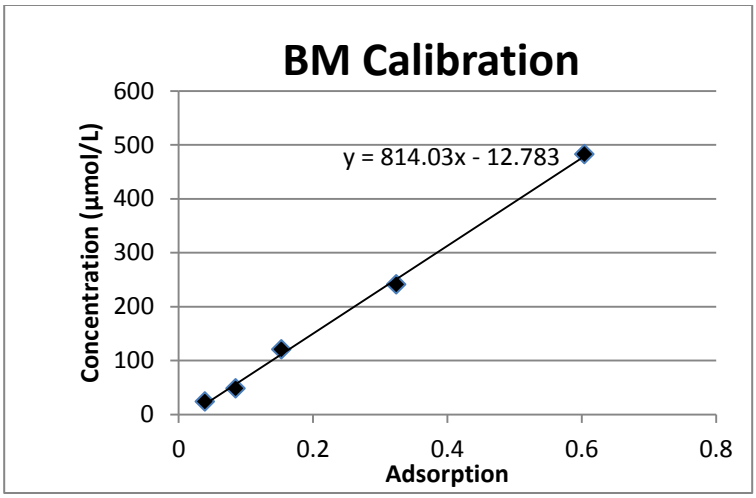


Figure AG.7. Standard Solution Calibration Curve BM 4 Weeks

Table AG.14. PBET/IVG Concentrations BM 4 Weeks

BM				
	Adsorption	Concentration (µmol/L)	Concentration (mg/L)	Concentration (mg/kg)
PBET 1	0.006	-18.8	-3.9	-79.3
IVG 1	0.003	-21.9	-4.5	-116.2
IVG 1	0.002	-22.9	-4.7	-121.7
PBET 2	0.013	-11.5	-2.4	-41.0
IVG2	0.002	-22.9	-4.7	-98.9
IVG 2	0.001	-23.9	-5.0	-103.4
PBET 3	0.007	-17.7	-3.7	-66.7
IVG 3	0.001	-23.9	-5.0	-110.3
IVG 3	0.007	-17.7	-3.7	-81.6
PBET 4	0.004	-20.8	-4.3	-93.8
IVG 4	0.011	-13.6	-2.8	-78.1
IVG 4	0.013	-11.5	-2.4	-66.1

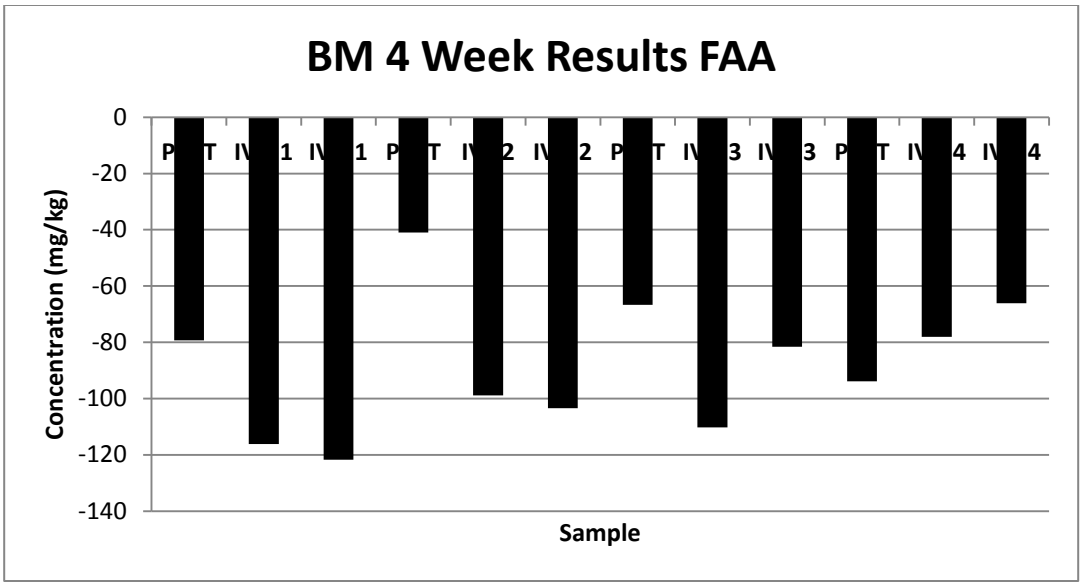


Figure AG.8. PBET/IVG Concentrations BM 4 Weeks

Table AG.15. GFAA PBET/IVG Concentrations BM 4 Weeks

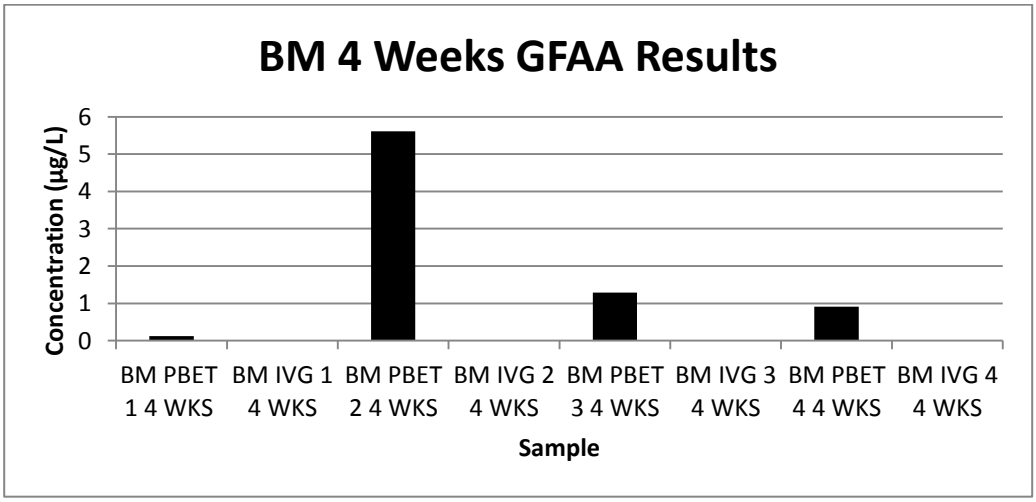


Figure AG.9. GFAA PBET/IVG Concentrations BM 4 Weeks

SAMPLE	Concentration (µg/L)	Concentration (µg/L)	Concentration (mg/L)	Concentration (mg/kg)	Std. Dev
BM PBET 1	0.12	0.12	0.012	0.33	0.01
BM IVG 1	BDL	0	0	0.00	0.39
BM PBET 2	5.61	5.61	0.561	15.58	0.04
BM IVG 2	BDL	0	0	0.00	0.01
BM PBET 3	1.29	1.29	0.129	3.58	0.03
BM IVG 3	BDL	0	0	0.00	0.06
BM PBET 4	0.91	0.91	0.091	2.53	0.08
BM IVG 4	BDL	0	0	0.00	0.02

APPENDIX H.

16 WEEK RESULTS

Table AH.1. pH from PBET/IVG Analysis PA 16 Weeks

	Bottle 1	Addition 1	Bottle 2	Addition 2	Bottle 3	Addition 3	Bottle 4	Addition 4
Time								
0 min	1.72	NA	1.72	NA	1.72	NA	1.72	NA
5 min	1.78	NA	1.77	NA	1.77	NA	1.76	NA
10 min	1.77	NA	1.76	NA	1.75	NA	1.74	NA
0.25	1.77	NA	1.75	NA	1.74	NA	1.73	NA
0.50	1.68	2 mL DI	1.67	3 mL DI	1.66	4 mL DI	1.64	6 mL DI
0.75	1.69	1 mL DI	1.67	3 mL DI	1.69	1 mL DI	1.71	NA
1.00	1.69	1 mL DI	1.72	NA	1.70	NA	1.70	NA
1.25	1.71	NA	1.73	NA	1.72	NA	1.70	NA
1.50	1.70	NA	1.73	NA	1.72	NA	1.71	NA
1.75	1.70	NA	1.73	NA	1.72	NA	1.71	NA
2.00	6.30	NA	6.28	NA	6.39	NA	6.34	NA
2.25	6.14	NA	6.13	NA	6.30	NA	6.16	NA
2.50	6.14	NA	6.11	NA	6.31	NA	6.13	NA
2.75	6.14	NA	6.09	NA	6.29	NA	6.09	NA
3.00	6.12	NA	6.06	NA	6.29	NA	6.09	NA
3.25	6.11	NA	6.04	NA	6.28	NA	6.08	NA
3.50	6.08	NA	6.05	NA	6.32	NA	6.08	NA
3.75	6.11	NA	6.06	NA	6.31	NA	6.06	NA
4.00	6.11	NA	6.04	NA	6.29	NA	6.04	NA
	Total liquid added (mL)	4 mL DI		6 mL DI		5 mL DI		6 mL DI

Table AH.2. Weight of Bile Salts and Pancreatin Addition PA 16 Weeks

	Bile Salt Weight (g)	Pancreatin Weight (g)	Actual Bile Salt Weight (g)	Actual Pancreatin Weight (g)
Bottle 1	2.10	0.21	2.08	0.19
Bottle 2	2.10	0.21	2.07	0.17
Bottle 3	2.10	0.21	2.09	0.20
Bottle 4	2.10	0.21	2.05	0.19

Table AH.3. Standard Solution Calibration Values PA 16 Weeks

PPM	Adsorption	Concentration (µmol/L)
1	0.004	4.8
5	0.014	24.1
10	0.023	48.3
25	0.051	120.7
50	0.099	241.3
100	0.18	482.6

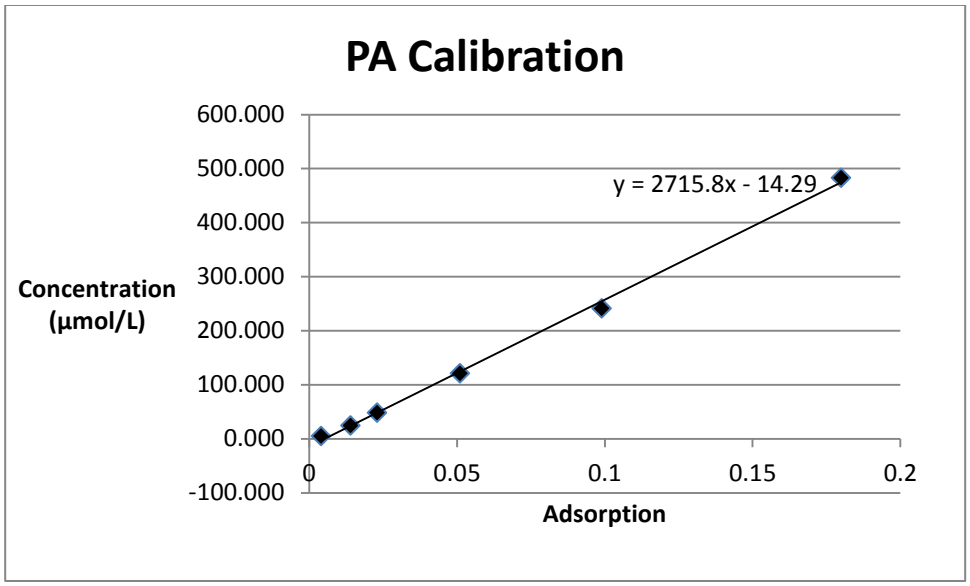


Figure AH.1. Standard Solution Calibration Curve PA 16 Weeks

Table AH.4. PBET/IVG Concentrations PA 16 Weeks

PA RESULTS				
	Adsorption	Concentration (µmol/L)	Concentration (mg/L)	Concentration (mg/kg)
PBET 1	0.008	5.6	1.2	26.4
IVG 1	0.003	-6.1	-1.3	-37.4
IVG 1	0.012	18.3	3.8	111.5
PBET 2	0.005	-3.2	-0.7	-14.5
IVG2	0.008	7.4	1.5	42.8
IVG 2	0.015	26.4	5.5	152.2
PBET 3	0.008	5.6	1.2	25.8
IVG 3	0.007	4.7	1.0	27.9
IVG 3	0.001	-11.6	-2.4	-68.5
PBET 4	0.006	-0.3	-0.1	-1.3
IVG 4	0.02	40.0	8.3	230.4
IVG 4	0.028	61.8	12.8	355.4

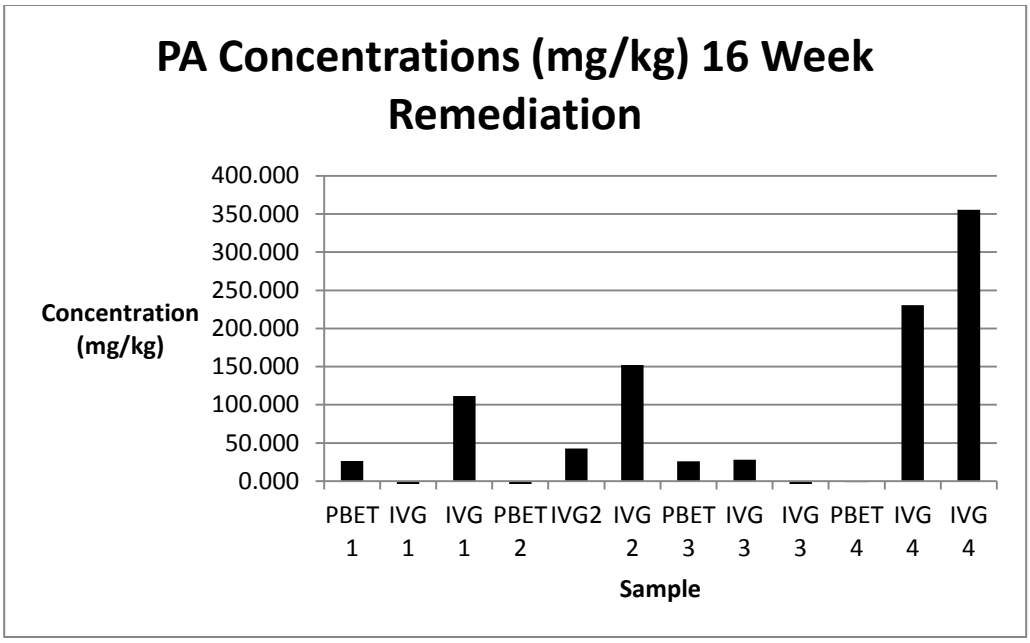


Figure AH.2. PBET/IVG Concentrations PA 16 Weeks

Table AH.5. pH from PBET/IVG Analysis TSP 16 Weeks

	Bottle 1	Addition 1	Bottle 2	Addition 2	Bottle 3	Addition 3	Bottle 4	Addition 4
Time								
0 min	1.74	NA	1.74	NA	1.74	NA	1.74	NA
5 min	1.62	10 mL DI	1.62	10 mL DI	1.59	15 mL DI	1.59	15 mL DI
10 min	1.74	NA	1.72	NA	1.76	NA	1.71	NA
0.25	1.72	NA	1.68	4 mL DI	1.73	NA	1.75	NA
0.50	1.70	NA	1.69	2 mL DI	1.69	2 mL DI	1.71	NA
0.75	1.69	2 mL DI	1.71	NA	1.71	NA	1.71	NA
1.00	1.71	NA	1.71	NA	1.71	NA	1.71	NA
1.25	1.71	NA	1.71	NA	1.72	NA	1.71	NA
1.50	1.72	NA	1.71	NA	1.71	NA	1.72	NA
1.75	1.71	NA	1.71	NA	1.71	NA	1.71	NA
2.00	6.10	NA	6.02	NA	5.96	NA	6.04	NA
2.25	6.08	NA	5.98	NA	5.93	NA	6.03	NA
2.50	6.08	NA	5.97	NA	5.93	NA	6.03	NA
2.75	6.08	NA	5.97	NA	5.93	NA	6.03	NA
3.00	6.09	NA	5.97	NA	5.93	NA	6.03	NA
3.25	6.08	NA	5.98	NA	5.94	NA	6.04	NA
3.50	6.10	NA	5.97	NA	5.96	NA	6.08	NA
3.75	6.14	NA	6.04	NA	5.99	NA	6.11	NA
4.00	6.13	NA	6.05	NA	6.04	NA	6.21	NA
	Total liquid added (mL)	12 mL DI		16 mL DI		17 mL DI		15 mL DI

Table AH.6. Weight of Bile Salts and Pancreatin Addition TSP 16 Weeks

	Bile Salt (g)	Pancreatin(g)	Actual Bile Salt (g)	Actual Pancreatin (g)
Bottle 1	2.10	0.21	2.07	0.18
Bottle 2	2.10	0.21	2.08	0.16
Bottle 3	2.10	0.21	2.10	0.20
Bottle 4	2.10	0.21	2.06	0.17

Table AH.7. Standard Solution Calibration Values TSP 16 Weeks

PPM	Adsorption	Concentration (µmol/L)
1	0.003	4.8
5	0.011	24.1
10	0.022	48.3
25	0.049	120.7
50	0.096	241.3
100	0.179	482.6

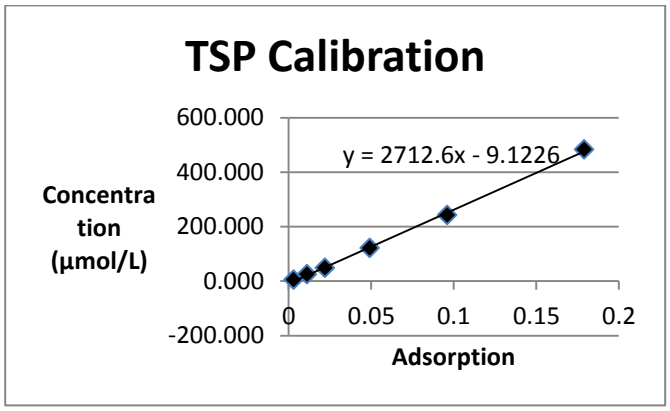


Figure AH.3. Standard Solution Calibration Curve TSP Four Weeks

Table AH.8. PBET/IVG Concentrations TSP 16 Weeks

TSP RESULTS				
	Adsorption	Concentration (µmol/L)	Concentration (mg/L)	Concentration (mg/kg)
PBET 1	0.008	5.5	1.1	21.7
IVG 1	0.013	26.1	5.4	128.9
IVG 1	0.026	61.4	12.7	302.9
PBET 2	0.006	-0.4	-0.1	-1.5
IVG2	0.007	9.9	2.0	44.4
IVG 2	0.01	18.0	3.7	81.1
PBET 3	0.006	-0.4	-0.1	-1.4
IVG 3	0.022	50.5	10.5	183.7
IVG 3	0.02	45.1	9.3	164.0
PBET 4	0.01	11.3	2.3	42.6
IVG 4	0.008	12.6	2.6	57.9
IVG 4	0.01	18.0	3.7	82.9

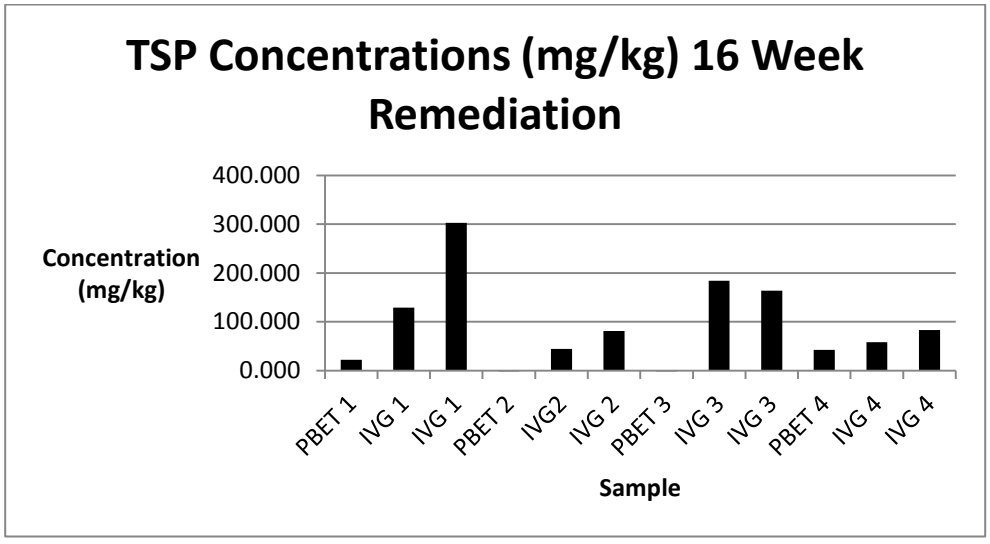


Figure AH.4. PBET/IVG Concentrations TSP 16 Weeks

Table AH.9. pH from PBET/IVG Analysis BM 16 Weeks

	Bottle 1	Addition 1	Bottle 2	Addition 2	Bottle 3	Addition 3	Bottle 4	Addition 4
Time								
0 min	1.70	NA	1.70	NA	1.70	NA	1.70	NA
5 min	1.62	10 mL DI	1.61	12 mL DI	1.60	15 mL DI	1.59	16 mL DI
10 min	1.71	NA	1.72	NA	1.75	NA	1.80	NA
0.25	1.70	NA	1.70	NA	1.73	NA	1.79	NA
0.50	1.71	NA	1.69	2 mL DI	1.73	NA	1.77	NA
0.75	1.71	NA	1.72	NA	1.74	NA	1.77	NA
1.00	1.72	NA	1.72	NA	1.76	NA	1.78	NA
1.25	1.72	NA	1.73	NA	1.77	NA	1.78	NA
1.50	1.74	NA	1.73	NA	1.77	NA	1.78	NA
1.75	1.75	NA	1.74	NA	1.78	NA	1.78	NA
2.00	6.38	NA	6.34	NA	6.12	0.5 mL NaCO3	6.08	0.5 mL NaCO3
2.25	6.39	NA	6.37	NA	6.09	NA	5.63	
2.50	6.41	NA	6.39	NA	6.11	NA	6.19	NA
2.75	6.29	NA	6.19	NA	6.11	NA	6.03	NA
3.00	6.41	NA	6.38	NA	6.16	NA	5.98	NA
3.25	6.44	NA	6.37	NA	6.18	NA	5.96	NA
3.50	6.44	NA	6.38	NA	6.14	NA	5.92	NA
3.75	6.43	NA	6.37	NA	6.16	NA	5.87	NA
4.00	6.45	NA	6.36	NA	6.13	NA		
	Total liquid added (mL)	10 mL DI		14 mL DI		15 mL DI		16 mL DI

Table AH.10. Weight of Bile Salts and Pancreatin Addition BM 16 Weeks

	Bile Salt Weight (g)	Pancreatin Weight (g)	Actual Bile Salt Weight (g)	Actual Pancreatin Weight (g)
Bottle 1	2.10	0.21	2.16	0.19
Bottle 2	2.10	0.21	2.18	0.18
Bottle 3	2.10	0.21	2.17	0.19
Bottle 4	2.10	0.21	2.16	0.16

Table AH.11. Standard Solution Calibration Values BM 16 Weeks

PPM	Adsorption	Concentration (µmol/L)
1	0.003	4.8
5	0.011	24.1
10	0.023	48.3
25	0.054	120.7
50	0.106	241.3
100	0.188	482.6

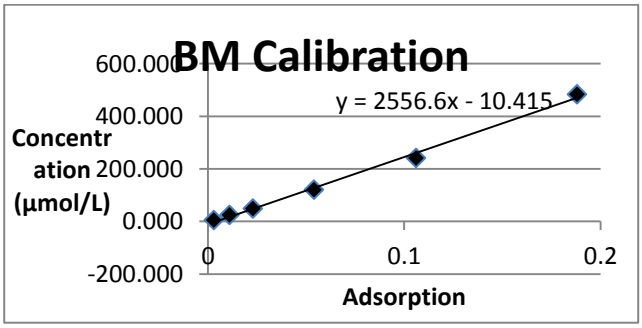


Figure AH.5. Standard Solution Calibration Curve BM 16 Weeks

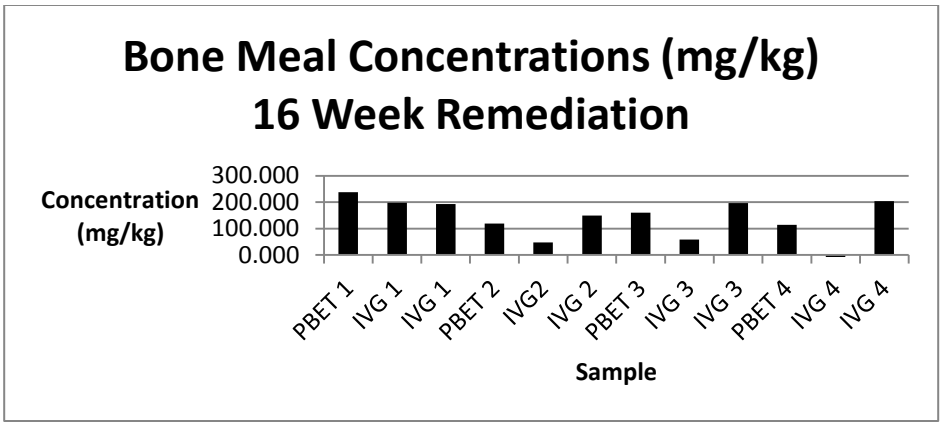


Figure AH.6. PBET/IVG Concentrations BM 16 Weeks

Table AH.12. PBET/IVG Concentrations BM 16 Weeks

BM RESULTS				
	Adsorption	Concentration (µmol/L)	Concentration (mg/L)	Concentration (mg/kg)
PBET 1	0.018	57.3	11.9	237.3
IVG 1	0.019	38.2	7.9	197.6
IVG 1	0.012	37.2	7.7	192.8
PBET 2	0.009	30.9	6.4	118.7
IVG2	0.008	10.0	2.1	47.2
IVG 2	0.01	31.8	6.6	149.7
PBET 3	0.013	42.6	8.8	160.6
IVG 3	0.009	12.6	2.6	58.0
IVG 3	0.014	42.7	8.8	196.4
PBET 4	0.009	30.9	6.4	114.5
IVG 4	0.003	-2.8	-0.6	-12.4
IVG 4	0.015	45.4	9.4	204.4

APPENDIX I.

20 WEEK RESULTS

Table AI.1. pH from PBET/IVG Analysis PA 20 Weeks

	Bottle 1	Addition 1	Bottle 2	Addition 2	Bottle 3	Addition 3	Bottle 4	Addition 4
Time								
0 min	1.7	NA	1.7	NA	1.7	NA	1.7	NA
5 min	1.75	NA	1.78	NA	1.81	NA	1.79	NA
10 min	1.8	NA	1.81	NA	1.79	NA	1.82	NA
0.25	1.76	NA	1.7	NA	1.7	NA	1.71	NA
0.50	1.75	NA	1.68	2 mL DI	1.71		1.7	NA
0.75	1.75	NA	1.73	NA	1.73	NA	1.69	2 mL DI
1.00	1.76	NA	1.71	NA	1.68	3 mL DI	1.72	NA
1.25	1.7	NA	1.68	2 mL DI	1.69	1 mL DI	1.7	NA
1.50	1.7	NA	1.7	NA	1.71	NA	1.68	2 mL DI
1.75	1.71	NA	1.72	NA	1.72	NA	1.7	NA
2.00	6.04	NA	6.29	NA	6.29	NA	6.4	NA
2.25	6.05	NA	6.29	NA	6.31	NA	6.09	NA
2.50	6.05	NA	6.32	NA	6.32	NA	6.05	NA
2.75	6.1	NA	6.35	NA	6.34	NA	6.03	NA
3.00	6.05	NA	6.38	NA	6.37	NA	6.06	NA
3.25	6.11	NA	6.35	NA	6.38	NA	6.08	NA
3.50	6.15	NA	6.38	NA	6.35	NA	6.11	NA
3.75	6.15	NA	6.42	NA	6.4	NA	6.17	NA
4.00	6.21	NA	6.42	NA	6.44	NA	6.18	NA
	Total liquid added (mL)	0 mL DI		4 mL DI		4 mL DI		4 mL DI

Table AI.2. Weight of Bile Salts and Pancreatin Addition PA 20 Weeks

	Bile Salt Weight (g)	Pancreatin Weight (g)	Actual Bile Salt Weight (g)	Actual Pancreatin Weight (g)
Bottle 1	2.10	0.21	2.08	0.19
Bottle 2	2.10	0.21	2.07	0.17
Bottle 3	2.10	0.21	2.09	0.20
Bottle 4	2.10	0.21	2.05	0.19

Table AI.3. Standard Solution Calibration Values PA 20 Weeks

PPM	Adsorption	Concentration (µmol/L)
1	0.004	4.8
5	0.01	24.1
10	0.017	48.3
25	0.04	120.7
50	0.084	241.3
100	0.162	482.6

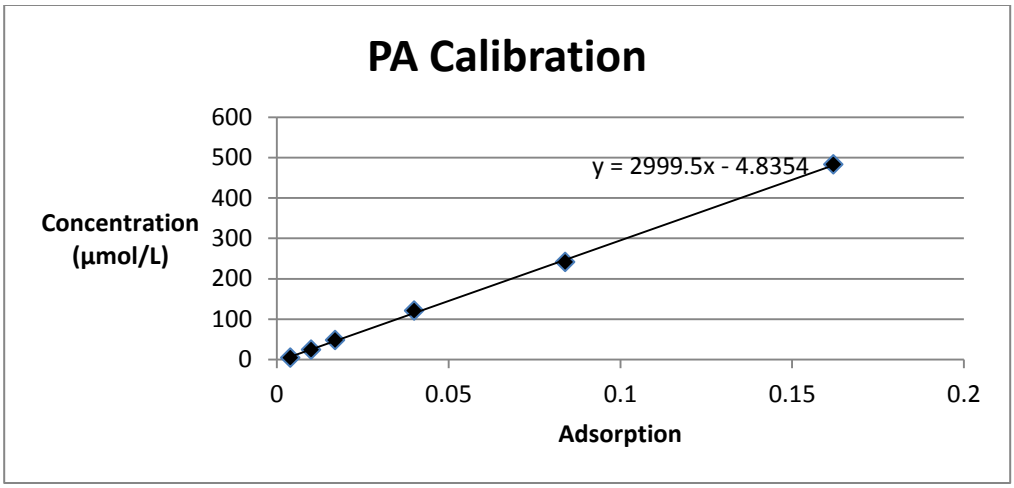


Figure AI.1. Standard Solution Calibration Curve PA 20 Weeks

Table AI.4. PBET/IVG Concentrations PA 20 Weeks

PA RESULTS				
	Adsorption	Concentration (µmol/L)	Concentration (mg/L)	Concentration (mg/kg)
PBET 1	0.005	10.2	2.1	52.6
IVG 1	0.004	7.2	1.5	49.5
IVG 1	0.003	4.2	0.9	28.8
PBET 2	0.008	19.2	4.0	90.2
IVG2	0.001	-1.8	-0.4	-11.5
IVG 2	0.004	7.2	1.5	45.0
PBET 3	0.006	13.2	2.7	62.0
IVG 3	0	-4.8	-1.0	-30.4
IVG 3	0.002	1.2	0.2	7.3
PBET 4	0.007	16.2	3.3	76.1
IVG 4	0.001	-1.8	-0.4	-11.5
IVG 4	0.003	4.2	0.9	26.1

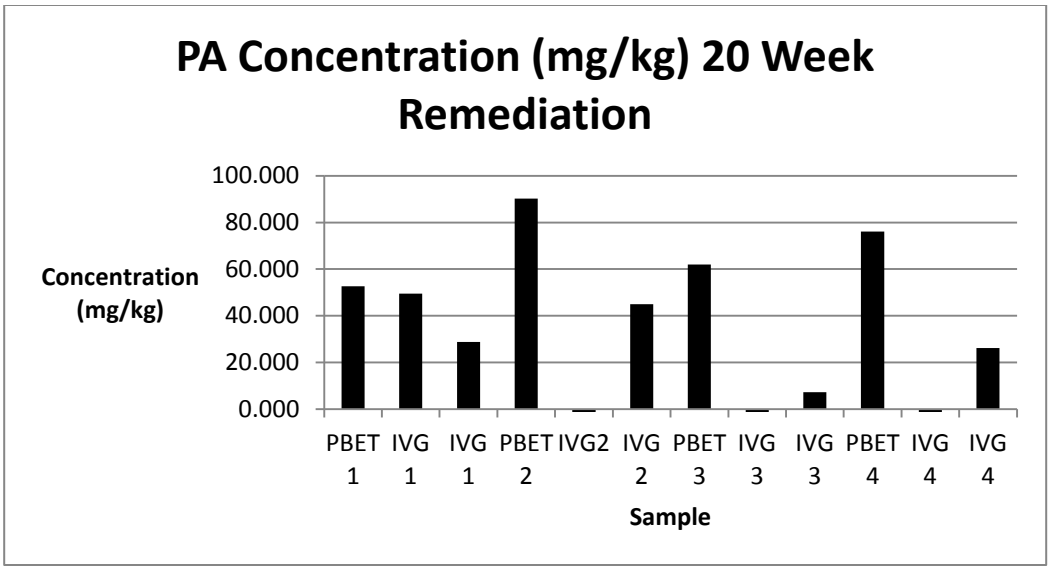


Figure AI.2. PBET/IVG Concentrations PA Four Weeks

Table AI.5. GFAA PBET/IVG Concentrations PA 20 Weeks

SAMPLE	Concentration (µg/L)	Concentration (µg/L)	Concentration (mg/L)	Concentration (mg/kg)	Std. Dev
PBET 1	44.76	44.8	4.5	124.3	0.47
IVG 1	BDL	0.0	0.0	0.0	0.08
IVG 1	9.64	9.6	1.0	26.8	0.15
PBET 2	15.74	15.7	1.6	43.7	0.03
IVG 2	8.83	8.8	0.9	24.5	0.18
IVG 2	1.54	1.5	0.2	4.3	0.11
PBET 3	11.44	11.4	1.1	31.8	0.08
IVG 3	3.65	3.7	0.4	10.1	0.02
IVG 3	1.71	1.7	0.2	4.8	0.08
PBET 4	64.97	65.0	6.5	180.5	1.3
IVG 4	1.02	1.0	0.1	2.8	0.02
IVG 4	6.21	6.2	0.6	17.3	0.03

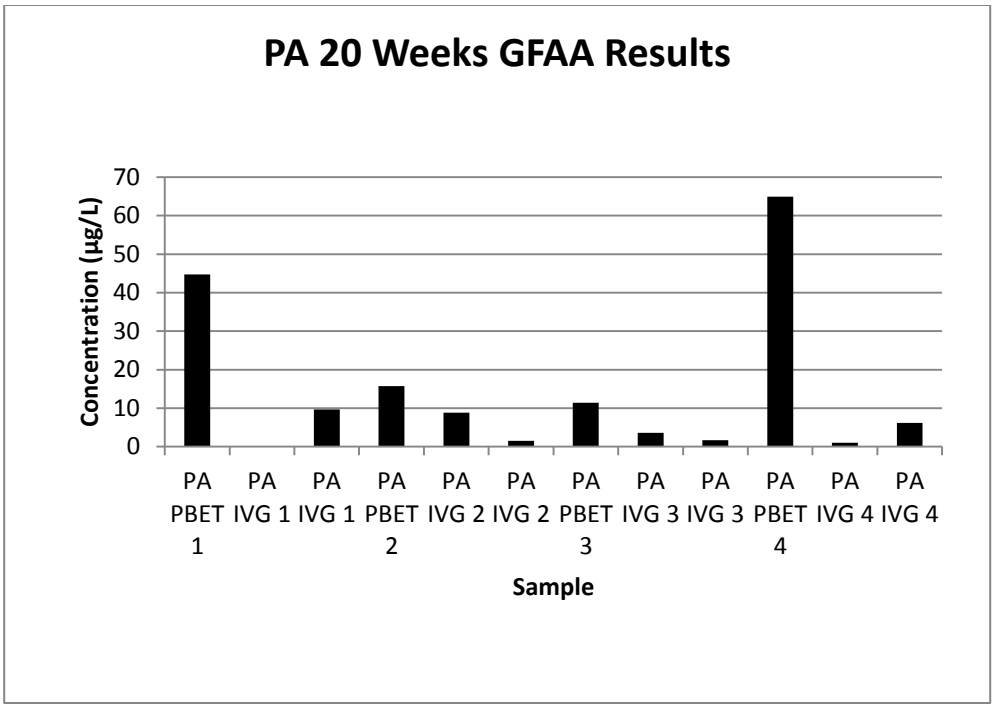


Figure AI.3. GFAA PBET/IVG Concentrations PA 20 Weeks

Table AI.6. pH from PBET/IVG Analysis TSP 20 Weeks

	Bottle 1	Addition 1	Bottle 2	Addition 2	Bottle 3	Addition 3	Bottle 4	Addition 4
Time								
0 min	1.62	NA	1.62	NA	1.62	NA	1.62	NA
5 min	1.68	NA	1.62	NA	1.62	NA	1.62	NA
10 min	1.67	NA	1.62	NA	1.62	NA	1.62	NA
0.25	1.65	NA	1.62	NA	1.62	NA	1.62	NA
0.50	1.67	2 mL DI	1.62	3 mL DI	1.62	4 mL DI	1.62	6 mL DI
0.75	1.67	1 mL DI	1.62	3 mL DI	1.62	1 mL DI	1.62	NA
1.00	1.67	1 mL DI	1.62	NA	1.62	NA	1.62	NA
1.25	1.67	NA	1.62	NA	1.62	NA	1.62	NA
1.50	1.67	NA	1.62	NA	1.62	NA	1.62	NA
1.75	1.67	NA	1.62	NA	1.62	NA	1.62	NA
2.00	1.67	NA	1.62	NA	1.62	NA	1.62	NA
2.25	1.67	NA	1.62	NA	1.62	NA	1.62	NA
2.50	1.67	NA	1.62	NA	1.62	NA	1.62	NA
2.75	1.67	NA	1.62	NA	1.62	NA	1.62	NA
3.00	1.67	NA	1.62	NA	1.62	NA	1.62	NA
3.25	1.67	NA	1.62	NA	1.62	NA	1.62	NA
3.50	1.67	NA	1.62	NA	1.62	NA	1.62	NA
3.75	1.67	NA	1.62	NA	1.62	NA	1.62	NA
4.00	1.67	NA	1.62	NA	1.62	NA	1.62	NA
	Total added (mL)	4 mL DI		6 mL DI		5 mL DI		6 mL DI

Table AI.7. Weight of Bile Salts and Pancreatin Addition TSP 20 Weeks

	Bile Salt Weight (g)	Pancreatin Weight (g)	Actual Bile Salt Weight (g)	Actual Pancreatin Weight (g)
Bot 1	2.10	0.21	2.08	0.19
Bot 2	2.10	0.21	2.07	0.17
Bot 3	2.10	0.21	2.09	0.20
Bot 4	2.10	0.21	2.05	0.19

Table AI.8. Standard Solution Calibration Values TSP 20 Weeks

PPM	Adsorption	Concentration (µmol/L)
1	0.002	4.8
5	0.008	24.1
10	0.015	48.3
25	0.038	120.7
50	0.075	241.3
100	0.148	482.6

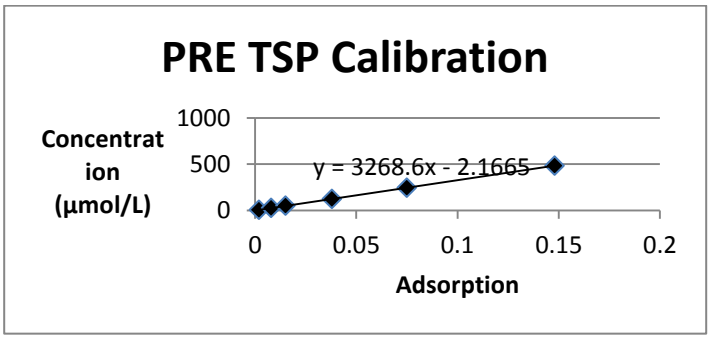


Figure AI.4. Standard Solution Calibration Curve TSP 20 Weeks

Table AI.9. PBET/IVG Concentrations TSP 20 Weeks

TSP RESULTS				
	Adsorption	Concentration (µmol/L)	Concentration (mg/L)	Concentration (mg/kg)
PBET 1	0.007	20.7	4.3	87.6
IVG 1	0.007	20.7	4.3	110.0
IVG 1	0.006	17.4	3.6	92.7
PBET 2	0.003	7.6	1.6	31.7
IVG2	0.003	7.6	1.6	39.6
IVG 2	0.002	4.4	0.9	22.6
PBET 3	0.004	10.9	2.3	52.6
IVG 3	0.005	14.2	2.9	89.0
IVG 3	0.003	7.6	1.6	48.0
PBET 4	0.01	30.5	6.3	117.1
IVG 4	0.004	10.9	2.3	51.4
IVG 4	0.005	14.2	2.9	66.8

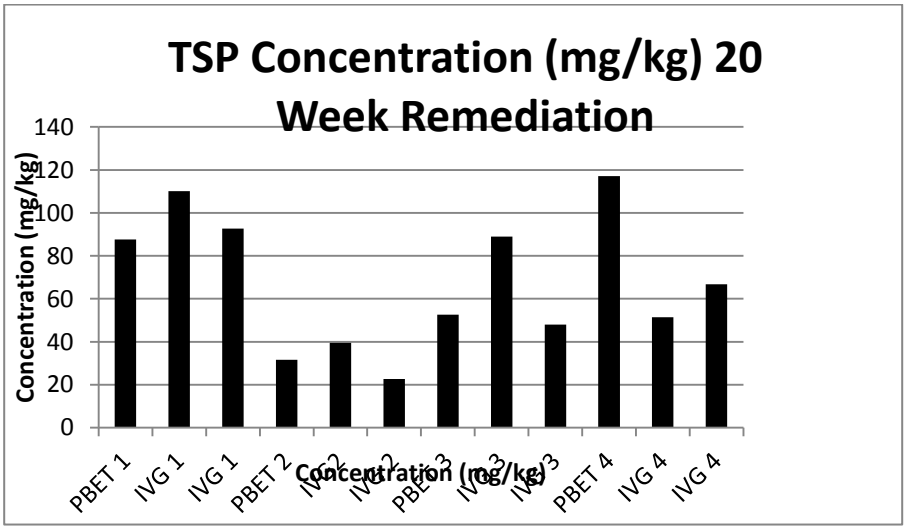


Figure AI.5. PBET/IVG Concentrations TSP 20 Weeks

Table AI.10. GFAA PBET/IVG Concentrations TSP 20 Weeks

SAMPLE	Concentration (µg/L)	Concentration (µg/L)	Concentration (mg/L)	Concentration (mg/kg)	Std. Dev
TSP PBET 1	71.03	71.0	7.1	197.3	0.01
TSP IVG 1	6.05	6.1	0.6	16.8	0.07
TSP IVG 1	8.53	8.5	0.9	23.7	0.11
TSP PBET 2	6.50	6.5	0.7	18.1	0
TSP IVG 2	5.16	5.2	0.5	14.3	0.04
TSP IVG 2	3.07	3.1	0.3	8.5	0.04
TSP PBET 3	7.06	7.1	0.7	19.6	0.06
TSP IVG 3	16.57	16.6	1.7	46.0	0.01
TSP IVG 3	BDL	0.0	0.0	0.0	0.32
TSP PBET 4	31.50	31.5	3.2	87.5	0.01
TSP IVG 4	4.55	4.6	0.5	12.6	0
TSP IVG 4	1.98	2.0	0.2	5.5	0.06

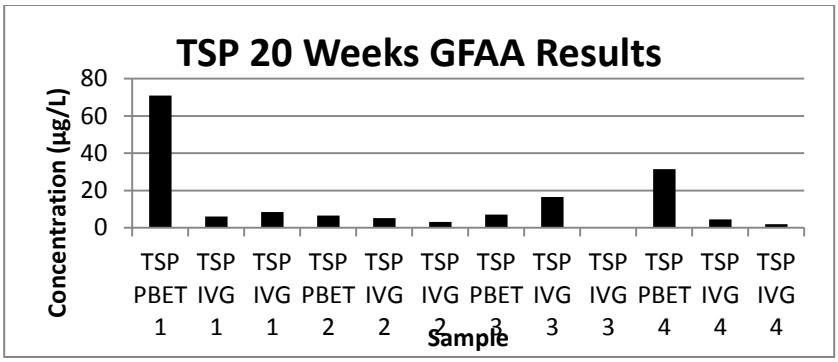


Figure AI.6. GFAA PBET/IVG Concentrations TSP 20 Weeks

Table AI.11. pH from PBET/IVG Analysis BM 20 Weeks

	Bot 1	Add 1	Bot 2	Add 2	Bot 3	Add 3	Bot 4	Add 4
Time								
0	1.62	NA	1.62	NA	1.62	NA	1.62	NA
5	1.68	NA	1.62	NA	1.62	NA	1.62	NA
10	1.67	NA	1.62	NA	1.62	NA	1.62	NA
0.25	1.65	NA	1.62	NA	1.62	NA	1.62	NA
0.50	1.67	2 mL DI	1.62	3 mL DI	1.62	4 mL DI	1.62	6 mL DI
0.75	1.67	1 mL DI	1.62	3 mL DI	1.62	1 mL DI	1.62	NA
1.00	1.67	1 mL DI	1.62	NA	1.62	NA	1.62	NA
1.25	1.67	NA	1.62	NA	1.62	NA	1.62	NA
1.50	1.67	NA	1.62	NA	1.62	NA	1.62	NA
1.75	1.67	NA	1.62	NA	1.62	NA	1.62	NA
2.00	1.67	NA	1.62	NA	1.62	NA	1.62	NA
2.25	1.67	NA	1.62	NA	1.62	NA	1.62	NA
2.50	1.67	NA	1.62	NA	1.62	NA	1.62	NA
2.75	1.67	NA	1.62	NA	1.62	NA	1.62	NA
3.00	1.67	NA	1.62	NA	1.62	NA	1.62	NA
3.25	1.67	NA	1.62	NA	1.62	NA	1.62	NA
3.50	1.67	NA	1.62	NA	1.62	NA	1.62	NA
3.75	1.67	NA	1.62	NA	1.62	NA	1.62	NA
4.00	1.67	NA	1.62	NA	1.62	NA	1.62	NA
	Total (mL)	4 mL DI		6 mL DI		5 mL DI		6 mL DI

Table AI.12. Weight of Bile Salts and Pancreatin Addition BM 20 Weeks

	Bile Salt Weight (g)	Pancreatin Weight (g)	Actual Bile Salt Weight (g)	Actual Pancreatin Weight (g)
Bottle 1	2.10	0.21	2.08	0.19
Bottle 2	2.10	0.21	2.07	0.17
Bottle 3	2.10	0.21	2.09	0.20
Bottle 4	2.10	0.21	2.05	0.19

Table AI.13. Standard Solution Calibration Values BM 20 Weeks

PPM	Adsorption	Concentration (µmol/L)
0.1	0	0.5
0.5	0.003	2.4
1	0.005	4.8
2.5	0.006	12.1
5	0.009	24.1
10	0.019	48.3
25	0.046	120.7
50	0.088	241.3
100	0.176	482.6

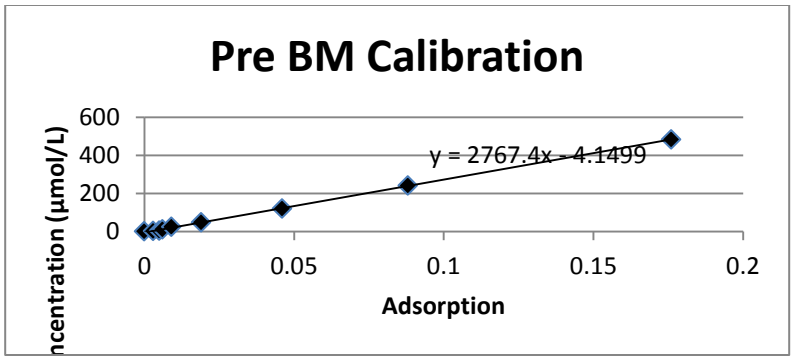


Figure AI.7. Standard Solution Calibration Curve BM 20 Weeks

Table AI.14. PBET/IVG Concentrations BM 20 Weeks

BM RESULTS				
	Adsorption	Concentration (µmol/L)	Concentration (mg/L)	Concentration (mg/kg)
PBET 1	0.006	12.5	2.6	46.9
IVG 1	0.005	9.7	2.0	44.6
IVG 1	0.005	9.7	2.0	44.6
PBET 2	0.007	15.2	3.2	60.7
IVG2	0.005	9.7	2.0	47.8
IVG 2	0.006	12.5	2.6	61.4
PBET 3	0.01	23.5	4.9	92.0
IVG 3	0.002	1.4	0.3	6.7
IVG 3	0.001	-1.4	-0.3	-6.7
PBET 4	0.012	29.1	6.0	102.1
IVG 4	0.002	1.4	0.3	5.9
IVG 4	0.003	4.2	0.9	17.6

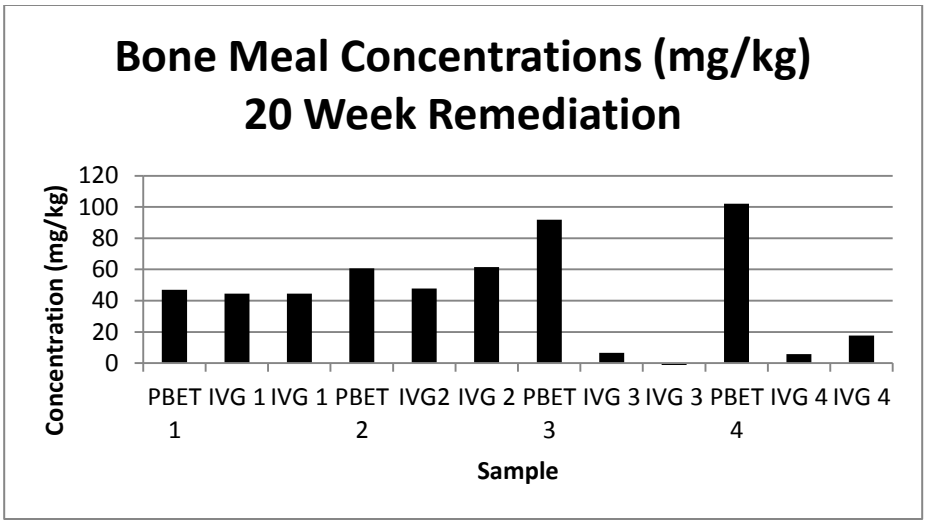


Figure AI.8. PBET/IVG Concentrations BM 20 Weeks

Table AI.15. GFAA PBET/IVG Concentrations BM 20 Weeks

SAMPLE	Concentration (µg/L)	Concentration (µg/L)	Concentration (mg/L)	Concentration (mg/kg	Std. Dev
BM PBET 1	35.87	35.9	3.6	99.6	0.6
BM IVG 1	18.63	18.6	1.9	51.8	0.4
BM IVG 1	8.25	8.3	0.8	22.9	0.2
BM PBET 2	20.67	20.7	2.1	57.4	0.2
BM IVG 2	2.32	2.3	0.2	6.4	0.1
BM IVG 2	18.45	18.5	1.8	51.3	0.9
BM PBET 3	75.56	75.6	7.6	209.9	0.1
BM IVG 3	33.46	33.5	3.3	92.9	0.5
BM IVG 3	3.44	3.4	0.3	9.6	0.1
BM PBET 4	48.78	48.8	4.9	135.5	0.0
BM IVG 4	6.63	6.6	0.7	18.4	0.1
BM IVG 4	14.84	14.8	1.5	41.2	0.1

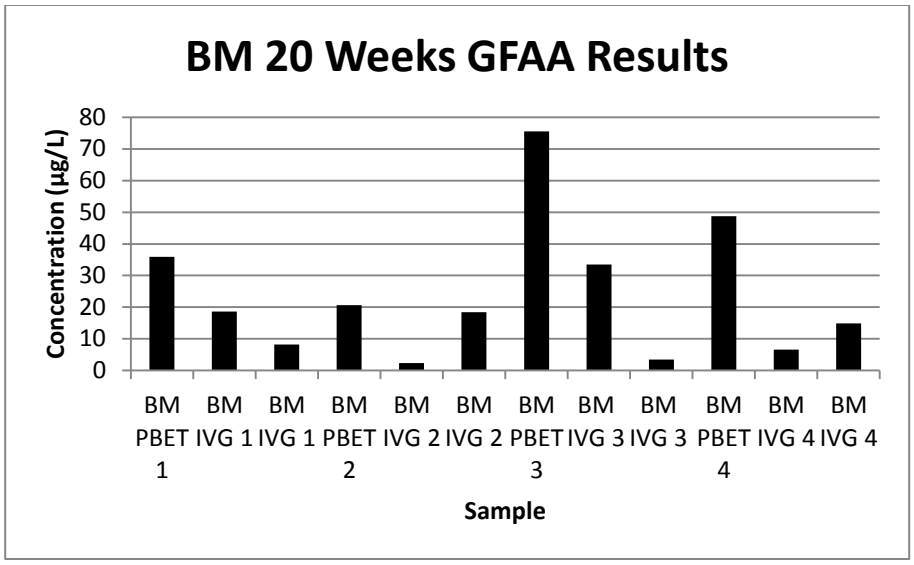


Figure AI.9. GFAA PBET/IVG Concentrations BM 20 Weeks

APPENDIX J.

SEM/XRD RESULTS

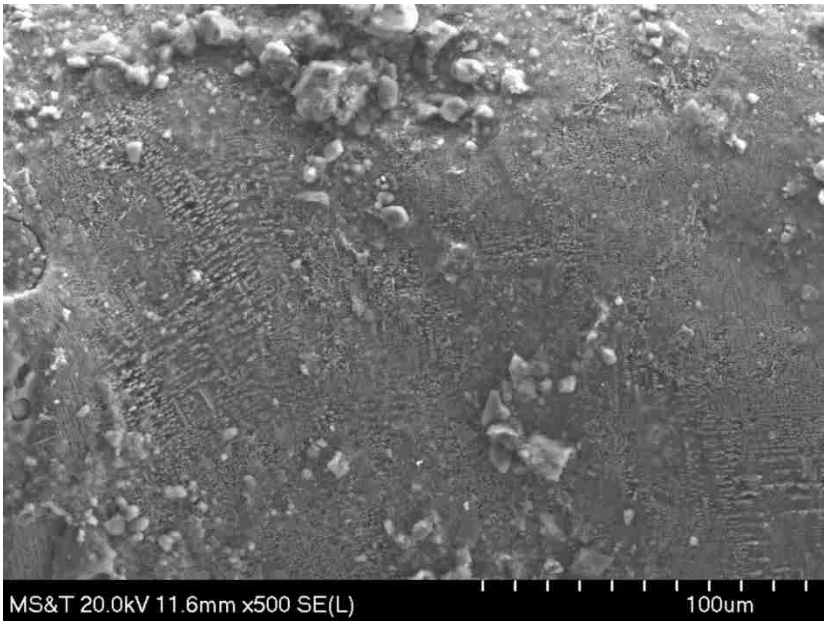


Figure AJ.1. Confirmed Lead Sulfide Formation in Control Sample 1 Using SEM

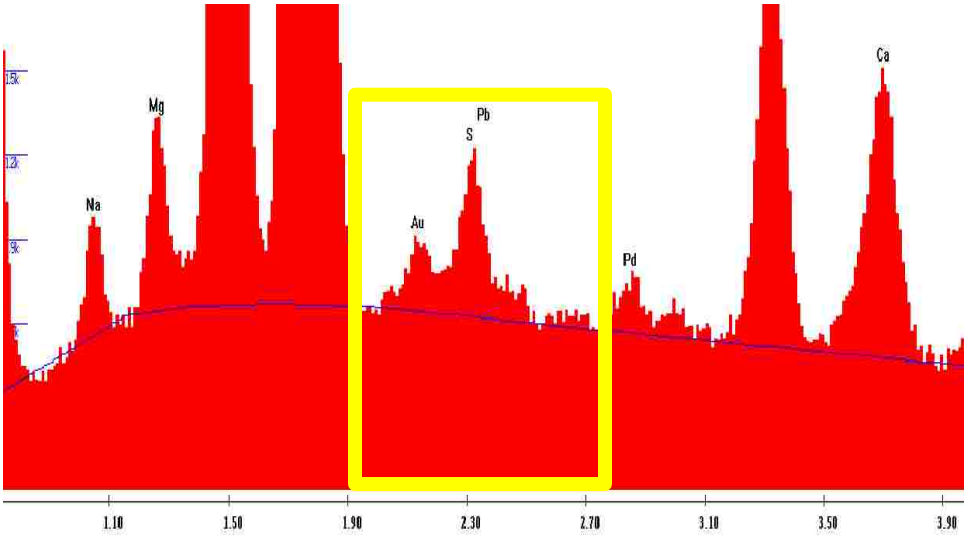


Figure AJ.2. Confirmed Lead Sulfide Peaks in Control Sample 1

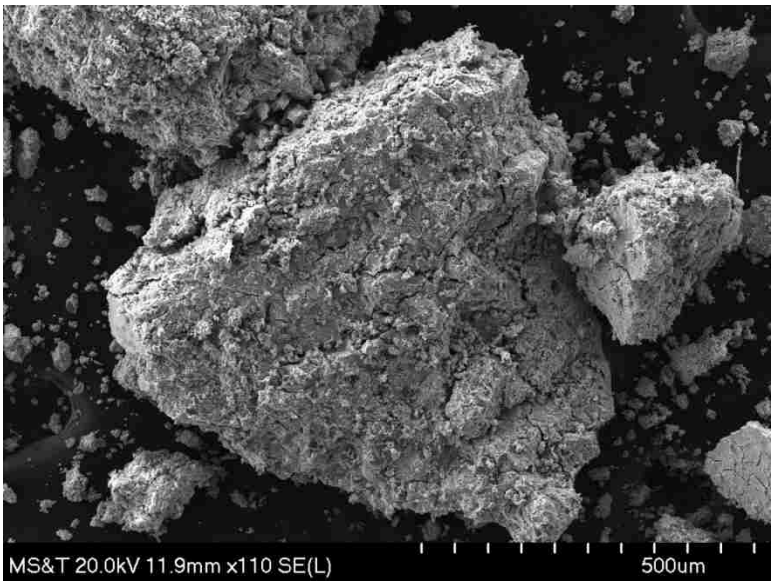


Figure AJ.3. Confirmed Lead Particles in Control Sample 2 Using SEM

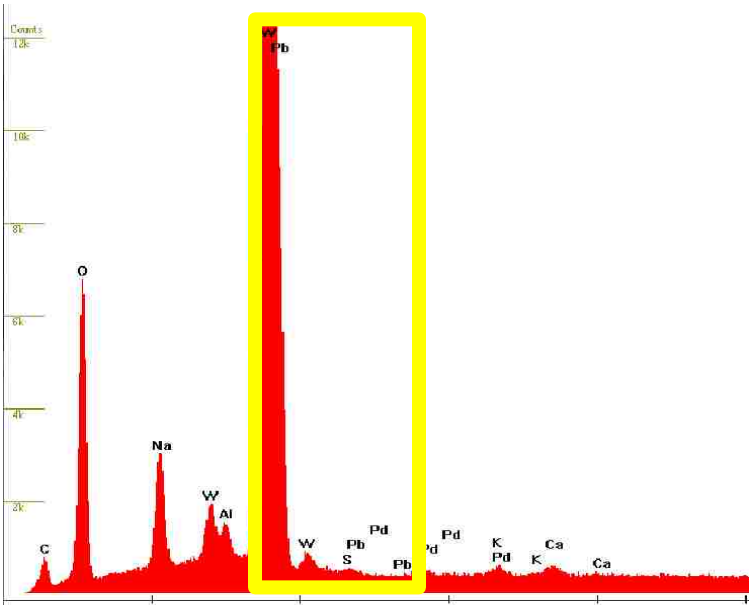


Figure AJ.4. Confirmed Lead Peaks in Control Sample 2

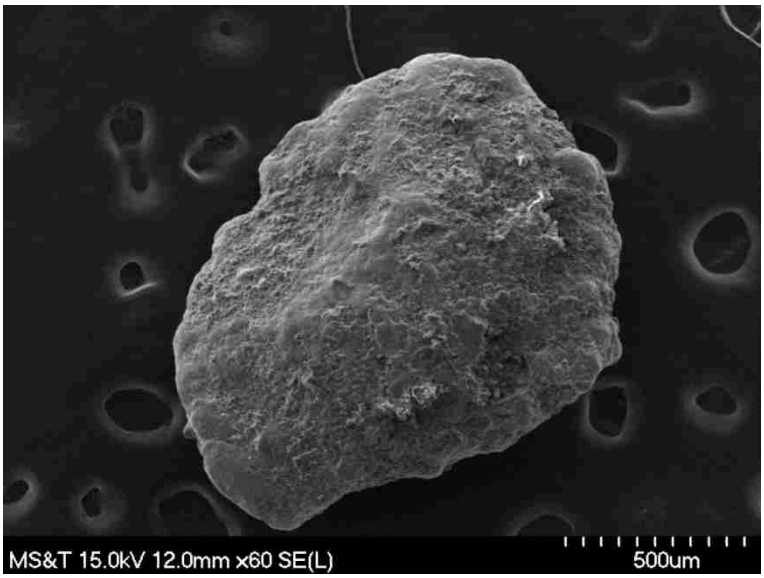


Figure AJ.5. Confirmed Lead and Calcium Phosphate Using SEM, 16 Week Remediation with TSP

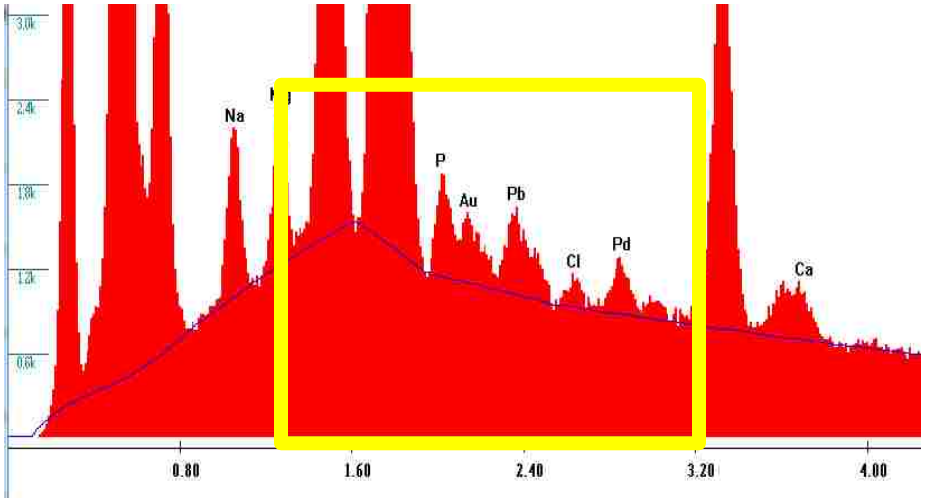


Figure AJ.6. Confirmed Lead and Calcium Phosphate Peaks, 16 week Remediation with TSP

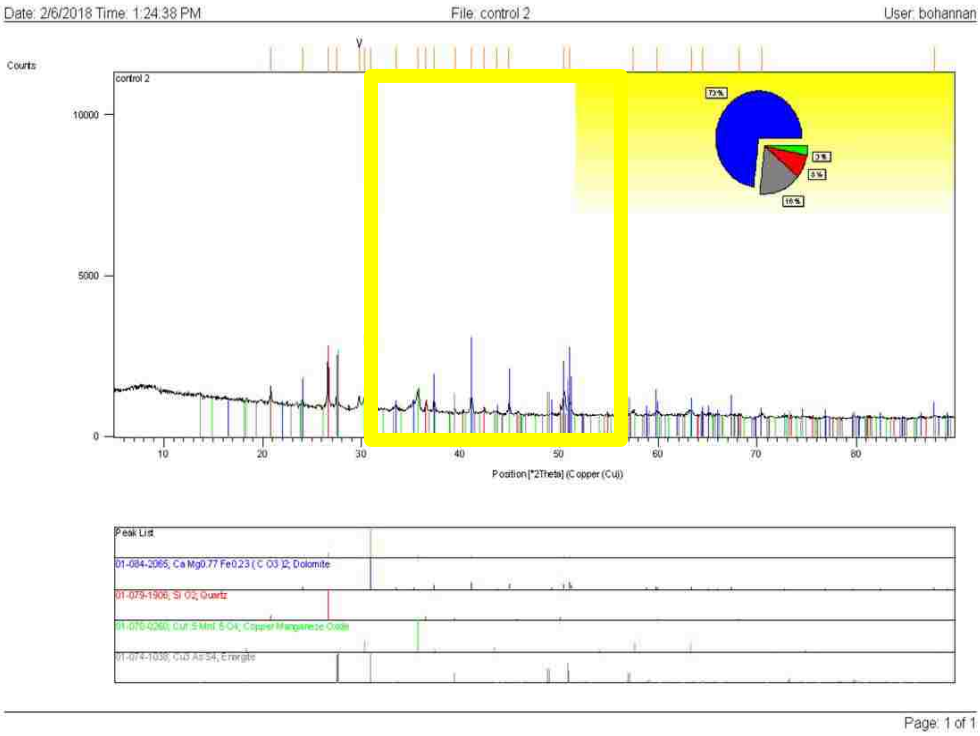


Figure AJ.7. XRD Confirmation of Quartz and Dolomite in Control Sample

As the rectangle indicates on the graph, the blue and red peaks indicate dolomite and quartz crystal peaks present in this sample. However, as shown above, during SEM-EDS, lead particles discovered in each of the control samples.

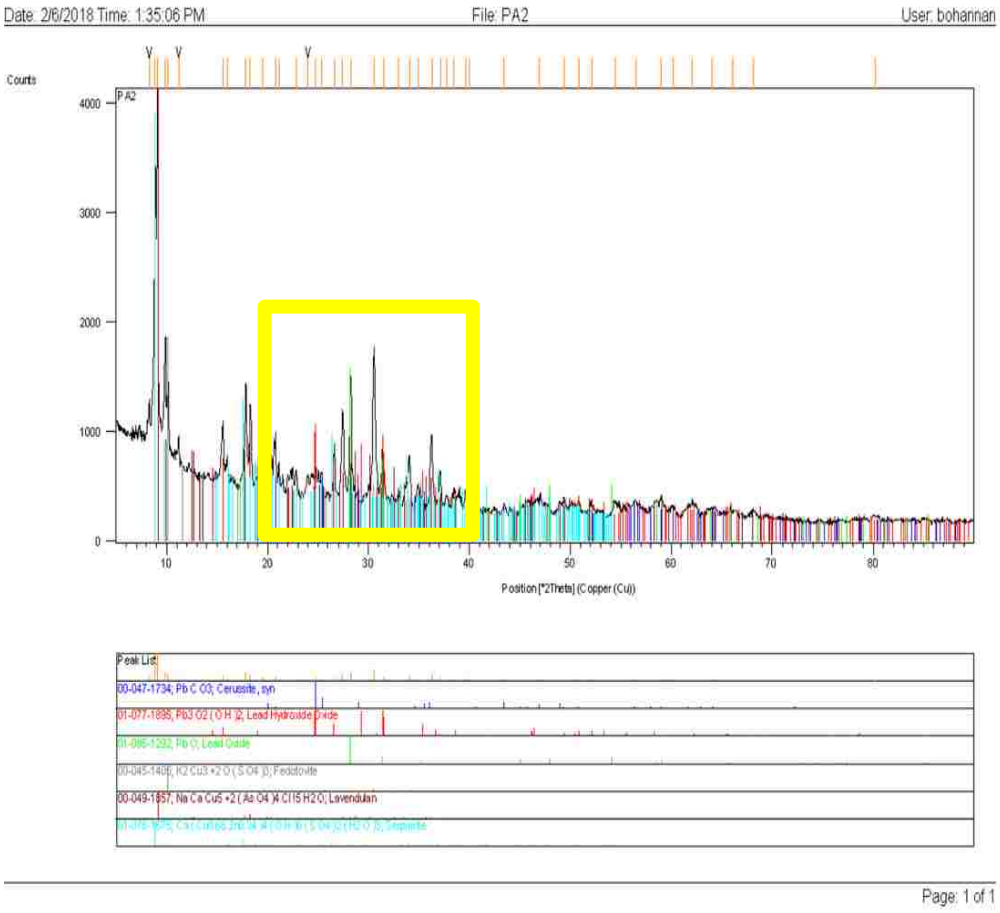


Figure AJ.8. XRD Reading on PA Sample 2 after 16 Week Remediation

As the rectangle on the graph indicates, lead hydroxide, and lead oxide may be present here. Lead hydroxide and oxide are shown in the red and green peaks within the highlighted rectangle. However, the diffraction peaks did not give a definite confirmation of lead compounds from this sample. This sample was not able to be analyzed using SEM-EDS because the particles analyzed using XRD were lost

when trying to transfer the material from the loading slide of the XRD back to the test vial.

Not all samples analyzed using SEM-EDS were analyzed using XRD. If all samples were analyzed using XRD, the risk of losing particles and the precision of analysis would likely occur.

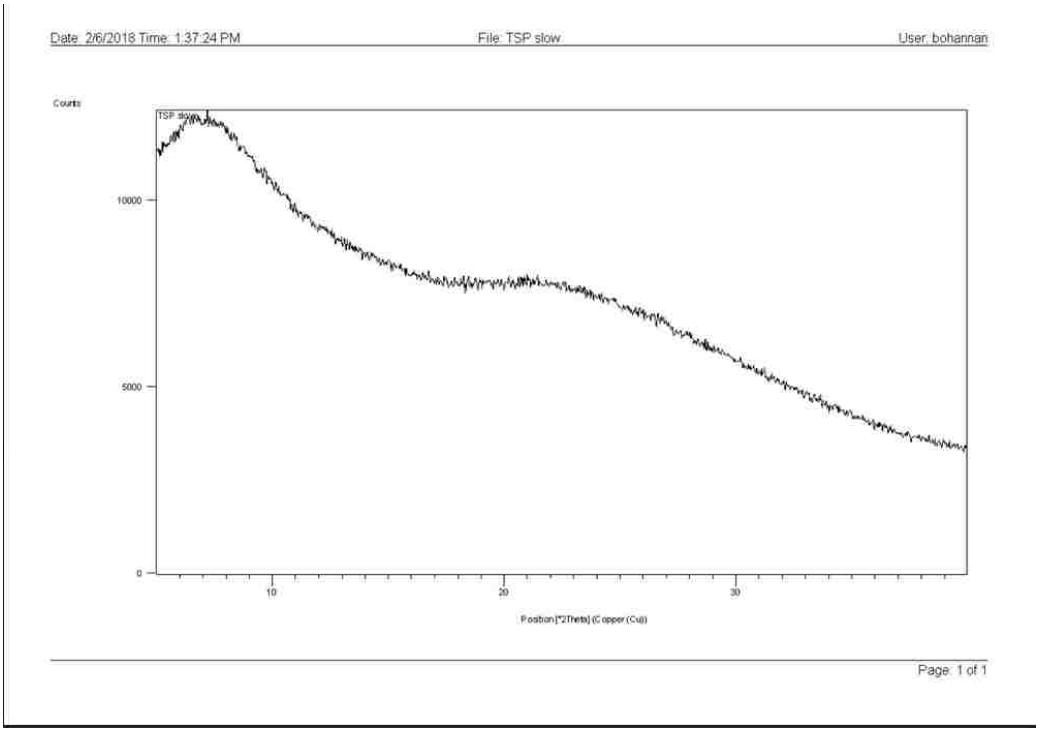


Figure AJ.9. XRD Reading on TSP 2 after 16 Week Remediation

This sample was analyzed to see if the XRD was able to record any peaks from the very small particle that was used. Results showed that there may have been a

small piece of lead oxide present within this sample, but there was not a definitive peak to confirm formation. However, analyzing this sample using SEM-EDS confirmed a lead and calcium phosphate compound present within this particle.

APPENDIX K.

KSP'S OF VARYING PH VALUES ANALYZED USING PHREEQC

Table AK.1. Ksp's of Chloropyromorphite from Varying pH Values and Phosphate Sources

pH	Phosphate Source	KSP from PHREEQC
1.50	H_3PO_4	$10^{-35.19}$
1.75	H_3PO_4	$10^{-36.68}$
2.00	H_3PO_4	$10^{-38.19}$
2.25	H_2PO_4-	$10^{-39.69}$
2.50	H_2PO_4-	$10^{-41.20}$
3.00	H_2PO_4-	$10^{-44.21}$
3.50	H_2PO_4-	$10^{-47.24}$
4.00	H_2PO_4-	$10^{-50.28}$
4.50	H_2PO_4-	$10^{-53.33}$
5.00	H_2PO_4-	$10^{-56.41}$
5.50	H_2PO_4-	$10^{-59.52}$
6.00	H_2PO_4-	$10^{-62.66}$
6.50	H_2PO_4-	$10^{-65.72}$
7.00	H_2PO_4-	$10^{-68.43}$

APPENDIX L.

CHEMICALS USED AND EXPIRATION DATES

Table AL.1. Chemicals and Expiration Dates

Chemical	Expiration Date
Acetic Acid	6/30/1994
Lactic Acid	6/10/2014
Hydrochloric Acid	3/30/2004
Nitric Acid	8/31/2005
Lead Nitrate	6/6/1995
Sodium Chloride	11/22/1999
Sodium Phosphate	7/30/2001
Malic Acid	6/10/2014
Citric Acid	6/14/1995
Bile Salts	NA
Pancreatin	NA
Sodium Carbonate	6/15/1995
Calcium Chloride	6/8/1995
Phosphoric Acid	8/27/2010
Triple Super Phosphate	NA
Organic Bone Meal	NA
Sulfuric Acid	6/30/1994
Silicate Based Sand	NA
Sodium Metatungstate	NA

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