

UNITED STATES DEPARTMENT OF INTERIOR
OFFICE OF SURFACE MINING RECLAMATION AND ENFORCEMENT
APPLIED SCIENCE PROGRAM COOPERATIVE AGREEMENT

**Low-cost, Green Technology to Improve Water Quality in Mining-Impacted
Ecosystems, Phase I – Model Development and Optimization**

OSM Cooperative Agreement No. S12AC20001

FINAL TECHNICAL REPORT

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September, 2016

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ABSTRACT

Acid mine drainage (AMD) and acid sulfate soils cause surface water pollution through direct discharge, leaching and erosion. The proposed study was aimed at developing two complementary green methods for passive treatment of AMD water and AMD impacted soil. One method utilized the metal adsorbing and acid-neutralizing property of drinking water treatment residuals (WTRs) to treat acidic mine water and impacted soil. The other green approach applied the metal accumulating properties (phytoremediation) of two fast-growing, plants/grasses; Vetiver (*Vetiviera zizanioides L.*) and Pokeweed (*Phytolacca americana L.*) to clean metal-rich mine water and metal-contaminated soil.

Illinois has an estimated 35,000 acres (≈ 55 square miles) of abandoned mine sites that generate acid mine drainage (AMD). Tab-Simco is home to an abandoned coal mine located six (6) kilometers southeast of Carbondale, Illinois. Historically, two coal beds were mined by an underground method during the 1890s – 1955s, followed by surface mining during the 1960s - 1970s. AMD from the old mine site has recorded low average pH (2.54-2.81), high metal (Fe, Al, Mn, Zn, etc.), sulfate concentration, and total acidity. AMD discharges at a rate of about 35,000 gallons per day which resulted in a significant impact on the nearby Sycamore Creek. In 1996, the Tab-Simco site was reported as one of the most highly contaminated AMD sites in the Mid-Continent Region of the Office of Surface Mining Reclamation and Enforcement (OSMRE). The AMD was consolidated by backfilling and grading of the area with the installation of a series of French drains that resulted in several AMD seeps at the site. In 2007, an anaerobic sulfate reducing bioreactor (SRB) was constructed at the site by the Illinois Department of Natural Resources (IDNR) with the assistance of the OSMRE-MCR to treat the AMD. The SRB was intended to partially treat the AMD removing all of the aluminum and part of the iron and other trace metals. The remainder of the iron was designed to be precipitated in the next cell; an oxidation pond and wetland as ferric hydroxide which adsorbs the remaining trace metals.

Tab-Simco was selected as a field site for this study not only due to its proximity to SIU campus, but also because of the availability of an operating SRB which would allow for performance comparison of the proposed methodologies/technologies. Strategically, two sets of AMD water, and AMD impacted-soil samples, including one from the Acid Seep area and the other from the Oxidation Pond area were collected for this study. The team intended to make use of the Oxidation Pond samples to investigate further possible remediation of the Tab- Simco site, whereas our intention was to make use of the findings from the Acid Seep samples for remediation of any other AMD site located at high sulfur coal mines in Illinois and elsewhere.

The suitability of three locally acquired water treatment residual (WTRs) samples for metal adsorption and acid-neutralization was evaluated in a batch sorption and equilibrium studies. The Fe- and Ca-based WTRs were found to adsorb more aluminum (Al), manganese (Mn), iron (Fe), zinc (Zn) and copper (Cu) metal ions. The initial heavy metal concentrations (mg/L) in the AMD were Al: 128.8, Mn: 31.13, Fe: 341.2, Zn: 2.65 and Cu: 0.03. Greenhouse hydroponic studies revealed significant uptake of Fe, Zn and Cu in Vetiver grass. Greenhouse column studies to evaluate the integrated remediation of contaminated soil using WTR and Vetiver or Pokeweed were performed. The results indicated significant soil acidity neutralization (pH >6) which was maintained throughout the 12-week study period. Analysis of metal concentration in the leachates

showed very low percent leachable concentrations following a leaching protocol that simulates Illinois rain conditions.

The total metal concentration of the Oxidation Pond area soils for a few metals, such as, Al, Fe and P non-metal were relatively higher irrespective of their proximity to the Oxidation Pond which receives SRB pre-treated water. Only a small fraction of the total metal concentration was leachable in ammonium oxalate. For example, although 3,100 ppm of Al was determined to be present in the Oxidation Pond soil (embankment material-regraded mine spoil with ferric hydroxide precipitate), only 45.12 mg/kg was leachable to the surroundings under acidic conditions. The leachability could be reduced to 15.4 mg/kg and 13.34 mg/kg by applying the combined green technologies proposed in this study. This amounts to 65.9% and 70.4% reductions in the Al leaching potential of the Oxidation Pond soil. The reductions were significantly higher for lead (Pb), nickel (Ni) and Fe, whereas the reductions with Mn and arsenic (As) leachabilities are minimal for the Oxidation Pond soil sample.

The total metal concentration in the Acid Seep soil was predictably higher than the Oxidation Pond soil. However, it was found that only a small fraction of the metal and non-metal concentrations were found to be leachable. For example, the non-metal phosphorus content in the AMD seep soil was 1,100 mg/kg; however only 20.88 mg/kg of phosphorous was leachable to the surrounding. The application of the proposed methods could potentially reduce the P leachability by another 48.46% and 42.14%, respectively. Similar metal leachability from the proposed method were also evident for Pb, As and Fe. For the current SRB is equally effective as the proposed methods in reducing chromium (Cr).

The results for Al, Mn, Ni and Cu indicated differences in the leachability of trace metals between the two soil samples. It appeared that a significant amount of these metals and non-metals are leachable in the Oxidation Pond soil. The increased leachability in metals and non-metals are believed to be related to the mineralogy of mineral precipitates within these soils and their ability to take in and retain trace elements. Minerals stable at very low pH (< 3.0) such as jarosite precipitates in the Acid Seep "soils" whereas minerals that precipitate at neutral pH (6.0-7.0) such as ferrihydrite or amorphous iron hydroxide are present in the Oxidation Pond "soils".

The major factors that were considered to investigate soil erosion and metal leaching in the simulated field study conducted in Southern Illinois' ambient environment included rate of precipitation, soil condition and planted grass density. It was observed that at high amount of rain in a short period, the water tends to run over the soil surface into the silt dams rather than leach into the soil. The texture of the Acid Seep area soil (13.9% clay, 59.4% silt and 26.7% sand) caused significantly dense mass following dry days in June 2016. Leachate analysis showed reduced metal concentrations in the WTR-treated-AMD-impacted soil with improved pH (sustained at neutral). There was better growth of Vetiver grass that was planted on the WTR treated soil than the untreated soil. The Vetiver grass growth helped to decrease erosion caused by the rain. The growth of Vetiver shoot and root was observed to be significantly different in the WTR treated and untreated soil. The growth in root and shoot lengths were higher in the treated soil. Nitrogen sufficiency index calculated from measured chlorophyll content was found to be comparatively higher in the Vetiver grass planted in the amended soil than the untreated soil. More research is recommended to identify the soil characteristics, initial soil exchangeable metal concentrations,

degree of slopes, rainfall intensities, and WTR rate application procedure that help to reduce offsite sediment transport.

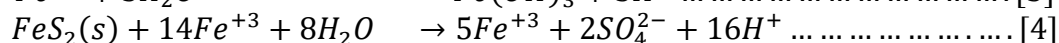
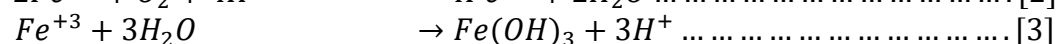
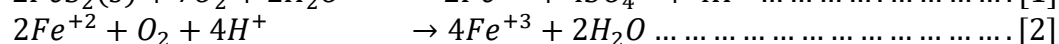
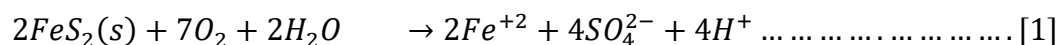
The simulated field hydroponic study investigated the metal uptake potential of two different plants: Vetiver grass and Pokeweed plant. The grass and plants, grown separately in untreated acidic water, were deceased by the end of the 30-day study period. The aluminum and iron uptake from the WTR treated water by the Vetiver were found to be similar and were about twice as high as the manganese uptake. The difference in the uptake capability of Vetiver was apparently due to the selective adsorption and the available concentrations of the metal ions in solution. Mn in the WTR treated water was reduced by 56.9%, Fe by 44.4% and cobalt (Co) by 55.7%. Other considered metals that exhibited varying levels of reduction from the Vetiver hydroponic system included Ni (54.1%), Cu (16.5%), Zn (28.4%) and As (8.6%). The percentage reduction can be seen to be influenced by the initial concentration of the metals in solution prior to the hydroponic treatment. The mean Mn, Fe and Al uptake (in mg per Vetiver plant) on dry weight basis was found to be 0.059, 0.115 and 0.104, respectively.

Pokeweed had difficult time adjusting to the hydroponic condition. However, Pokeweed shoot and root were found to contain more P, Mn and Fe than that of the Vetiver grass. The metal and phosphorus translocation in the Pokeweed was less than 1 except for manganese which was 1.6. Though very high Mn translocation has been reported in Pokeweed in literature, the low translocation and uptake observed in our study was apparently due to the low growth rate exhibited by the Pokeweed set used in this study. The adsorption of metals by Pokeweed, however, was relatively significant. The dissolved Mn in the WTR-treated-AMD used was further reduced by 31.8% whilst the concentration of Fe was reduced by 48.7%. Significant reduction was also measured in Co (26.1%), Ni (24.8%) with the least amount of reduction in Cu (3.5%). Nonetheless, the mean Mn, Fe and Al accumulation (mg per Pokeweed plant) was found to be as low as 0.003, 0.007 and 0.001 respectively.

1.0 INTRODUCTION AND BACKGROUND

Mining activities provide basic raw materials needed for infrastructure development and economic growth world-wide. However, it is well recognized that mining activities have been the cause of a variety of environmental pollutions via production of acidic mine drainage (AMD), and generation of metal contaminated soils. Water seeping out of an old abandoned mine is generally characterized by low-pH with heavy loads of dissolved sulfates (SO_4) and metal species such as, Fe, Al Mn, Zn. Other elements (i.e. Cd, As, Cr, Ni, Pb, Se and Cu) dissolved in mine water of environmental concern are categorized as toxic metals. Anthropogenic activities such as mining and metallurgical processes, chemical and transportation systems release high amounts of heavy metals into surface and ground water, soils and ultimately to the biosphere (Pantelica et al., 2008; Jantschi et al., 2008; Schutze et al., 2007). Currently, there are between 20,000 to 50,000 abandoned mines in the United States, many of which produce acidic metal contaminated water. Several environmental problems have been reported to be associated with these mined out sites such as heavy metal poisoning, loss of vegetation, air pollution (Boulanger & Gorman, 2004). These abandoned mines generate acid mine drainage (AMD) that impacts and degrades the ecosystem in many different ways.

AMD is formed when sulfide minerals such as pyrites reacts with oxygen in the presence of water (Kalin et al., 2005; Ziemkiewicz, 2003). The oxidation of sulfide minerals occurs through combinations of abiotic and biotic processes, which leads to formation of low pH level and high concentrations of dissolved metals and sulfate ions. This process of oxidation involves several reactions steps (Rimstidt and Vaughan, 2003) and begins with oxidation of sulfide minerals by oxygen as shown in Equation 1 (Nordstrom, 1982; Garrels et al., 1960; Kalin et al., 2005). This process oxidizes the sulfide minerals such as pyrite and releases ferrous ion (Fe^{+2}), and sulfate ion along with hydrogen ions (H^+). The ferrous ion oxidizes to ferric ion following Equation 2. However, the conversion of ferrous to ferric ion is a slow process at low pH and in the absence of lithotrophic bacteria. On the other hand, in a natural environment at higher pH, iron oxidizing bacteria such as *Acidithiobacillus Ferrooxidans* (syn-thiobacillus ferrooxidans) can accelerate the rate of the reaction by factor greater than 106 (Singer et al., 1970; Zagury et al., 1997; Brown et al., 2002). At pH levels around 3 and 3.5, Fe^{+3} is unstable and reacts with water to form ferric hydroxides (Perez- Lopez et al., 2007b; Hedin et al., 1994), which precipitates into red to yellow compound commonly known as “yellow boy”. This process also releases three moles of (H^+) into solution for each mole of Fe^{+3} , as indicated in Equation 3. At pH around 3- 3.5, Fe^{+3} remains in solution and can oxidize FeS_2 according to the reaction shown in Equation 4 (Stumm and Morgan, 1996; Rimstidt and Vaughan, 2003; Hedin et al.,1994; Kalin et al.,2005). During the reaction, for every mole of FeS_2 , sixteen moles of H^+ are released into solution. The rate of FeS_2 oxidation by Fe^{+3} is much higher than the rate of pyrite oxidation by O_2 (Neculita et al., 2007). The overall pyrite oxidation under aerobic conditions is shown by the reaction (Bonnissel –Gissinger et al., 1998) suggested in Equation 5.



Factors that control AMD generation include type of sulfide minerals, mineral surface area environmental conditions (pH level, temperature and dissolved oxygen) and bacterial activity (Berghom et al., 2001). The heavy metals in AMD impacted soil affect wildlife, plant growth etc. (Cojcaru et al., 2006; Popescu et al., 2009; Ene et al., 2009). The general difficulty with the elimination of metals from the environment is because, upon their introduction and subsequent contamination of the surrounding environment, they do not biodegrade except for mercury and selenium which can be transformed and volatilized by microorganisms. As most heavy metals are not biodegradable, they are continuously deposited and incorporated in water, thus causing long term heavy metal pollution. Moreover, bio-concentration could lead to high toxicity of these metals in organisms, even when the exposure level is low.

The major problem associated with conventional AMD remediation methods is cost, which may vary depending upon the type of contaminants in the soil. Therefore, there is a need for low cost green technologies to remediate the AMD-contaminated soils. To intervene AMD production, capping of affected sites with a layer of non-sulfidic topsoil is widely used during revegetation (Bell, 2001). However, this technique has had limited success with prevention of long-term acid generation and eventual production of acidic heavy metal runoff. Alkaline addition is a frequently used practice to effectively and rapidly raise the pH of soils. Several different neutralizing agents, including lime (calcium oxide), hydrated lime (calcium hydroxide), limestone (calcium carbonate), sodium carbonate, sodium hydroxide, and magnesium oxide and hydroxide have been used for this process. Currently, there has been increasing efforts directed at the application of industrial residues and other waste materials for remediation of heavy metal contamination and degraded soils (Babel and Kurniawan, 2003; Bailey et al., 1999; Lin et al., 2002; Lombi et al., 2001). The beneficial use of industrial residues can also assist these industries in reducing the costs associated with waste disposal and gives a second life to these materials instead of simply landfilling them. In this study we have made use of the waste products generated from the water treatment plants in combination with phytoremediation techniques for AMD remediation.

1.1 Water Treatment Residuals (WTR)

Drinking water treatment processes utilized to produce potable water generate a wide variety of residual products depending on the raw water source, chemicals used for purification, and types of unit operations used. In the conventional coagulation-filtration treatment process, suspended solids and natural organic matter are removed from the raw water supply by the addition of aluminum and iron salts as coagulants, resulting in the production of water treatment residues (WTR) (National Drinking Water Clearinghouse, 1998; Butkus et al., 1998) which are readily available at no cost. Major components of WTR are soil separates, organic materials, and Al and Fe hydrous metal hydroxides (Ippolito et al., 2011; O'Connor et al., 2001), depending on the metal salt used for coagulation. Alum [$KAl(SO_4)_2 \cdot 12H_2O$] is the most commonly used coagulant in the United States and Canada (Elliott et al., 1990); the iron salts $FeCl_3$ and $Fe_2(SO_4)_3$ are also used. Lime is used in water treatment for precipitation, to raise the pH, and to adjust the hardness of water. More than 2 million tons of WTRs are generated from public water treatment facilities in the U.S. every day (Prakash and Sengupta, 2003) and they are usually disposed of in landfills. Water treatment residuals tend to be amorphous in nature (Ippolito, 2001; Ippolito et al., 2009; Yang et al., 2006) and have been shown, via scanning electron microscopy (SEM), to be of various shapes and sizes (Makris et al., 2004) and are highly porous (Ippolito et al., 2003; Babatunde et al., 2008). The inherent porosity, amorphous nature and the presence of the (hydr)oxides of some major ions (Al

and Fe) enable WTRs to adsorb significant quantities of other ions. Past research has demonstrated the high affinity of WTRs for other contaminants of environmental and public health concern, such as perchlorate, phosphate, dichromate, and arsenate (Makris et al., 2004; Makris et al., 2006a; Makris et al., 2006b, Hardy et al., 2007). Other studies have investigated the effectiveness of WTRs to irreversibly remove heavy metals from acid mine drainage and impacted soils, which suggest long-term stable immobilization of WTR-bound metals (Codling, 2008; Gallimore et al., 1999; Birikorang et al., 2009; Oladeji et al., 2009). One major concern of WTR use in soil; which was studied as part of this study at SIU is the rate of application or co-application of constituent materials. This is because excessive WTR application can induce some nutrient deficiency in crops. The lime derived residues (Ca-based WTRs), with their high CaCO_3 content, was found to be an excellent low-cost liming agent to raise the pH of acid sulfate soils which would also reduce the mobility of heavy metals.

1.2 Tab-Simco Site Description

Coal underlies $\approx 95,830$ square kilometers (37,000 square miles) of Illinois, accounting for about 65 percent of the state's surface cover. Over 200 years of pre-regulatory coal mining in Illinois affected land and water use in various parts of the state. Since the introduction of the Surface Mining Control and Reclamation Act (SMCRA) in 1977, the Illinois Department of Natural Resources and Office of Surface Mining Reclamation and Enforcement (IDNR and OSMRE) through the Abandoned Mine Land Reclamation Program has recovered coal mine impacted land and aquatic ecosystems by the application of various remediation technologies. One such abandoned mining area that was impacted is the Tab-Simco site located about 6 km (3.7 miles) South-East of Southern Illinois University at Carbondale, Illinois. The site consists of approximately 12 ha (29.7 acres) of land with underlying underground mine works in two coal seams; the 2 meters (6.6 ft.) thick Murphysboro coal seam and the overlying Pennsylvanian-age Mt. Rorah coal seam. The site was reported in 1996 as one of the highly contaminated AMD sites in the mid-continent region (Smith, 2002). The floodplain adjacent to the Tab-Simco site received a relatively consistent base flow of approximately 150 cubic meter per day (39,600 gallons per day) from acid mine drainage emanating from seeps with a pH ranging between 2.3 to 2.9 (Smith, 2002). The AMD discharge flowed across small floodplain and created a 3.6 ha (9 acre) area devoid of vegetation, referred to as the "kill zone" shown in the Figure T1.1.

In response to the severity of the AMD impact, a 0.3 ha (0.75 acre) passive anaerobic sulfate reducing bioreactor (SRB) was constructed in 2007 at the abandoned Tab-Simco surface and underground mine site by the Illinois Department of Natural Resources (IDNR). The treatment system treated an average 4.86 cubic meter per hour (1,284 gallons per hour) of acid mine drainage (Behum et al., 2010; Behum, 2011; Behum, 2012; and Lewis, 2008). High concentration of major contaminants detected in the AMD included dissolved iron (900 mg/L), aluminum (200 mg/L), manganese (40 mg/L), sulfate (5,000 mg/L), along with an average pH and total acidity of 2.7 and 1,816 (mg/L calcium carbonate equivalent, CCE) respectively (Behum et al., 2011; Lefticariu, 2015). The treatment system concentrates AMD from the main seep through an open limestone channel to promote calcite (CaCO_3) dissolution and bicarbonate (HCO_3^{2-}) formation, to subsequently cause a pH increase before entering the bioreactor. It was however noted that at the time of this study, the limestone was passivated by iron coatings and low-pH precipitates. Several seeps along the drain had slightly lower metal content which dilutes the main acid seep discharge. The pH of the acidic water during the study period actually dropped some more before it enters

the SRB due to Fe hydrolysis in the drainage way. The bioreactor is constructed in three layers: a shallow acid impoundment, an underlying thick layer of compost, and limestone with embedded drain pipes. Oxidation wetlands follow the bioreactor unit to precipitate most of the remaining metals before discharge into the adjoining Sycamore Creek.



Figure T1.1 Photograph of acidic iron oxide (ochre) sediment deposited in a floodplain at the Tab-Simco AML site in southern Illinois prior to remediation. Location of the main AMD seep is upstream to the left. (Photo from Behum, 2004).

During the first two years of its operation, the SRB recorded significant metal removal capability of 75.6 % Fe, 99.6 % Al, 97.1 % Ni, and 93.4 % Zn (Behum et al., 2010). Beyond five years of operation, metal and SO_4^{2-} removal efficiency declined along with the pH of the bioreactor effluent. The significant decline in the performance of the SRB was possibly due to several contributing factors which included contaminant retention time, exhaustion of organic substrates, precipitate induced reduction of reactive surface area, and degeneration of system permeability (Blowes et al., 2009; Lindsay et al., 2011; Neculita et al., 2008; Zagury et al., 2007). The SRB system therefore underwent rehabilitation through excavation and replacement of the component materials. About 95% of the soil area previously devoid of vegetation at the site has been reclaimed to high-capability yield standards.

For the present study, the soil sampling areas at the Tab-Simco site from where contaminated soils were collected are sub-areas with soil characteristics that do not represent the revegetated areas of the Tab-Simco site. By design, the Acid Seep soil was intended to remain in contact with the AMD funneled from the buried French drains. The sampled soil area at the inner part of the Oxidation Pond embankments by design was intended to represent AMD treatment precipitates. The specific soil characteristics at the two sampling points were intended to present different AMD impact levels that can be found at various abandoned mine lands across the state and beyond. Shown in Figure T1.2 is the schematic of the layout of the Tab-Simco site showing the existing two-stage AMD treatment prior to discharge into the nearby Sycamore Creek. A major portion of the AMD seep goes through the above mentioned bioreactor, as shown in Figure T1.2. Only the excess offsite water during rainy season is allowed to bypass the bioreactor inlet and is shunted directly to the

Oxidation Pond. The water treated by the bioreactor is collected in the Oxidation Pond, from where it is released to the nearby creek.

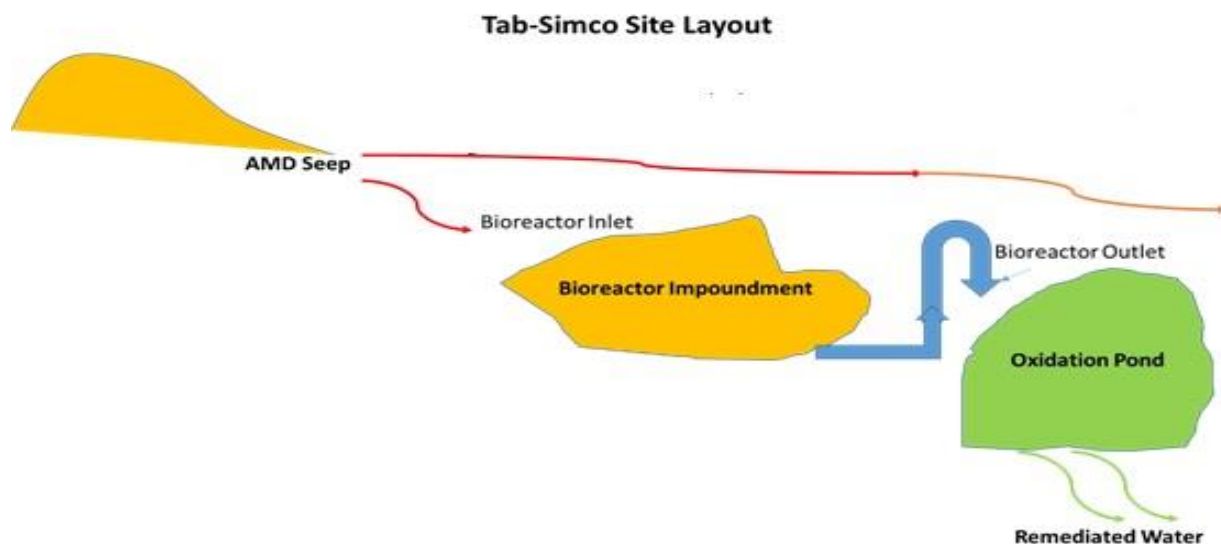


Figure T1.2 The topographical layout of the Tab-Simco acidic mine water seepage site

1.3 Phytoremediation

Phytoremediation refers to the use of green plants such as grasses to clean up contaminated soil or water. In essence, selected plants can uptake heavy metals from soil/water to their shoots through their root system, a process called phyto-extraction. Two different plant/grass species, *Vetiveria Zizanioids* (commonly referred as Vetiver) and *Phytolacca Americana Linn* (commonly known as Pokeweed) were examined in the present study for their potential application in AMD remediation

1.3.1 Vetiver Grass: Vetiver grass, also known as *Chrysopogon zizanioides*, is a graminaceous plant native to tropical and subtropical India (Dalton et al., 1996). It is reported that Vetiver grows 0.5–2 meters high, with stiff stems in large clumps from a much branched, massive root stock. These are considered very valuable characteristics of the Vetiver grass in our application (Truong, 2000; Pichai et al., 2001; Erskine, 1992; Truong, 1999; Hellin and Haigh 2002). The Vetiver root system is reported to reach depths up to 3 meters extending deep enough in the soil to provide the grip and anchorage needed to prevent surficial slip in the event of heavy prolonged rainstorm (Hengchaovanich, 1999). The World Bank (1990) and The Vetiver Network (Paul Truong's personal communication, www.Vetiver.org) stand on these advantages of this grass species to promote its use for slope protection against erosion. Vetiver grass applications include soil and water conservation systems in agricultural environment, slope stabilization, rehabilitation of mines, contaminated soil and saline land, as well as wastewater treatment (Truong and Loch, 2004). Vetiver grass applications include soil and water conservation systems in agricultural environment, slope stabilization, rehabilitation of mines, contaminated soil and saline land, as well as wastewater treatment (Truong and Loch, 2004). Because this species propagates by small offsets instead of stolon's (horizontal stems) and does not bear seeds, it is classified as noninvasive by the United States Department of Agriculture (USDA Plant Guide) and can easily be controlled.

1.3.2 Pokeweed: During the preliminary sample characterization of dissolved metals using inductively coupled plasma mass spectrometry (ICP-MS), it was found that high concentrations of manganese was present in the AMD solution. Low manganese removal has been reported at the SRB at the Tab-Simco site and other similar operating sites. Segid (2001) reported dissolved manganese reduction of only ~15.1% of the ~3.6 kg/day loading from the AMD in the bioreactor at Tab-Simco. Low manganese removal rates by sulfate-reducing bioreactors, similar to that of the Tab-Simco bioreactor, are widely reported in many previous bioreactor performance studies (Neculita et al. 2008a; Zaluski et al., 2003; Hallberg et al., 2005; Kuyucak et al., 2006). From this background, a plant species known for its hyper-accumulation characteristics of dissolved Mn in water and in soil was included in the present study to be investigated alongside the Vetiver grass. This manganese hyper-accumulator; Pokeweed, is a perennial weed with large biomass and fast growth rate that occurs worldwide. Previous research results have shown that Pokeweed can accumulate large amounts of heavy metals in its aerial tissues, especially Cd and Mn (Peng et al., 2006, 2008), with concentrations reaching 10,700 and 408 mg/kg, respectively. It was found to have a high potential in co-accumulating high concentrations of Mn and Cd in shoots at contaminated sites in China (Yuan et al., 2007; Min et al. 2007; Peng et al., 2006). A known disadvantage of the use of Pokeweed is the high toxicity of its berries. Therefore, cultivation of Pokeweed in or near populated areas is not encouraged; however, most abandoned mine lands, like Tab-Simco are not expected to be in populated areas.

2.0 RESEARCH OBJECTIVES & APPROACH

The overall goal of this investigation was to develop low-cost green technologies to remediate and restore AMD-impacted soil and water at abandoned coal mine sites. Waste materials generated at drinking water treatment plants, having excellent metal ion adsorbing capacities was used for AMD-impacted soil amendment and also in a filtration system to clean AMD water. The other green technology investigated in this study is phytoremediation, using two different varieties of hyper-accumulating plants/grasses. The AMD water and soils collected from the Tab-Simco abandoned coal mine site were used in the present study; however, the findings from this study will be equally applicable to all other AMD producing coal mine sites in Illinois and elsewhere. In light of the above discussions, the specific research objectives to satisfy the overall project goal were as follows:

- Identification of the most suitable WTR and its application rate for pH neutralization and the high efficiency reduction of the heavy metal content of the AMD and impacted soils.
- Identification of the most suitable plant type for the maximum uptake of metal ions from AMD and impacted soils.
- Performance comparison of the proposed green technologies with that of the SRB currently operating at the Tab-Simco site.

It was desired to conduct this study in such a way that the findings would be useful not only for the remediation work needed at the Tab-Simco site, but also for any other high sulfur coal mine sites in Illinois and elsewhere. Consequently, AMD and two separate sets of AMD-impacted soil samples were collected: 1) from the main Acid Seep (termed Acid Seep) and the Oxidation Pond

near the discharge of the SRB (termed the Oxidation Pond). Both locations are marked in the layout of Figure T1.2. The samples from the Acid Seep site would represent the AMD quality of most other abandoned coal mine sites in Illinois, whereas the Oxidation Pond site samples would be specific to the Tab-Simco site and other passive treatment systems that employ an Oxidation Pond to follow-up an alkalinity enhancement cell such as a SRB to remediate metal-laden AMD. Water treatment residual (WTR) samples were collected from three water treatment plants (WTPs) nearest to the Tab-Simco site. After initial lab tests with each WTR sample, the most suitable sample was used for most of the subsequent soil amendment and water purification studies. The conventional tube-filter design proved to be difficult to employ due to extremely low permeability of the WTR filter bed as a result of very fine size of the particles comprising the filter bed. A proprietary reverse flow was designed to filter large quantities of AMD water needed for the hydroponic studies. The overall project exercise comprising of laboratory studies, green-house studies and simulated field studies was subdivided into six different project tasks. The procedure followed and the results obtained from each project task are discussed in detail in the following sections.

3.0 EXPERIMENTAL RESULTS AND DISCUSSIONS

3.1 Task 1: Samples Collection and Characterization

3.1.1 AMD Impacted Soil: The soil used in the study was obtained as a composite (pooled) sample of top soil (0-20 cm depth). Sampling areas with grass were cut down by 5 cm before digging down to take samples. Several sampling trenches were made within 20 feet radius of each sampling area identified in Figure T1.2. Soils were collected in plastic buckets and transported to SIU's Energy Development Park in Carterville for air drying and further processing. The air-dried samples were sieved through a 2-mm sieve. Various physical/chemical properties including the pH of the soil types were measured. The Electrical Conductivity (EC) of the soil gives a measure of the ability of the soil in a solution to conduct electricity. The EC indicates the presence or absence of salts, though it does not indicate which salts might be present. The AMD impacted soil samples were tested to determine the amount of salts (electrolytes) therein and to assess their suitability to support crop growth. The measured conductivity was used to calculate the Salt Index of the soil (Hanlon et al., 1993).

Air dried soil collected from the Acid Seep (SS) was dark brown whilst the Oxidation Pond site (OS) was yellowish brown. Both soils were then identified as acidic soils based on their pH. These two soil samples were expected to vary in metal concentrations and compositions with the SS expected to contain higher metal concentrations due to its mostly direct contact with the AMD from the main seep. The OS soil was from the Oxidation Pond containing AMD water treated by the SRB at the Tab-Simco site. A list of physicochemical properties of the soil samples is provided in Table T1.1. It may be noted that up to a total of 20 metal content analysis was done on each sample that included the RCRA-8 (the eight metals considered hazardous by the Resource Recovery & Conservation Act at even very low concentrations).

Representative samples of both soils were analyzed for total elemental concentrations by ICP/MS-aqua regia where a 0.5g sample is digested in aqua regia at about 90°C in a microprocessor controlled digestion block for 2 hours. The suite of metals analysis was performed by the Acme Labs (Bureau Veritas) commercial laboratory (<http://acmelab.com>). Table T1.2 shows that soil

composition of the RCRA 8 metals as well as other major and trace elements and vary in concentrations in both soils.

Table T1.1 Physico-chemical data obtained from analysis of experimental soils.

Soil	pH*	EC* ($\mu\text{s}/\text{cm}$)	Particle density (g/cm^3)	Salt index (ds/m)	C %	N %	Org. matter content %	Soil Texture (%)		
								Sand	Silt	Clay
OS*	3.1	539	2.49	4.31	0.165	0.03	1.84	23.4	53.62	22.98
SS*	2.92	767	2.51	6.13	0.387	0.1	5.54	26.7	59.37	13.92

pH = 1:2.5(soil: water), EC = 1:5 (soil: water); SS= Acid Seep area soil, OS= Oxidation Pond area soil



Figure T1.3 Plan view of Tab-Simco site and material sampling points (©Google earth)

The concept of soil quality is based on the ability of the soil to perform specific functions which includes the ability to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation (Karlen et al., 1997). Given the wide scope of functions listed, it would be difficult to directly assess the quality of the Tab-Simco soil. The suitability of current state of the Tab-Simco soil was assessed based on some common soil quality indicators that include a combination of physical, chemical and biological properties, suggesting

that for a soil to function effectively, all three components must be addressed (Ditzler and Arlene, 2002).

Table T1.2 ICP-MS total concentration of RCRA 8 and other selected elements in Oxidation Pond area soil (OS) and Acid Seep soil (SS) by aqua regia digestion.

Metals (RCRA)	Mean concentration in ppm (mg/kg) ± S.D								
<i>Soil sample</i>	As	Ba	Cd	Cr	Pb	Se	Hg	Ag	
OS	29.30	61.30	0.06	9.40	22.91	5.60	0.08	0.15	
SS	21.5 ±0.346	47.03 ±0.057	0.37	15.67 ±0.231	14.70 ± 0.115	4.53 ± 0.057	0.05 ± .002	0.26 ± .006	
Elements	Mean concentration in ppm (mg/kg) ± S.D						(%)		
<i>Soil sample</i>	Cu	Mn	Ni	Zn	Co	Li	Fe	Al	
OS	14.59	74	8.4	28.5	2.6	2.2	5.38	0.31	
SS	32.67 ± 0.652	250 ± 20.7	24.2 ± .519	81.7 ± 2.428	7.13 ± 0.23	6.73 ± .23	6.50 ± 0.115	0.6	
Elements	Mean concentration in % ± S.D								
<i>Soil sample</i>	Mg	P	Ti	Na	K	Ca			
OS	0.04	0.074	0.01	0.04	0.14	0.17			
SS	0.12	0.11 ± 0.003	0.006	0.024	0.16	0.38 ± 0.005			

* RCRA = Resource Recovery and Conservation Act.

Soil pH is considered as a major variable in soils as it controls many chemical processes that take place. It specifically affects plant nutrient availability by controlling the chemical forms of the nutrient. The optimum pH range for growth of most plants is neutral or slightly acid soil (between 5.5 and 7.0). The United States Department of Agriculture Natural Resources Conservation Service (NRCS), classifies soils pH ranges < 3.5 as ultra-acidic soil. The soils collected at both sampling areas at the Tab-Simco site had measured pH < 3.5. This clearly indicates that the soil at the Tab-Simco site is highly acidic. Soil pH directly affects the solubility of many of the nutrients in the soil needed for proper plant growth and development. In very low soil pH conditions, nutrients, in their dissolved states, may be taken up by the plant system too rapidly. The excess nutrition cannot be processed fast enough thus overloading a plant's system and causing it to deteriorate in health or die. In other conditions, as soil pH decreases, bio-availability of nutrients, such as phosphorus, usually decreases because of precipitation reactions with iron and aluminum (Hanlon, 1993). For this reason, most plant species do not thrive in highly acid soil conditions such as that found at the Acid Seep and Oxidation Pond embankments at Tab-Simco site. However, there are a few plants that have adapted to such acidic extremes.

The electrical conductivity (EC) of a solution is a measure of the ability of the solution to conduct electricity. The presence of metal ions (salts) is indicated by high EC readings of the solution. The EC of the Tab-Simco soil as measured was an equivalent of 540 $\mu\text{s}/\text{cm}$ and 770 $\mu\text{s}/\text{cm}$ for the OS and SS respectively. Hanlon (1993), however, reported that when soils EC exceed 250 $\mu\text{s}/\text{cm}$ (or

0.25 x 8 = 2.0 dS/m salt index), many plants experience stress due to salts. Both soils (OS and SS) at the Tab-Simco site were classified as saline soils. Saline soils have excessive levels of soluble, high enough to negatively affect plant growth, resulting in reduced crop yields and even plant death under severe conditions. The primary effect of excessive soluble salts on plants is to limit the ability of plant roots to absorb soil water even under wet soil conditions. Because soil water flows from higher osmotic potential (low salt concentration) to lower osmotic potential (high salt concentration), a soil solution with low osmotic potential due to the higher concentration of soluble salts compared to the plant cells, will not allow plant roots to extract water from soil, causing drought-like symptoms in the plants (Seelig, 2000). That process is called “osmotic effect”.

Soil texture has been reported to have a large influence on water holding capacity, water conducting ability and chemical soil properties. Both soil samples contained more than 50% silt materials. The OS soil was, however, found to be more clayey than the SS (22.98% vs. 13.92%). Based on the high amount of silt and comparatively lower clay particles of the SS soil than the OS soil, it was expected that the SS soil will be free-draining whilst the OS will have a much higher water retaining capacity. The reported concentrations of total metal ions in the soil was studied to evaluate the level of soil contamination and to assess potential release of these contaminant metals into the environment. The concentrations of the eight heavy metals listed under the Resource Recovery and Conservation Act (RCRA) lists, each of which is extremely toxic at even small concentrations, was critically looked in the assessment of Tab-Simco soil contamination. The respective USEPA allowable limits of the RCRA 8 metals are shown in Table T1.3.

Table T1.3 Allowable limits (in mg/kg) of the RCRA 8 metals

Heavy Metal	As	Ba	Cd	Cr	Pb	Hg	Se	Ag
EPA Hazardous Waste #	D004	D005	D006	D007	D008	D009	D010	D011
US EPA Allowable Limits (ppm)	5.0	100.0	1.0	5.0	5.0	0.2	1.0	5.0

From the total metal concentrations analysis, both soils were found to contain varying concentrations of the RCRA 8 metals, however, the potential of their leachability based on the TCLP estimates as explained above shows that the RCRA 8 metal concentrations are all well within their allowable set limits. Both soil samples also showed considerably high concentrations of iron, aluminum, manganese and zinc. The Tab-Simco soil (SS and OS) was therefore concluded to be acidic, saline and polluted with several heavy metals. The results from the characterization therefore justify the need for a remedial measure to neutralize the acidity, reduce the salinity and to permanently immobilize and/or adsorb the metals through the application of appropriate dosages of the proposed WTR and the phytoremediation process with the hyper-accumulator plants identified for use in this study.

3.1.2 AMD Water: Sampling of the acidic water from the Tab-Simco site was done on an as needed basis to reduce the AMD storage time, since long term storage of the raw AMD has been reported to cause possible changes in water chemistry. The sampling point of this water used in all experiments in this study is shown in Figure T1.2. Several strategic locations were identified for

sample collection as marked previously in Figures T1.1 and T1.2. Contaminated water samples were collected at the Acid Seep, SRB impoundment, the SRB output in the Oxidation Pond. Dissolved metal and pH analysis of these samples showed that, the AMD at the seep area was more contaminated in terms of metal concentration, as expected. The lower metal concentration and higher pH of the Oxidation Pond was expected due to the presence of the SRB.

The seep area sampling point (Figure T1.3) had a constructed weir system built to impound the AMD and increase hydrologic head for a project previously sponsored by the OSM. Samples were stored in 5 gallons size plastic containers. Sample pH, EC and ORP was quickly measured on reaching the laboratory at Southern Illinois University which is 15 min. drive away. Collected samples were filtered with 0.45 micron filter paper and acidified and stored in a refrigerator for subsequent ICP-MS analysis. Sample acidification was necessary to prevent metals sticking to the walls of the sample container, halting all biological activity and breaking apart colloids and homogenizing the sample.



Figure T1.1 AMD sampling at Tab-Simco

The mean sulfate concentration of the Acid Seep AMD was 6,100 mg/L. The concentrations of relevant metals in the AMD collected from strategic locations that make up the Tab-Simco treatment system is shown in Table T1.4. The low pH of the AMD prior to the SRB suggests that most of the metals remain dissolved, mobile and that results in increased water toxicity to biological organisms and surrounding soil. The samples collected from the sites upstream of the SRB showed high concentrations of heavy metals and sulfates. The Acid Seep sample pH and metal concentrations clearly indicate that the generated Tab-Simco AMD water needs a treatment system prior to being released to the surrounding area. The data shown in Table T1.4 also indicates that the SRB that has been treating most of the AMD water seeping from the Tab-Simco site is effectively treating the dissolved metal pollutants, however, Mn concentration still remains a concern. The influent pH was greatly increased at the end of the treatment system as shown by the pH at the oxidation pond sample. Similar concentration reduction can also be seen for sulfates between the Acid seep and Oxidation Pond samples.

Table T1. 4 A-B. Chemical analysis (in mg/L) of the AMD water samples collected from strategic locations of the treatment system at the Tab-Simco mine site.

A.

Sample pt.	Al	Mn	Fe	Co	Ni	Cu	Zn	As
Acid Seep	130	32.4	327	0.426	1.72	0.035	2.88	0.00346
Impoundment	64.1	27.8	158	0.288	0.977	0.017	1.64	0.00217
SRB effluent	BDL	16.5	0.682	0.004	0.008	0.002	BDL	0.00004
Oxidation Pond	BDL	9.92	0.186	0.003	0.008	0.004	BDL	0.00003

* *BDL = Below detectable limit.*

B.

Sample pt.	pH	ORP (mV)	Conductivity (μ S/cm)	DO (mg/L)	Sulfate (mg/L)
Acid Seep	2.9	400.4	4963	15.7	4300
Impoundment	2.54	516.8	3812	6.7	2200
SRB effluent	6.43	-45	3591	3.5	1900
Oxidation Pond	6.7	-58.7	3609	4.8	1050

3.1.3 Water Treatment Residuals (WTR): Three WTR samples were obtained from large water treatment works in the state of Illinois. The WTR from the City of Carbondale Water Treatment Plant in Jackson county, IL was an Alum $[Al_2(SO_4)_3 \times 14H_2O]$ coagulant residue with high amounts of Al. The WTR from Rend Lake Conservancy District Water Treatment Plant in Franklin County, IL was a $FeCl_3$ as well as CaO residue from lime-softening. The third WTR was collected from the Saline Valley Conservancy District Water Treatment Plant in Saline County, IL which produces a lime-softening residue with high amounts of CaO. Figure T1.4 shows the county map of Illinois State with the WTR source counties shaded.

Figures T1.5 and T1.6 show the collection of the WTR from their storage ponds. The air-dried samples of iron and calcium based WTRs and the oven-dried aluminum-based WTR were crushed and sieved through 2 mm size openings. The -2 mm samples of each WTR type are shown in Figure T1.7.



Figure T1.2 County map of the State of Illinois. Counties where WTRs were sampled are shaded.



Figure T1.3 Sampling Aluminum-based WTR from the sludge pond.



Figure T1.4 Sampling Calcium-based WTR from the sludge pond.



Figure T1.5 Dry Aluminum (Al)-WTR, Iron (Fe)-WTR and Calcium (Ca)-WTR

The pH of the WTR samples were measured in distilled water using a two-point calibrated Pasco pH probe with an accuracy of ± 0.1 and resolution of 0.01. A WTR: deionized water ratio of 1:2.5

was used, and the mix left to stand for 45 minutes with occasional stirring with a glass rod. The electrical conductivities were measured using a Pasco conductivity probe, 10X (PS-2571) in a 1:5 WTR: water solution (United States Salinity Laboratory Staff, 1954). Expressed as the ratio of the total mass of the solid particles to their total volume, excluding pore spaces between particles; the particle densities of the WTR and soil samples were determined using standard test for soil solids by water pycnometer (ASTM D 854-00).

Analysis of the physical and chemical properties of the three WTRs is shown in Table T1.5. The conducted pH in water of the WTR samples ranged from 6.48 to 8.85 as indicated in Table T1.5. Two of the three WTR samples were alkaline, with only the Al-WTR having a slightly below neutral pH. Electrical conductivity ranged from 3213 to 6755 $\mu\text{S}/\text{cm}$. It was observed that the Ca-WTR and the Fe-WTR are made up mainly of silt and clay. The effect of drying on the particle size characteristics of the fresh wet Al-WTR sample was found to be significant as dried samples hardened and formed hard stable coarse sizes. The particle densities of the dry WTRs ranged from 1.54 to 2.47 g/cm^3 .

Table T1.5 Relevant properties of the three southern Illinois water treatment residues samples

Chemical/Physical Property	AL-WTR	Ca-WTR	Fe-WTR
pH	6.48	8.85	8.3
Electrical conductivity ($\mu\text{S}/\text{cm}$)	4120	3213	6755
Particle density (g/cm^3)	1.54	2.47	2.32
Sulfur (%)	0.65	0.12	0.05

The WTRs were analyzed for total elemental concentrations by ICP/MS-aqua regia as was conducted for the AMD soil. Table T1.6 shows the total metal analysis of the WTRs. The dominant element in each WTR that defines its name can be seen from the table where the Al-WTR contains more aluminum; an order of magnitude more than the other two types. Ca concentrations were low in the Fe-WTR, while the other WTRs showed considerably higher Ca concentrations, the highest being recorded for the Ca-WTR as was expected. The same is seen with the amount of iron in the WTRs with the Fe-based WTR having the most constituent irons. However, the amount of calcium in the Fe-based WTR is more than the iron content of the WTR. Magnesium concentrations were consistently lower than the Ca concentrations, with very low concentrations recorded for the Al-WTR. The Ca:Mg ratio of all samples ranged from 24.53 to 5.07. Total sulfur concentrations were low for all WTRs. Schmitt and Hall (1974) on examining a sediment basin residue from the Oak-Ridge Water Treatment Plant (Tennessee, USA) for 72 elements found Si, Ca, Mg, K, Fe and Ti to be generally the most abundant elements in the WTRs. Titshall and Hughes (2005), however, found that the elements in the six (6) WTRs they characterized from different South African water treatment plants varied in abundance based primarily on the treatment chemicals used. In the case of the Ca-WTR collected from the Saline County in southern Illinois, use of high amounts of CaO in the treatment process is reflected in high Ca concentrations in the residue produced. A similar situation exists for the Fe-WTRs, where use of a Fe-salt in the treatment process has resulted in a higher Fe concentration in the residues than the other WTRs. The plant that employs Alum coagulant in their treatment process was seen to have the most amount of total Aluminum in its residues.

Table T1.6 Heavy metal concentration in the WTR samples, S.D (Standard deviation)

Metals (RCRA)	Mean concentration in ppm (mg/kg) \pm S.D							
WTR sample	As	Ba	Cd	Cr	Pb	Se	Hg	Ag
Al-WTR	69.6 \pm 3.25	163.85 \pm 6.01	0.09 \pm 0.014	5.35 \pm 0.07	5.84 \pm 0.12	1.45 \pm 0.21	0.02 \pm 0.001	0.029 \pm 0.001
Ca-WTR	18.1 \pm 0.28	96.05 \pm 1.76	0.18 \pm 0.007	2 \pm 0.14	0.36 \pm 0.02	0.20	<0.005	0.005 \pm 0.001
Fe-WTR	9.15 \pm 0.21	126.15 \pm 2.75	0.12 \pm 0.007	20.25 \pm 0.77	7.04 \pm 0.12	0.45 \pm 0.07	0.01 \pm 0.009	0.028 \pm 0.002
Elements	Mean concentration in ppm (mg/kg) \pm S.D						(%)	
WTR sample	Cu	Mn	Ni	Zn	Co	Li	Fe	Al
Al-WTR	46.23 \pm 2.36	5283 \pm 42.42	16.65 \pm 0.35	47.25 \pm 3.74	3.65 \pm 0.21	8.3 \pm 0.7	0.58	>10
Ca-WTR	1.38 \pm 0.84	202.5 \pm 2.12	0.6 \pm 0.14	9	0.85 \pm 0.07	0.35 \pm 0.07	0.605 \pm 0.007	0.035 \pm 0.007
Fe-WTR	18.24 \pm 0.57	2638.5 \pm 40.3	14.6	26.5	5.85 \pm 0.21	3.45 \pm 0.07	2.92 \pm 0.056	0.625 \pm 0.007
Elements	Mean concentration in % \pm S.D							
WTR sample	Mg	P	Ti	Na	K	Ca	S	
Al-WTR	0.07	0.373 \pm 0.01	0.01	0.016 \pm 0.0007	0.03	0.355 \pm 0.007	0.65	
Ca-WTR	1.345	0.015 \pm 0.0007	0.002	0.04 \pm 0.0014	<0.01	33.005 \pm 0.51	0.12	
Fe-WTR	1.765	0.051 \pm 0.001	0.008	0.012 \pm 0.0007	0.04	15.975 \pm 0.20	0.05	

The pHs of two of the three WTR samples fall out of the higher range of the typical pH range of 5.10 to 8.00 reported by Basta (2000), and Titshall and Hughes (2005). The very high pH of the Ca-WTR, and to a lesser extent the Fe-WTR give these WTRs moderately high acid neutralizing potential. The high concentrations of Ca and Mg shown in the ICP-MS data in Table T1.6 gives a strong indication that the Ca and Fe-WTRs have a very reactive acid neutralizing component, probably in the form of Ca and Mg carbonates (Titshall and Hughes, 2005). The textural composition of the residues exhibits high clay and silt fractions in the dried samples of the Ca and Fe-WTRs. It is worth noting however that, since the residues are formed by the coagulation of fine particles into larger stable aggregates, they do have the ability to exhibit a coarse texture once dried as it is in the case of the Al-WTR, this depending on the strength of the bonds between the particles (Skene et al., 1995; Ahmed et al., 1997). There is an unmistakable possibility of a dry WTR disintegrating to its constituent fractions when in constant contact with water for a longer period. Such scenarios can lead to clogging of soil pores reducing hydraulic conductivity and water retention in affected soils. According to Titshall and Hughes (2005) this may increase the reactive sites for release of potentially toxic elements from the WTR into the soil system. To avert such situations, the amount of WTR application was carefully selected.

The high pH of the WTRs (in particular the Ca-WTR and Fe-WTR) would enable them to increase the pH of the acid mine drainage impacted soil collected from the Tab-Simco site. The very low organic matter content of the soil may be improved by the addition of manure. Subsequently, the soil is expected to be low on valuable plant nutrient concentration which may be overcome with the application of fertilizer to replenish low valuable elements. One major reported constraint with WTR application and provision of plant nutrient has to do with phosphorus. Numerous research have reported on the high P-sorbing capacity of WTRs and the potential of these WTRs to reduce P-uptake by plants grown even in mixtures of soil and WTR (Elliott and Singer, 1988; Heil and Barbarick, 1989; Skene et al., 1995; Ahmed et al., 1997; Basta et al., 2000; Codling et al., 2002). However, field experiments have shown that plant uptake of P is apparently not adversely affected by additions of WTR (Grabarek and Krug, 1987; Geertsema et al., 1994; Buyeye, 2003). Though the current research did not focus on phosphorus sorption as part of its objectives, the WTR application rates applied in this research was minimal enough to avoid issues of WTR phosphorus sorption.

Although the Ca-WTR had very high concentrations of total Ca (Table T1.6), it is clear that this Ca is in an unavailable form. Generally, however, extractable Ca and Mg appear to be at adequate plant available concentrations. Of some concern was the high Mn concentration of the Al and Fe-WTRs, which may lead to symptoms of toxicity in plants or perhaps cause antagonistic effects in the uptake of other elements (Ca, Mg, Fe). However, according to Tiller (1989), use of total element or metal content as presented in does not give the correct indication of the toxicity of a waste as it does not reflect the labile or available fraction. It is only good at giving an indication of the worst potential toxicity of an element assuming complete release of that element. Preventing heavy metal pollution is critical because cleaning contaminated soils is extremely expensive and difficult. The United States Environmental Protection Agency has set regulatory limits for the application of industrial waste or sludge to the soil.

Table T1.7 summarizes the maximum permissible concentration of some of the metals measured in the WTR being investigated in this study. Considering the limits set by the USEPA for soil, it is evident that in the worst case of total metal availability, the concentrations of the metals in all 3 WTRs lie below the maximum permissible limit. In most cases, by so many order of magnitude that the fear of contamination from these WTRs is negligible. The above table can therefore be used as a guide to calculate the maximum amount of sludge that can be applied based on the constituent elemental concentration. For example, to apply the maximum amount of sludge (kg/ha) on some agricultural land, the annual pollutant-loading rate for zinc is 140 kg/ha/yr (from Table T1.7). Assuming that the sludge shows a very high zinc concentration of 7500 mg/kg. The required amount of sludge to be applied (tons/acre) without exceeding the 140 kg/ha/yr will be 8.3 tons/acre. In a similar manner, using the concentration of Zn (47.25 mg/kg) in the Al-WTR (maximum possible of all 3 WTRs), under the same application rate requirements, the amount of sludge that will be required for application without exceeding the annual loading will be in excess of 1300 tons/acre. However, very low application rates were used in this study.

Table T1.7 Regulatory limits on heavy metals applied to soils (USEPA, 1993).

Heavy metal	Maximum concentration in sludge (mg/kg or ppm)	Annual pollutant loading rates		Cumulative pollutant loading rates	
		(kg/ha/yr)	(lb/A/yr)	(kg/ha)	(lb/A)
Arsenic	75	2	1.8	41	36.6
Cadmium	85	1.9	1.7	39	34.8
Chromium	3000	150	134	3000	2,679
Copper	4300	75	67	1500	1,340
Lead	420	21	14	420	375
Mercury	840	15	13.4	300	268
Molybdenum	57	0.85	0.80	17	15
Nickel	75	0.90	0.80	18	16
Selenium	100	5	4	100	89
Zinc	7500	140	125	2800	2500

3.1.4 Task 1 Summary Findings:

- As expected, the soil and water samples collected from the Acid Seep (SS) area were highly acidic (pH<3)
- Although the water collected from the Oxidation Pond (OS) had normal pH (~7) showing the acid neutralization effectiveness of the SRB in place at the Tab-Simco site, the soil from this area was found to be highly acidic.
- Both SS and OS soil samples were found to be saline as indicated by their high electrical conductivity of 770 $\mu\text{s}/\text{cm}$ and 540 $\mu\text{s}/\text{cm}$, respectively.
- High contamination of almost all RCRA8 metals along with Fe, Al, Mn and Zn were found in both AMD water and the impacted soil.
- The SRB appears to be effective in neutralizing and removing the metal ions from the AMD water with the exception of Mn ions. This fact led us to look for an Mn hyper-accumulator and evaluate it for its potential application at the Tab-Simco site.
- The chemical analysis of the water treatment residuals (WTR) collected from three different water treatment plants operating in southern Illinois were found favorable for their proposed applications.

3.2 Task 2: Greenhouse Phytoremediation Study: Soil Erosion and Metal Leaching Control

The available heavy metal in soils is of great concern to toxicity of plants and animals. Thus, it is critical to reduce this fraction in contaminated soils to curb the negative effects of heavy metals in soil media. The known benefits of WTR soil application, as discussed before, includes, maintenance of organic carbon in soil, soil structure improvement, increased water holding capacity of the soil among others (Elliot et al., 1990; Bugbee and Frink, 1985). This project task was designed to investigate the adsorption and long-term immobilization of metal ions in AMD impacted soil by WTR amendments of AMD soils. The acidic pH of the soils is also an inhibition factor for plant growth, hence, the acid neutralization capacity of WTR was also studied. Therefore, to evaluate WTR's AMD remediation capabilities, appropriate amount and treatment

composition that raises the pH of the soil, adsorbs metal ions effectively and support plant growth without much environmental impacts was investigated

3.2.1 Preliminary AMD Soil/WTR Equilibration Test: Preliminary tests were performed to investigate the optimum incubation period that will ensure maximum metal sorption and pH increase in the main incubation tests prior to the column study. WTR amendment compositions: Al-WTR, Fe-WTR and different ratios of Al:Fe was used to amend the AMD impacted soil. The Ca-WTR is considered a liming agent in this task. Incubation periods of 6 days (Dayton and Basta, 2005), 7 days (Silveira et al., 2006) and 10 days (Makris et al., 2004) have been reported in literature with emphasis on phosphorus adsorption. To confirm, preliminary tests were performed at application rates of 50 and 100 g/kg to monitor changes in pH, conductivity, and iron and manganese adsorption. The treatment mixes were thoroughly homogenized, moistened to 80% of field capacity and allowed to equilibrate at room temperature in plastic bags as shown in Figure T2.1.



Figure T2.1 AMD impacted soil/WTR equilibration test setup.

Analytical tests were conducted weekly to monitor changes in the considered parameters (conductivity and pH) by air drying collected sub-samples. The samples are also analyzed for deionized water leachable iron and manganese for samples collected on days 14 and 21. Weekly pH and conductivity results of the above set up is shown in Figures T2.2 and T2.3.

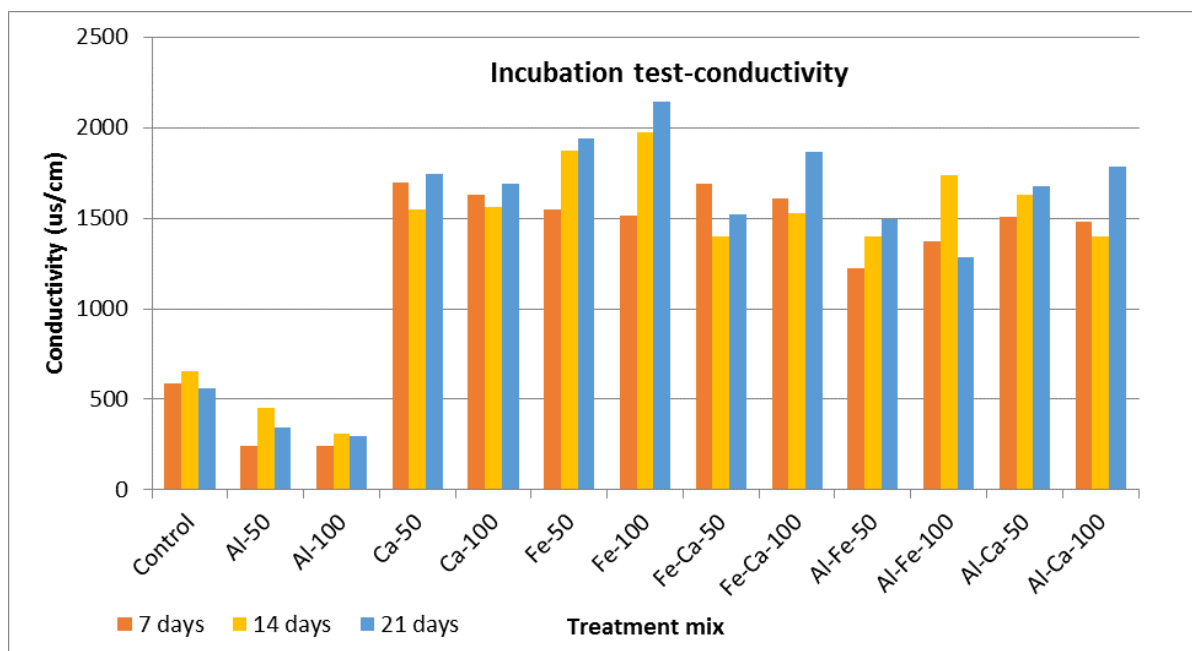


Figure T2.2 Weekly Conductivity analysis of AMD soil and WTR equilibration test.

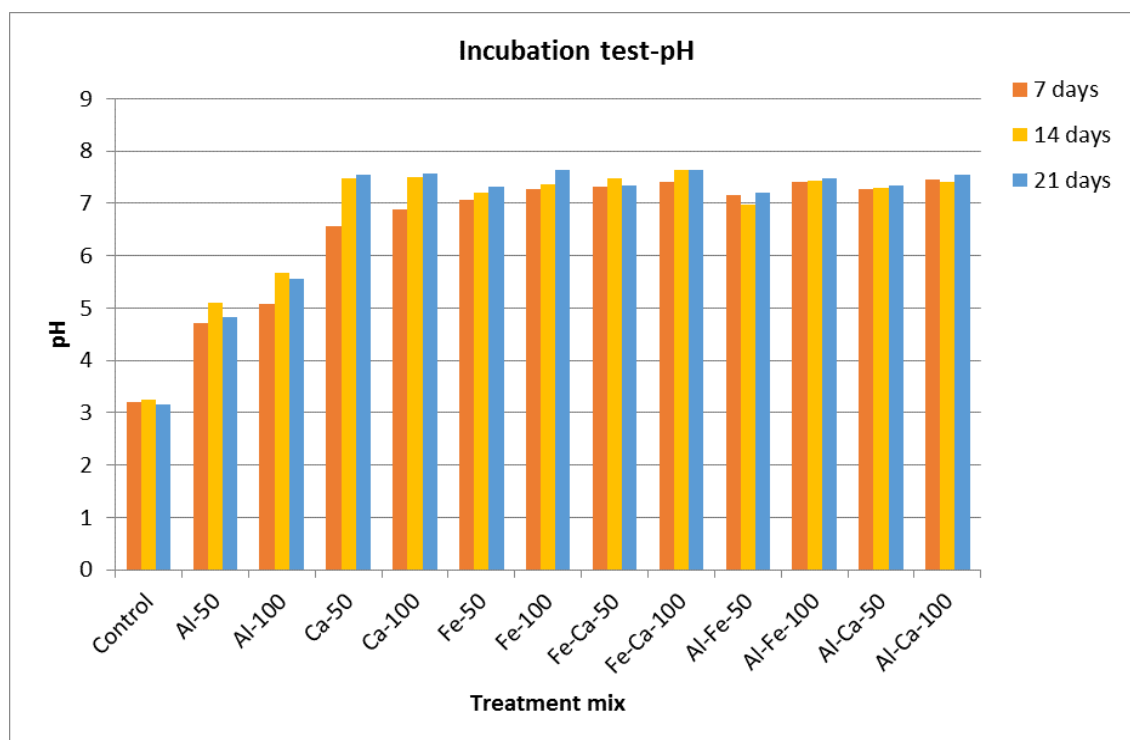


Figure T2.3 Weekly pH analysis of AMD soil and WTR equilibration test.

Figure T2.2 shows visible fluctuations in conductivity of the experimental samples over the 3-week period. Both concentrations of Al-WTR have consistently showed lower conductivities whereas the other mixes show conductivities higher than the control. It was worth noting, however, that conductivity is a blanket parameter and does not give much information on the target metals. The pH on the other hand shows more consistency across the study period, as illustrated in Figure T2.3. This gave an indication of the immediate neutralization impact of the WTR application on the acidic soil. The saved samples were analyzed for metal content changes to further reinforce the decision on the residence time for the incubation tests. It was found that the metal concentration decreased between day 14 and 21. For the 50 g/kg application rate, the recorded mean leachable Fe was 2.27 ppm (at 14 days) and 1.25 ppm (at 21 days) representing a difference of about 46%. Manganese leaching in deionized water for the same sample was 0.35 ppm and 0.325 ppm for Fe and Mn respectively, representing only 7% increase in reduction between the days. A similar trend was observed with the 100 g/kg application rate samples where percent difference was comparatively higher at a much lower concentrations of leachable Fe and Mn. Based on the outcome of this test, incubation or equilibration period of 21 days was chosen to be used in the column test. Since the Al-WTR under-performed in neutralizing the pH of the soil, the Fe-WTR was rather preferred to be used in the amendment design together with Ca-WTR which acts as an alkaline agent.

3.2.2 Column Incubation test: For the greenhouse column experiment, the two AMD-impacted soils collected from the Tab-Simco site were studied for the possibility of remediation through WTR amendments. The soils were collected from two locations namely the seep area (SS) and the Oxidation Pond area (OS). The collected soils from the two locations were air-dried, sieved to -2 mm, and thoroughly homogenized and stored in air-tight, plastic bags at room temperature at our high-bay facility in preparation for the incubation tests. At the onset of incubation, the air-dried soils were slowly moistened with DI and maintained at 80% field capacity and allowed to equilibrate for twenty-one (21) days at room temperature in plastic bags. Following equilibration, subsamples were collected and air dried for further metal analysis, from which the effects or contribution of the 21-days incubation period was evaluated. The remaining samples were used for the leaching and erosion control in the proposed column study. Figure T2.4 shows the incubation test samples in plastic bags.



Figure T2.4 Incubation test set at high-bay

Results of the conducted column studies using an integrated technique of WTR and two phytoremediation plants (Vetiver and Pokeweed) are presented. Vetiver sets that were set in the Oxidation Pond soil are identified as “OV” whilst the column set treating seep area soil with Vetiver was identified as “SV”. Prior to the column study, the contaminated soils were equilibrated with Fe-WTR for 21 days to assess the capability of the WTR to neutralize the acidic nature of the Tab-Simco soils sampled. Sub samples of soil were collected at the end of the incubation test. The air-dried soil samples were ground and passed through a 2-mm sieve before further analysis. The samples were analyzed for pH changes and Oxalate-extractable heavy metals were determined by extraction with 0.2 M oxalic acid with 0.2 M ammonium oxalate solution at pH 3 (McKeague et al., 1971). The suspension was equilibrated for 4 hours (in the dark) with continuous shaking, centrifuged, filtered through a 0.45- μ m filter, and analyzed by inductively coupled plasma spectrometry (ICP-MS). Analysis of pH was conducted using the procedure explained previously.

At the end of the 21 days of AMD-impacted soil and WTR incubation period, the average pH of the seep area soil and the Oxidation Pond area soil prior to incubation was found to be 2.92 and 3.10 respectively in deionized water. It can clearly be inferred from figure T2.5 that though all WTR amendment rates could dramatically increase the soil pH, the higher application rates (test runs 5-8) performed better in improving the soil pH to neutral. The lower initial pH of the seep area soil may be a responsible factor in the higher percent change in pH (121.8 - 140.3%) than as observed in the Oxidation Pond soil (99.6 - 112.4%). This confirms that the application rate directly affects the pH response of the soil after incubation and that the Fe-based WTR (average pH= 8.3) prior to lime application was able to neutralize the acidic soil.

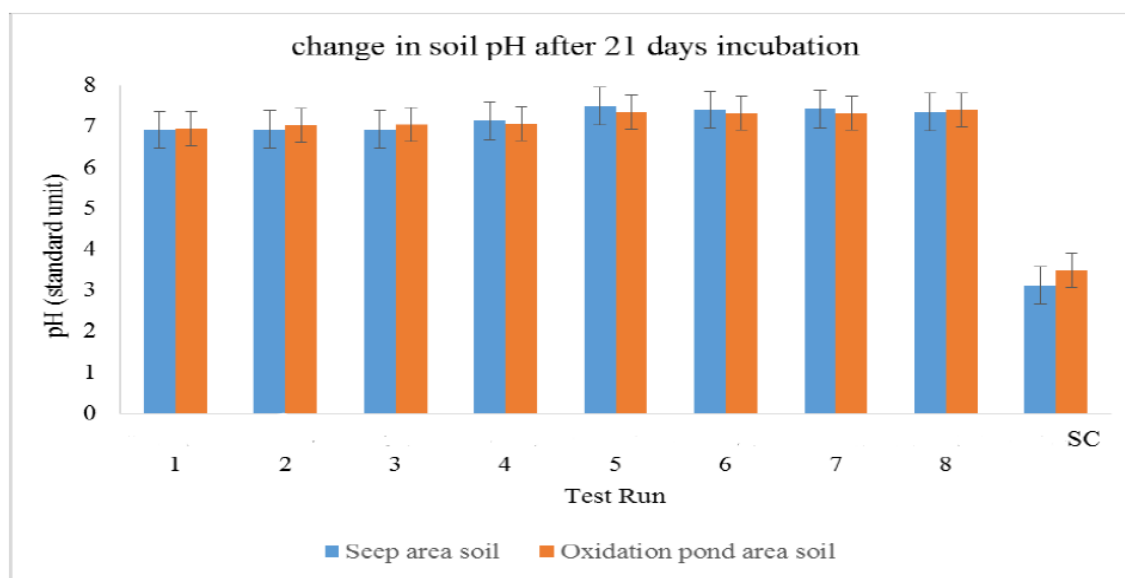


Figure T2.5 pH change after 21-day incubation period

Assessment of further pH change at the end of the 3-month column study was performed to study the stability of the WTR amendment over a longer time period. Figures T2.6 and T2.7 show the change in soil pH from incubation and completion of column study in the seep area and Oxidation

Pond area soils respectively. It can be inferred that a similar trend of pH increases and stabilization occurred in both soils. This indicates that the response of both soils to WTR treatment in terms of pH was not significantly different. Both Soils showed further increases of pH beyond neutral at the end of the column study irrespective of the amendment given. This is a clear indication of the long term acid neutralization capacity of WTR.

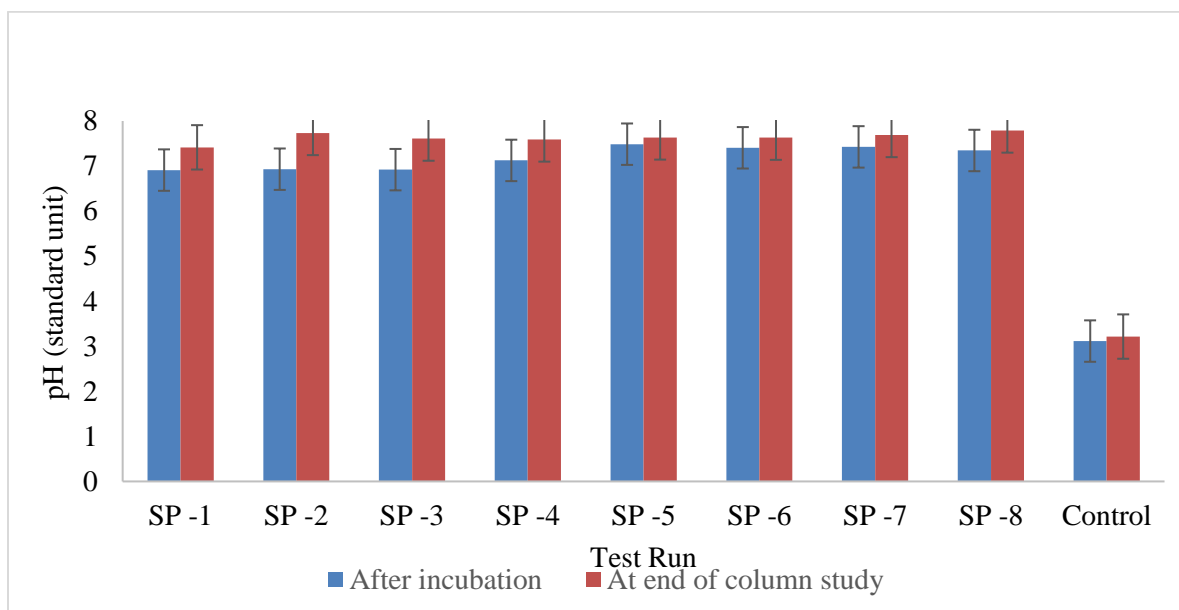


Figure T2.6 Change in soil pH from incubation and completion of column study (Acid Seep soil)

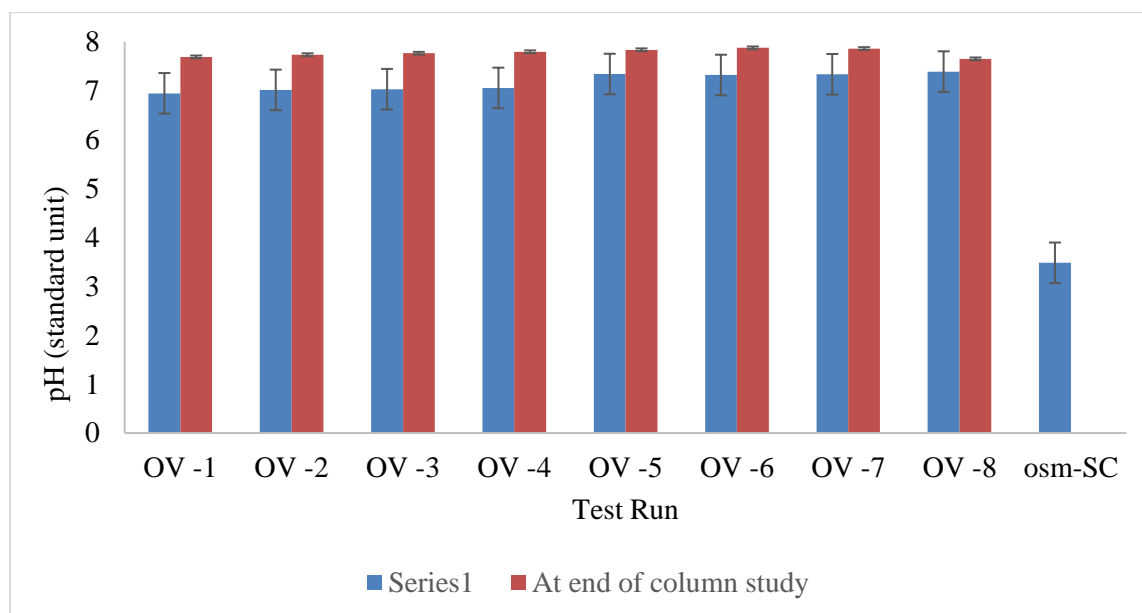


Figure T2.7 Change in soil pH from incubation and completion of column study (Oxidation Pond area soil).

3.2.3 Column studies: Prior to deciding on the levels of the main factors of influence, several amendment rates were evaluated in the preliminary experiments discussed above. This helped in narrowing the wider range of the respective application rates. A two-way factorial experimental design by considering 2 levels of iron based WTR, calcium based WTR (considered as a liming agent) and compost was used to compare and evaluate the effectiveness of WTR soil remediation techniques on both contaminated soils. Table T2.1 shows the designed test runs and three controls that were used in the column study. Thus, each of the two soil mixes (Acid Seep soil; SS and Oxidation Pond soil; OS) received two Fe-WTR amendment rates (50 and 100 g of WTR/kg of soil) equivalent to field application rates of 50 tons/acre (112 Mg ha⁻¹), and 100 tons/acre (224 Mg ha⁻¹) incorporated to a soil depth of 15 cm. Two levels (10 and 15 tons/acre) of the liming and manure materials were also used. The application rates of WTR were informed based on literature studies (Elliott et al., 2002; O'Connor et al., 2005). The test columns were set in duplicates. Primarily, this task was aimed at studying the extractability of dominant metals in two contaminated soils amended at different rates of water treatment residues, manure and complementary phytoremediation to evaluate the effectiveness of the treatment on the uptake and immobilization of metals under controlled leaching conditions, and plant growth in a controlled environment.

Table T2.1 Three factor two level experimental design (equivalent field application rates)

Run	WTR (tons/acre)	Lime (tons/acre)	Manure (tons/acre)
1	50	10	10
2	50	10	15
3	50	15	10
4	50	15	15
5	100	10	10
6	100	10	15
7	100	15	10
8	100	15	15
Control 1	0	10	15
Control 2	0	15	15
Control 3	0	0	0

3.2.4 Column preparation: Drain holes were perforated on the lower side of the column and connected with a flexible hosing. A clean airtight bottle was connected to the drain to collect leachate water samples for onward analysis during the leaching tests. The drainage hole at the lower side was covered with geotextile membrane to prevent soil loss, glass marbles (Figure T2.8) were packed at the bottom for easy water flow into the leachate bottle. The glass marbles were first washed carefully in an acid solution to prevent surface contamination or reaction when in

contact with the leached solution. A geotextile membrane was layered on the glass marble to reduce its top layer (sand) from blocking the drain. Samples (WTR-amended and control samples) were uniformly packed into the columns to a depth of 15 cm at an estimated bulk density of approximately 1.33 gcm^{-3} . Samples were gently transferred into the columns to ensure uniform distribution and hydraulic conductivity.



Figure T2.8 Glass marbles at the base of the column during construction

3.2.5 Column setup with Vetiver grass: Vetiver grasses were imported from Agriflora Tropicals in Puerto Rico. On receiving the Vetiver seedlings, they were potted and nursed in the green house with sufficient watering under controlled environmental conditions. The Vetiver was maintained in the greenhouse till development of new root and shoot system was observed to ensure good health of the grass before starting the column test.

Careful washing (Figure T2.9a) of potting soil from Vetiver roots prior to transplanting into the column media was performed to reduce material carry over into the system from the root. Initial measurement of the root and shoot lengths were also done (Figure T2.9b). Similar measurement at the end of the study was taken to estimate the net growth in shoot and root. The net weight of biomass was also evaluated from the difference between the recorded initial dry weight of the cleaned grass and the weight at the end of the study.



Figure T2.9 (a) Careful washing of Vetiver roots (left) and (b) measuring the root and shoot system of Vetiver (right).

The columns were set on raised prepared platforms so that leachate could flow out of the system under gravity into the collecting bottles. Containers were firmly secured and airtight to reduce metal ion speciation in the presence of oxygen. Collected leachate was filtered and analyzed for pH and oxidation reduction potential at the green house on collection. The collected leachate samples were filtered with 0.45 μm filter paper and spiked with pure dilute H_2NO_3 before storing in refrigerator in waiting for metal concentration analysis.

3.2.6 Column setup with Pokeweed: Pokeweed seeds were ordered from the Sand Mountain Herbs in Alabama. For the best germination of the acquired Pokeweed seeds, they were soaked in DI water overnight after which seeds that remained afloat were discarded. The remaining seed that sunk in the water were then soaked in concentrated sulfuric acid for 5 minutes to imitate the process of deterioration of the seed coat in a bird's stomach. The pretreated seeds were then sowed in a potting soil in the greenhouse. Figure T2.10 shows the highly networked, mesh-forming root system of Pokeweed which is capable of reaching bioavailable metals in the soil and to help control downward erosion.



Figure T2.10 Root system of Pokeweed

Prior to transferring the nursed Pokeweed seedlings into the amended soils in the columns, the roots were washed off of potting soil. Length measurement of the washed roots and shoot system of each Pokeweed; as shown in figure T2.11, was performed to enable proper assessment of the plant growth at the end of the study period.

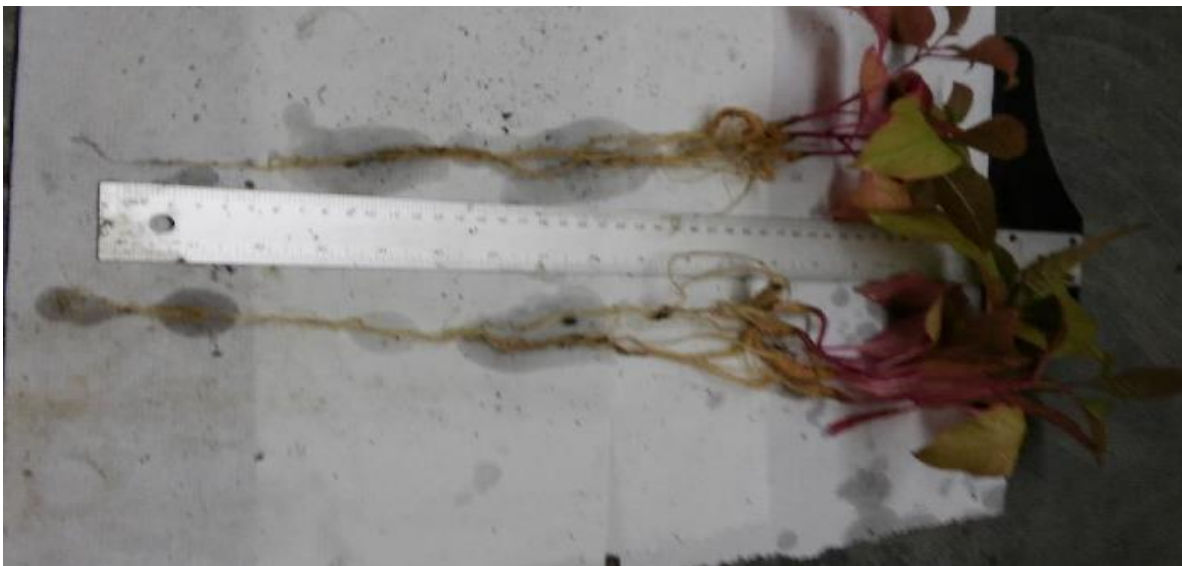


Figure T2.11 Measuring root and shoot length of Pokeweed prior to transplanting into columns.

The columns were prepared in the same way as the setup for Vetiver discussed above. The lower side of the column was perforated and connected with a flexible hosing to drain leachate into a connected clean airtight bottle as shown in Figure T2.12.

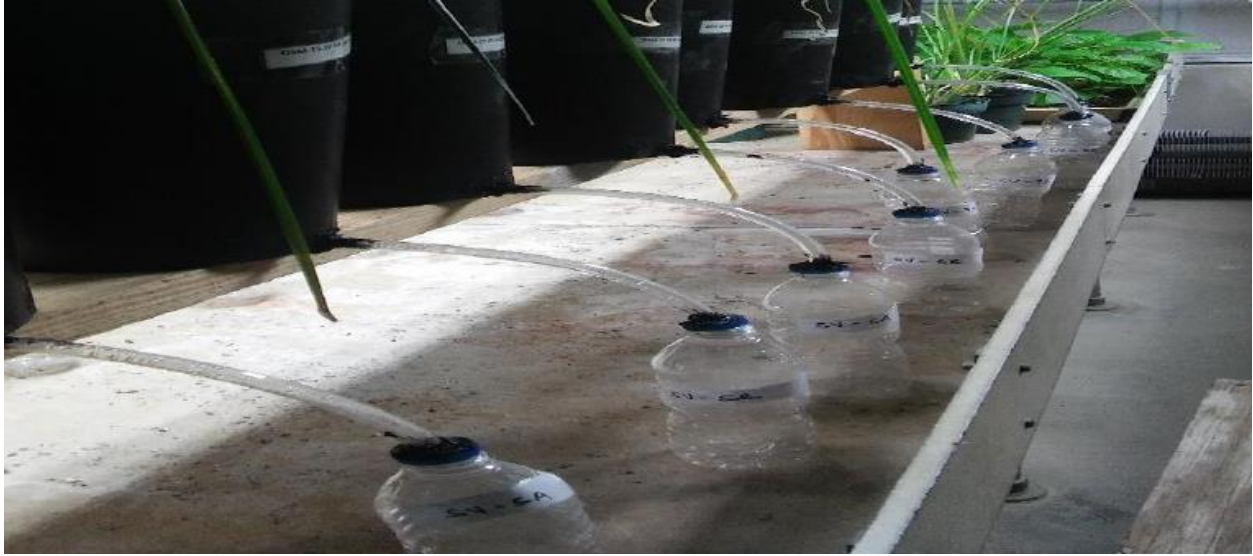


Figure T2.12 Leachate collection from the bottom of the column

Samples (WTR-amended and control) were uniformly packed into the columns to a depth of 15 cm at an approximate bulk density of 1.33 g cm^{-3} . Samples were gently tapped into the column with the plants to mimic field conditions and to ensure efficient hydraulic conductivity. Figures T2.13 and T2.14 shows the column study setup for Pokeweed under controlled conditions at one of SIU's green house facilities.



Figure T2.13 Column study experimental setup (Pokeweed) at SIU's green house facility



Figure T2.14 Column study experimental setup at SIU's green house facility

3.2.7 Leaching and analyses of leachates: Accumulated leachates after the first week of the experiment was collected for pH and ORP analysis. Leachates were also collected on the 1st, 6th, 8th and 12th week of the experiment. On the 8th and 12th week, deionized water was adjusted to pH = 4.4 to simulate the average rain water pH of Illinois. Equal amount of the simulated rain water was applied to each of the columns. Leachate samples were collected into airtight containers. Volumes of leached water samples were collected and measured with a graduated cylinder. Green house measurements of pH, and oxidation reduction potential (ORP) were conducted on each leachate sample with a Hach Co. (Loveland, CO) Model HQ-40 professional-grade multi-parameter meter. Collected water and soil sample analyses for this study was statistically analyzed using Microsoft® Excel 2013.

Each collected effluent sample from the columns was filtered through Whatman 0.45 μm membrane filter, acidified with Nitric acid to pH < 2 and stored for analyses of the metals reported. Leachates were analyzed by ICP-MS. The mean pH, ORP and metal concentration of the duplicate samples are reported for each experiment run. Figures T2.15 and T2.16 shows the mean duplicate leachate pH change over time for seep area and Oxidation Pond area contaminated soils with Vetiver grass respectively.

It can be seen from Figure T2.15 that the leachate pH of all amendments were reduced between the first week and the 12th week. However, this reduction cannot be considered to be very significant since most of the pH still remain neutral. Control test columns that were not amended with WTR also maintained their near neutral pH as a result of the lime application. The difference of the effects of the lime application rates of the control test columns (SC-1 and 2) in terms of pH is not significant. The control test column (SC-3) which did not receive any amendment of WTR, lime or manure can clearly be seen to leach out acidic water which also shows a reduction in its

pH in the long term. Similar trend of pH change can be observed in the Oxidation Pond area from Figure T2.16.

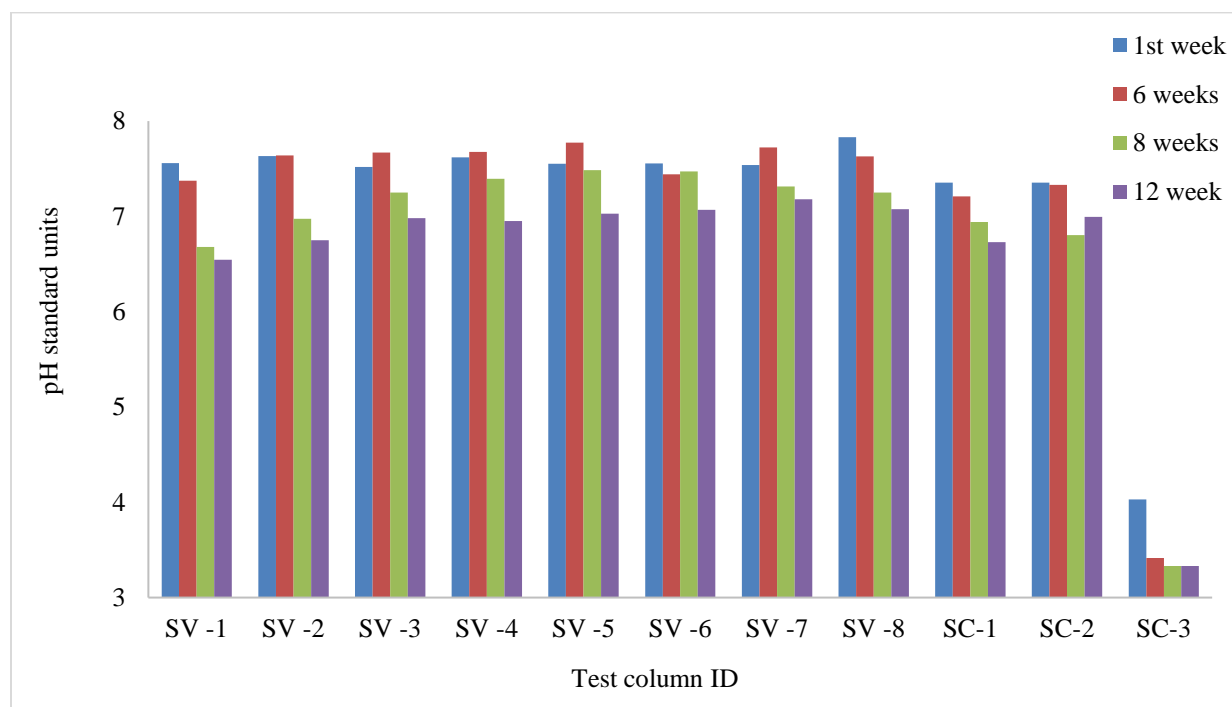


Figure T2.15 Leachate pH over time of Acid Seep soil amended with Vetiver (SV)

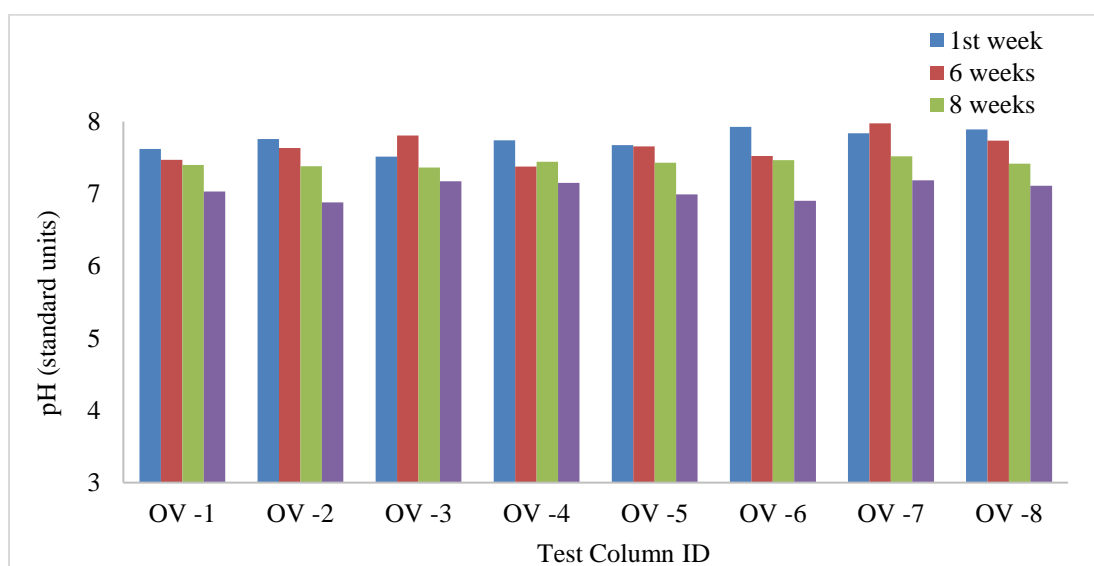


Figure T2.16 Leachate pH over time of Oxidation Pond area soil amended with Vetiver (OV)

The redox status in the column leachate was monitored. The oxidation reduction potential of the leachates for both soil types is shown in Figures T2.17 and T2.18. The ORP measurement of the seep area soil can be seen to slightly reduce over the 12 weeks period in both soils. The control

columns, however, do show significant leachate ORP changes. Control column SC-1 shows a steady drop in the ORP whilst SC-2 (amended Acid Seep soil) shows a reduction in the 6th week leachate and rises in the 8th week. The pattern is, however, different in the Oxidation Pond area soil column (Figure T2.18) where there is an observed reduction across all columns on the 6th week and further gain in ORP as observed on the 8th week. Column SV-1, however, shows an exception with a lower measured ORP on the 8th day.

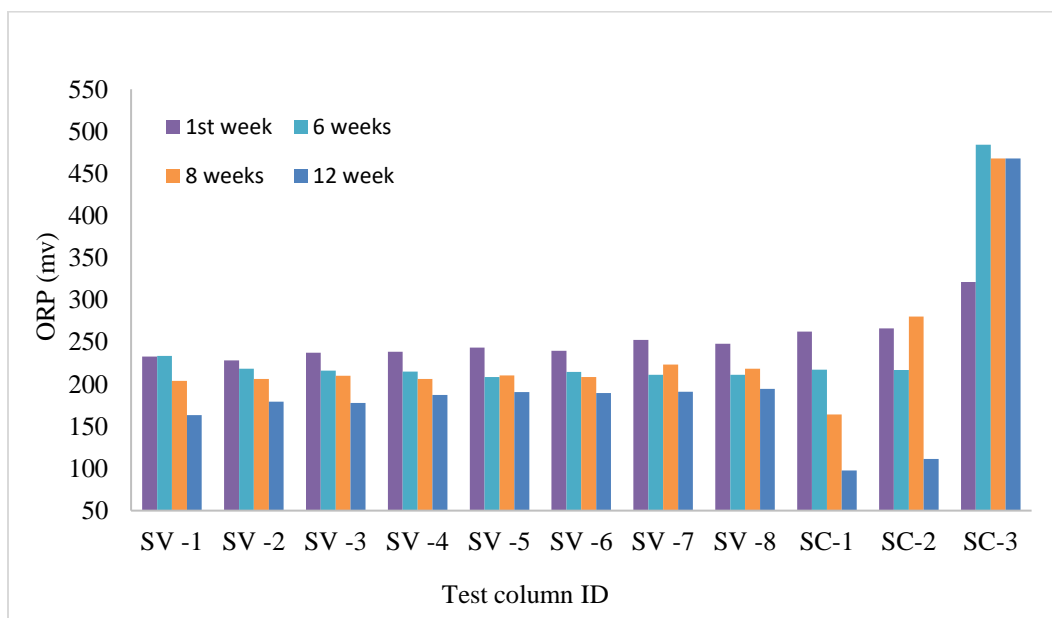


Figure T2.17 Leachate ORP (mV) over time of Acid seep soil amended with Vetiver (SV)

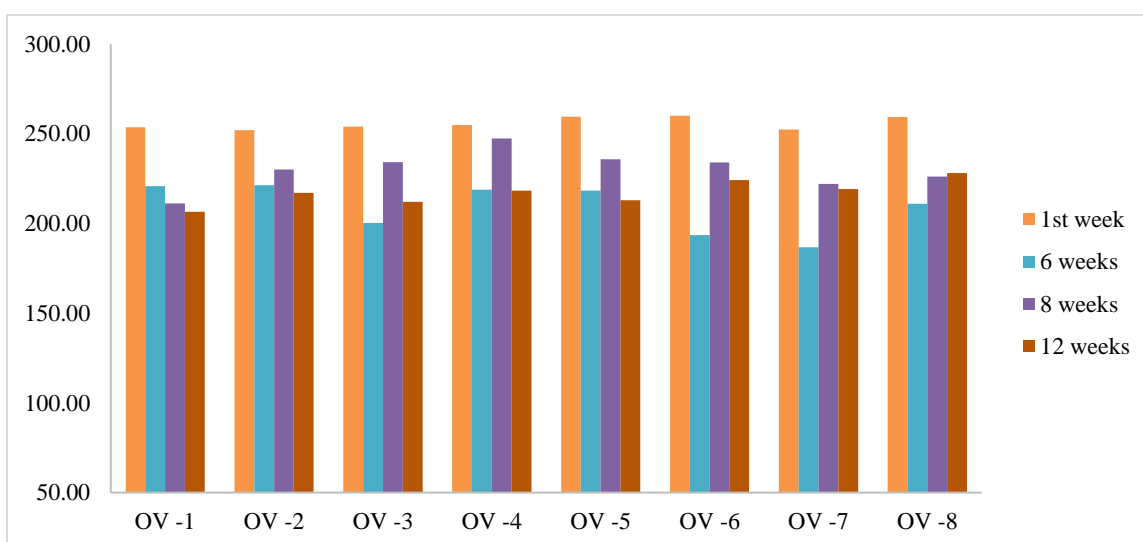


Figure T2.18 Leachate ORP (mV) over time of Oxidation pond area soil amended with Vetiver (OV)

Figures T2.19 and T2.20 show the leachate pH change for SS and OS soil types respectively in columns planted with poke weed. The pH trend was not significantly different from what was observed with the Vetiver grass set. The results confirm that, in a soil environment, the phytoremediation plants do not play significant role in altering the growth medium pH. The leachate ORP as observed in the poke weed column set followed the same trend as for the Vetiver grass (plot not shown to avoid redundancy).

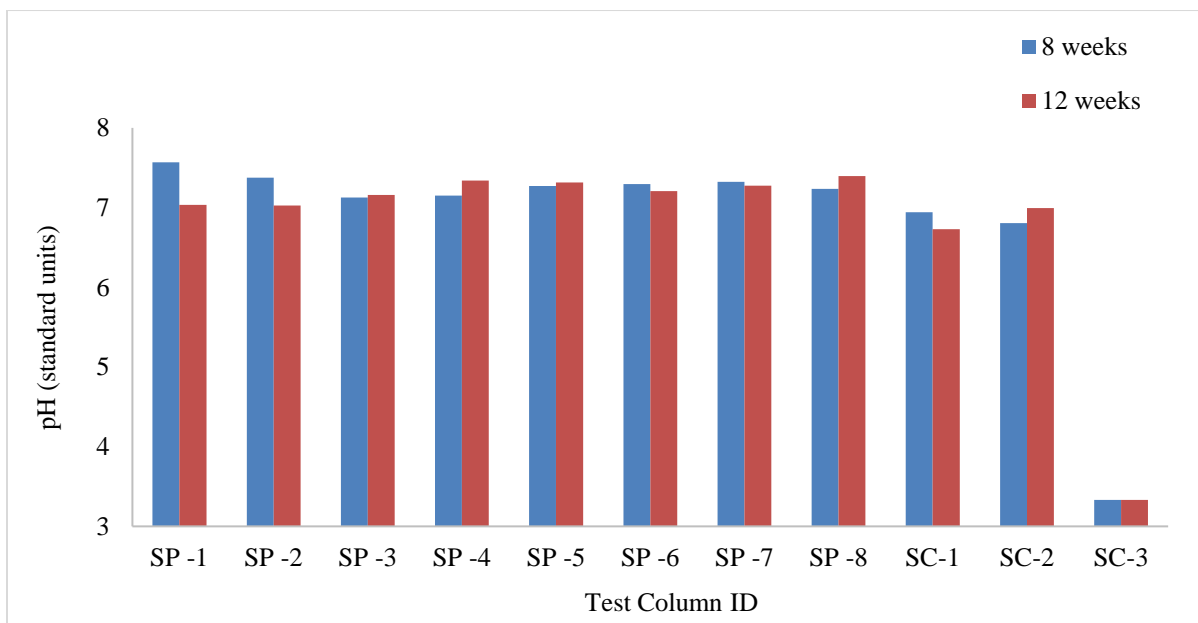


Figure T2.19 Leachate pH over time of Acid Seep area soil amended with Pokeweed (SP)

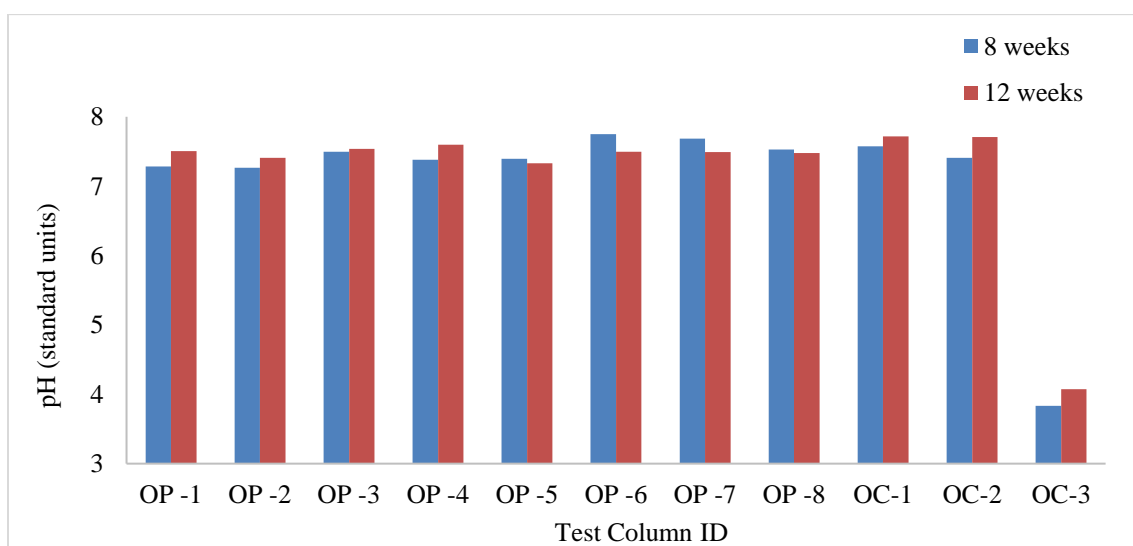


Figure T2.20 Leachate pH over time of Oxidation Pond area soil amended with Pokeweed (OP)

3.2.8 Metal extraction in leachates: Shown in Tables T2.2 to T2.5 are major heavy metals (Al, Mn, Fe, Zn, Cu, As and Pb) monitored during the 3-month greenhouse column study. Of these, Fe and Al were the ones comparatively leached in moderate concentrations. Results of the Vetiver columns in both soils are shown in tables T2.2 and T2.3. It can be seen from both tables that the concentrations of the various metals in the leachates are all within common range in soils (Deuel and Holliday, 1998; Lindsay, 1979). However, in the control columns for both soils (SC3 and OC3), the metal concentrations were fairly higher in the leachate collected on the 8th week with manganese at a high of 15.62 ppm in the seep area soil. The SC3 showed higher concentrations in the considered metals except for Zn, As and Pb on the 8th week. Pb concentration in all leachates were below equipment detection limit (≈ 0). Zinc detection was below detection limit for all columns with Vetiver in seep soil. Copper concentrations were also below detection in SV treated columns. There were moderate changes in the metal concentrations in the 8th and 12th week leachates of the control columns without any treatment. The columns with low levels of added WTR (SV-1 to SV-4) showed reductions in their metals concentrations except for column SV-1 which had the lowest levels of all treatment materials. Prominent in the columns with higher application rates of the WTR were several instances of increased leachate concentrations (mostly Fe and Mn) after 12 weeks in the seep soil with Vetiver.

Table T2.2 Concentrations* of metals, pH and ORP of leachates in treatment and control columns (SV).

Sample	Leaching Period	pH	ORP	Fe	Mn	Al	Zn	Cu	As	Pb
SV -1	Wk 8	6.68	204	0.722	28.8	0.258	BDL	BDL	8.19	BDL
	Wk 12	6.55	163	0.765	41.6	0.289	BDL	BDL	8.81	BDL
SV -2	Wk 8	6.98	206	0.448	8.50	0.095	BDL	BDL	10.4	BDL
	Wk 12	6.75	179	0.131	8.82	0.062	BDL	BDL	11.0	BDL
SV -3	Wk 8	7.25	210	0.225	12.7	0.074	BDL	BDL	10.1	BDL
	Wk 12	6.98	178	0.172	12.0	0.061	BDL	BDL	3.90	BDL
SV -4	Wk 8	7.40	206	0.181	11.1	0.130	BDL	BDL	8.51	BDL
	Wk 12	6.95	187	0.164	11.6	0.301	BDL	BDL	9.43	BDL
SV -5	Wk 8	7.49	210	0.389	20.5	0.120	BDL	BDL	13.6	BDL
	Wk 12	7.03	191	0.699	17.7	0.255	BDL	BDL	4.22	BDL
SV -6	Wk 8	7.47	209	0.861	48.0	0.233	BDL	BDL	11.5	BDL
	Wk 12	7.07	190	0.270	22.1	0.106	BDL	BDL	7.78	BDL
SV -7	Wk 8	7.32	223	0.320	21.8	0.096	BDL	BDL	3.98	BDL
	Wk 12	7.18	191	0.804	42.8	0.288	BDL	BDL	4.96	BDL
SV -8	Wk 8	7.25	218	0.574	29.1	0.188	BDL	BDL	6.10	BDL
	Wk 12	7.08	194	1.320	67.6	0.467	BDL	BDL	7.41	BDL
SC-1	Wk 8	6.94	164	0.488	1304	0.136	BDL	8.35	14.6	BDL
	Wk 12	6.73	97.8	8.53	1204	0.131	BDL	5.11	10.5	BDL
SC-2	Wk 8	6.81	280	0.004	74.8	0.003	BDL	1.27	8.17	BDL
	Wk 12	7.00	111	2.23	82.0	1.78	BDL	5.62	8.35	BDL
SC-3	Wk 8	3.33	468	2.11	15623	43.5	525	94.2	3.89	BDL
	Wk 12	3.33	468	1.51	8095	29.0	157	54.8	3.83	BDL

BDL = below detection limit; Concentration* in mg/L (for Fe & Al) and $\mu\text{g/L}$ for other metals

In the Oxidation Pond area soil with Vetiver, treatment columns with low level application rates consistently showed reduction in metal concentrations. Critical evaluations of the metal concentrations showed that treatment columns with run 3 applications (SV-3 and OV-3) were more appropriate for the columns with Vetiver integration. In column SV-3, Mn declined from an average of 0.225 mg/L in the initial 8-week period to an average of 0.172 µg/L in the remaining test period. Similarly, As declined from an average value of 10.0 to 3.90 µg/L.

Table T2.3 Concentrations* of metals, pH and ORP of leachates in treatment and control columns (OV).

Sample	Leaching Period	pH	ORP	Fe	Mn	Al	Zn	Cu	As	Pb
OV -1	Wk 8	7.40	211	0.751	172	0.196	BDL	BDL	7.02	BDL
	Wk 12	7.03	207	0.401	8.97	0.073	BDL	7.37	3.19	BDL
OV -2	Wk 8	7.38	230	1.39	38.5	0.218	BDL	BDL	6.10	BDL
	Wk 12	6.88	217	0.509	20.9	0.133	1.62	8.92	4.63	BDL
OV -3	Wk 8	7.37	234	0.883	29.9	0.199	BDL	BDL	7.06	BDL
	Wk 12	7.18	212	0.453	11.1	0.105	BDL	10.6	9.32	BDL
OV -4	Wk 8	7.45	247	0.503	17.4	0.087	BDL	BDL	5.94	BDL
	Wk 12	7.15	218	0.443	12.4	0.098	10.03	54.6	5.81	BDL
OV -5	Wk 8	7.43	236	0.807	26.8	0.295	BDL	BDL	6.35	BDL
	Wk 12	6.99	213	0.649	23.8	0.146	BDL	10.1	4.12	BDL
OV -6	Wk 8	7.47	234	0.676	64.5	0.113	BDL	BDL	14.3	BDL
	Wk 12	6.91	224	0.440	12.9	0.054	8.32	12.2	6.82	BDL
OV -7	Wk 8	7.52	222	0.943	46.9	0.134	BDL	BDL	7.06	BDL
	Wk 12	7.19	219	1.75	119	0.111	BDL	11.9	9.39	BDL
OV -8	Wk 8	7.42	226	0.317	129	0.063	BDL	BDL	13.5	BDL
	Wk 12	7.11	228	0.929	90.3	1.35	52.5	15.7	8.67	BDL
OC-1	Wk 8	7.58	213	0.490	18.1	0.104	6.27	4.58	5.92	BDL
	Wk 12	7.72	122	0.013	28.9	BDL	BDL	5.70	7.21	BDL
OC-2	Wk 8	7.41	215	0.723	9.98	0.099	2.32	4.77	6.53	BDL
	Wk 12	7.71	128	0.018	3.33	BDL	4.27	11.6	3.34	BDL
OC-3	Wk 8	3.84	388	2.50	2930	5.18	824	20.1	4.20	BDL
	Wk 12	4.08	377	0.144	1480	2.44	1104	13.6	3.51	BDL

BDL = below detection limit; Concentration in mg/L (for Fe & Al) and µg/L for other metals*

Considering column amendment with Pokeweed showed in tables T2.4 and T2.5, it was interesting to know that the amount of Mn in all treatment columns reduced in the 12-week leachate samples, unlike in some cases of Vetiver treatment. As was revealed in the columns treated with Vetiver, higher application rates showed higher propensity to release more metals into solution. This could be as a result of possible release of metals from the WTRs into the leachate. Comparative analysis of the metal leaching or immobilization in the columns showed treatment run which incorporates 50 tons/acre of Fe-WTR, 15 tons/acre each of liming agent (Ca-WTR) and manure material to be more efficient in reducing metal mobility and maintaining neutral pH.

Table T2.4 Concentrations* of metals, pH and ORP of leachates in treatment and control columns (SP).

Sample	Leaching Period	pH	ORP	Fe	Mn	Al	Zn	Cu	As	Pb
SP -1	Wk 8	7.57	212	0.306	31.9	0.203	64.5	23.9	8.78	BDL
	Wk 12	7.04	144	0.028	21.2	BDL	51.6	25.5	11.9	BDL
SP -2	Wk 8	7.38	262	0.308	23.2	0.136	89.2	17.2	6.80	BDL
	Wk 12	7.03	173	0.020	12.5	BDL	198.4	27.2	7.37	BDL
SP -3	Wk 8	7.13	282	0.177	22.7	0.074	63.4	13.5	4.08	BDL
	Wk 12	7.16	148	0.007	13.4	BDL	45.3	12.2	5.75	BDL
SP -4	Wk 8	7.15	289	0.407	25.4	0.148	97.6	14.3	4.79	BDL
	Wk 12	7.34	148	0.006	5.89	BDL	74.5	7.9	4.73	BDL
SP -5	Wk 8	7.27	284	0.625	59.2	0.210	107	13.7	3.41	BDL
	Wk 12	7.32	145	0.016	5.37	BDL	98.5	12.8	6.02	BDL
SP -6	Wk 8	7.30	278	0.579	46.1	0.217	98.1	14.2	4.04	BDL
	Wk 12	7.21	152	0.017	5.95	BDL	126	16.6	4.63	BDL
SP -7	Wk 8	7.33	282	0.358	17.9	0.114	106	11.8	2.40	BDL
	Wk 12	7.28	145	0.017	9.13	BDL	11.7	20.9	6.50	BDL
SP -8	Wk 8	7.24	276	0.146	13.6	0.045	15.4	14.1	3.70	BDL
	Wk 12	7.40	137	0.036	5.17	BDL	34.8	19.9	9.39	BDL
SC-1	Wk 8	6.94	164	0.490	18.1	0.104	6.27	4.58	5.92	BDL
	Wk 12	6.73	97.8	0.013	28.9	BDL	BDL	5.70	7.21	BDL
SC-2	Wk 8	6.81	280	0.723	9.98	0.099	2.32	4.77	6.53	BDL
	Wk 12	7.0	111	0.018	3.33	BDL	4.27	11.5	3.33	BDL
SC-3	Wk 8	3.33	468	2.50	2930	5.18	824	20.1	4.20	BDL
	Wk 12	3.33	468	0.144	1480	2.44	1104	13.6	3.51	BDL

BDL = below detection limit; Concentration in mg/L (for Fe & Al) and µg/L for other metals*

Table T2.5 Concentrations* of metals, pH and ORP of leachates in treatment and control columns (OP).

Sample	Leaching Period	pH	ORP	Fe	Mn	Al	Zn	Cu	As	Pb
OP -1	Wk 8	7.29	259	0.300	12.8	0.051	52.7	5.18	1.46	BDL
	Wk 12	7.51	136	0.024	9.42	BDL	89.9	23.9	12.6	BDL
OP -2	Wk 8	7.27	249	1.08	40.8	0.193	47.5	12.8	3.66	BDL
	Wk 12	7.41	144	0.015	25.0	BDL	12.7	31.5	20.0	BDL
OP -3	Wk 8	7.50	227	0.418	18.6	0.060	22.1	14.0	4.78	BDL
	Wk 12	7.54	139	0.031	10.4	BDL	35.6	16.6	13.3	BDL
OP -4	Wk 8	7.38	223	0.731	27.3	0.222	30.5	10.8	2.95	BDL
	Wk 12	7.60	147	0.017	4.92	BDL	41.8	17.6	6.79	BDL
OP -5	Wk 8	7.40	222	0.276	16.1	0.033	64.9	18.1	4.69	BDL
	Wk 12	7.33	138	0.005	9.02	BDL	108	29.1	5.48	BDL
OP -6	Wk 8	7.75	215	0.604	38.5	0.101	85.2	19.5	8.69	BDL
	Wk 12	7.50	143	0.125	13.4	0.031	191	21.7	4.72	BDL
OP -7	Wk 8	7.69	210	0.635	37.4	0.096	10.1	6.42	3.46	BDL
	Wk 12	7.49	126	0.003	7.66	BDL	18.0	9.77	4.06	BDL
OP -8	Wk 8	7.53	211	0.631	36.5	0.083	12.0	6.97	4.98	BDL
	Wk 12	7.48	120	0.054	6.96	BDL	122	5.60	9.10	BDL
OC-1	Wk 8	7.58	213	0.490	18.1	0.104	6.27	4.58	5.92	BDL
	Wk 12	7.72	122	0.013	28.9	BDL	7.67	5.70	7.21	BDL
OC-2	Wk 8	7.41	215	0.723	9.98	0.099	2.32	4.77	6.53	BDL
	Wk 12	7.71	128	0.018	3.33	BDL	4.27	11.6	3.33	BDL
OC-3	Wk 8	3.84	388	2.50	2930	5.18	824	20.1	4.20	BDL
	Wk 12	4.08	377	0.144	1480	2.44	1104	13.6	3.51	BDL

BDL = below detection limit; Concentration* in mg/L (for Fe & Al) and µg/L for other metals

3.2.9 Plant growth and health (Chlorophyll/Sufficiency Index): When agricultural soils are contaminated by heavy metals beyond acceptable limits through various anthropogenic processes, plant foliage is damaged and growth rate is also retarded (Adriano, 1986). To assess the current condition of the Vetiver grass, the concept of tissue testing was applied to provide an assessment of crop Nitrogen status. Research indicates a close link between leaf chlorophyll content and leaf Nitrogen content since the majority of leaf nitrogen is contained in chlorophyll molecules. The Minolta chlorophyll meter (model SPAD 502) was used to measure the chlorophyll content. Reference chlorophyll meter reading was recorded from a Vetiver grass which had been grown separately in rich potting soil. After recording average (N=4) meter readings from each column and reference grass, a Nitrogen sufficiency index was calculated from equation 2.1 and shown in table T2.6.

$$\text{sufficiency Index} = \frac{\text{average chlorophyll meter reading}}{\text{average reference reading}} \times 100 \dots \dots \dots [2.1]$$

Table T2.6 Vetiver grass nitrogen sufficiency index analysis

Seep area soil					Oxidation Pond area soil				
Column ID	Plant density	Average meter reading (N=12)	SD	S.I	Column ID	Plant density	Average meter reading (N=12)	SD	S.I
SV -1A	3	42.5	2.18	99.3	OV -1A	3	40.6	2.14	94.7
SV -1B	3	41.0	5.19	95.7	OV -1B	3	41.8	2.84	97.6
SV -2A	3	41.3	1.92	96.5	OV -2A	3	40.7	1.81	95.0
SV -2B	3	42.5	4.77	99.1	OV -2B	3	41.7	1.34	97.4
SV -3A	3	42.4	3.11	99.0	OV -3A	3	45.2	2.49	105.4
SV -3B	3	42.8	3.85	99.8	OV -3B	3	46.2	3.18	107.9
SV -4A	3	38.7	4.36	90.3	OV -4A	3	39.8	3.81	92.8
SV -4B	3	44.9	3.07	104.9	OV -4B	3	37.3	1.76	87.1
SV -5A	3	45.8	2.08	106.9	OV -5A	3	36.5	10.00	85.2
SV -5B	3	44.3	3.11	103.5	OV -5B	3	40.7	4.91	95.0
SV -6A	3	41.8	2.98	97.5	OV -6A	3	38.3	1.66	89.3
SV -6B	3	43.0	2.29	100.5	OV -6B	3	43.8	3.99	102.3
SV -7A	3	40.5	2.75	94.5	OV -7A	3	42.0	2.73	97.9
SV -7B	3	43.5	2.07	101.6	OV -7B	3	41.2	2.48	96.1
SV -8A	3	41.0	2.86	95.0	OV -8A	3	40.3	3.69	94.1
SV -8B	3	39.1	3.17	91.15	OV -8B	3	39.2	3.16	91.6

3.2.10 Vetiver grass harvesting and growth estimation: The Vetiver grass was harvested (Figure T2.21) at the end of the column study. The soil on the roots was carefully washed off. Measurement of the root length and shoot height were conducted and recorded for growth estimation during the period of study. The difference between the shoot and root lengths of the grass prior to transplanting into the columns and after harvesting gives an indication of the extra length gain in the root and shoot system.

Each harvested grass was cut and separated into root and shoot and bagged for storage as shown in figure T2.22a. They were subsequently oven dried (figure T2.22b), ground and underwent high temperature combustion to produce ash which was acid digested in order to perform ICP-MS analysis to identify the adsorbed concentrations of constituent metals from the contaminated soil. Analysis of the plant tissue translocation of adsorbed metals were also be evaluated. The analytical results will determine the efficiency of our proposed phytoremediation and WTR metal immobilization models.



Figure T2.21 Vetiver freshly harvested Vetiver at end of the column study.



Figure T2.22 (a) Bagged Vetiver grass.

(b) Oven drying of harvested Vetiver grass

The shoot length was measured as the length of the longest shoot in the center area of the grass. The longest root in a horizontally stretched position was recorded as the root length for all the grass. The estimated change in Vetiver and Pokeweed root and shoot length indicates normal growth. There was no observed loss in grass root or shoot, though some of the plants showed signs

of stress visible by discoloration of the leaves. Table T2.7a shows the mean root and shoot growth of duplicate columns of each run. Vetiver plant growth was observed across the columns, with the aforementioned best treatment mix (run 3) column recording the most shoot growth in SS (71 cm) and OS (63.8 cm) during the study period. Corresponding shoot growth of 23 cm and 19.3 cm was recorded for Vetiver in the SS and OS respectively.

Table T2.7b shows that the shoot and root growth for the same mix for Pokeweed in SS was 10.7 cm and 7.7 cm respectively. Similarly, the shoot and root growth for Pokeweed in OS was 11.3cm and 17.8cm respectively.

Table T2.7a Root and shoot growth of Vetiver

Column ID	Number of Plants	Increase in root (cm)	SD	Increase in shoot (cm)	SD
SV -1	6	23.3	12.4	49.0	11.6
SV -2	6	41.7	17.7	82.8	17.9
SV -3	6	23.0	8.6	71.0	16.2
SV -4	6	31.5	6.6	65.3	27.6
SV -5	6	34.7	6.6	51.5	15.8
SV -6	6	25.0	8.5	38.0	14.8
SV -7	6	42.7	20.6	56.7	24.2
SV -8	6	25.8	6.3	45.3	13.4
OV -1	6	32.8	11.2	48.3	19.8
OV -2	6	28.2	5.2	49.8	19.8
OV -3	6	19.3	14.5	63.8	25.8
OV -4	6	39.2	20.6	53.5	16.0
OV -5	6	19.8	10.1	38.8	10.7
OV -6	6	35.2	22.2	46.5	20.1
OV -7	6	19.5	7.4	32.0	13.0
OV -8	6	27.5	17.5	27.7	22.1

Table T2.7 b Root and shoot growth of Pokeweed

Column ID	Number of Plants	Increase in root (cm)	SD	Increase in shoot (cm)	SD
SP-1	6	20.4	6.9	23.6	20.3
SP-2	6	14.0	6.8	19.7	16.8
SP-3	6	7.7	9.6	10.7	14.1
SP-4	6	16.2	11.2	10.3	14.4
SP-5	6	13.7	7.5	4.3	12.8
SP-6	6	10.0	4.1	15.7	5.8
SP-7	6	12.7	12.4	11.5	12.0
SP-8	6	15.7	12.5	19.8	5.6
OP-1	6	22.0	7.9	12.8	8.3
OP-2	6	15.3	3.9	13.7	10.5
OP-3	6	17.8	10.1	11.3	9.5
OP-4	6	19.3	8.8	5.0	4.3
OP-5	6	5.0	10.1	10.2	11.3
OP-6	6	15.3	14.6	10.5	4.3
OP-7	6	13.3	5.5	7.0	7.7
OP-8	6	12.3	9.4	0.7	4.5

3.2.11 Phytoremediation plants tissue analysis: Several past studies have demonstrated the effectiveness of plants in cleaning up contaminated soil (Wenzel et al., 1999). The favored application of phytoremediation stems from its being aesthetically pleasing as well as several fold more cost efficient than other physical or chemical remediation methods since it depends on sun light, requires minimal to no maintenance once established and it is performed in situ (Hooda, 2007). To assess the efficiency and contribution of Vetiver and Pokeweed to the metal removal from the soil, the plants were harvested for metal analysis. A Multi-element, ICP-dry ash method (Munter and Grande, 1981) was used to prepare the Vetiver plant samples for the simultaneous total heavy metals analysis of by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Dried plant roots and shoots were oven dried and dry-ground with a blender. 500 mg sample of dried plant material was weighed into a 20 mL high form silica crucible and dry ashed at 485°C for 10 – 12 hours. (Crucibles were covered during the ashing as a precaution against contamination.) The ash was equilibrated with 5 mL of 20% HCl at room temperature for ½ hour. Then 5 mL of deionized water was added, gently swirled and allowed to settle for 3 hours. The solution was decanted into 15 ml plastic disposable tubes for direct determination by ICP-MS.

3.2.12 Task 2 Summary Findings:

- The 21-day incubation tests of acidic soil and Fe-WTR, showed a higher acid neutralization capacity of the WTRs. The acidity of the OS soil (pH≈3.48) was increased to between 6.95 to 7.39 by the various application rates. Likewise, the seep soil (pH≈3.12) was neutralized to between 6.91 to 7.49.

- The soil pH at the end of the 12-week column leaching study showed further increase, which proves the longer term soil pH neutralization potential of the proposed amendment technique.
- Analysis of leachate following leaching protocol with deionized water adjusted to simulate rain water conditions in Illinois showed very low concentrations of heavy metals from both soil samples integrated with either Vetiver or Pokeweed phytoremediation.
- In all cases of the WTR/lime/manure amendments, the leachate pH over the study period remained near neutral.
- Comparative analysis of the metal leaching or immobilization in the soil columns showed treatment run which incorporates 50 ton/acre of Fe-WTR, 15 tons/acre each of liming agent and manure to be most efficient in reducing metal mobility and maintaining neutral pH.
- Prior to harvesting Vetiver grass, a Minolta chlorophyll meter (Model SPAD 502) was used to measure the chlorophyll content from which estimates of the nitrogen sufficiency index (SI) of the grass were made. The minimum SI estimate was 87.1 with over 81% of the columns having SI>95.0.
- Vetiver plant growth was observed across the columns, with the aforementioned best treatment mix column recording the most shoot growth in SS (71 cm) and OS (63.8 cm) during the study period. The corresponding growth of 23 cm and 19.3 cm was recorded for shoots in the SS and OS respectively.
- Shoot and root growth for the same mix for Pokeweed in SS was 10.7 cm and 7.7 cm respectively. Similarly, the shoot and root growth for Pokeweed in OS was 11.3 and 17.8 respectively.

3.3 Task 3: Simulated Field Study: Soil Erosion and Metal Leaching Control

Task 3 of this study was a follow-up of the greenhouse column study of Task 2 on a larger scale in the natural environment in southern Illinois weather condition. Erosion and sediment transport occurs when rain falls and impacts on the soil surface resulting in the dislodging of soil particles which may be transported by the rain splash or overland flow. Efforts at reducing soil erosion have long been a priority in various conservation projects. Common conventional approaches to controlling soil erosion, example construction of berms to detain or direct runoff, are mostly expensive and use up a lot of available space. The proposed application of Vetiver grass and WTR application is a relatively low cost erosion and sediment control technology which can be presented as a promising alternative to conventionally constructed berms. Due to its high tolerance level to heavy metals toxicity (e.g. Al and Mn), Vetiver has been applied successfully for phytoremediation and erosion control in acid sulfate soils (pH around 3) in different parts of the world (Truong et al. 2003). Water treatment residuals have been used in buffer strips down slope from soils containing elevated metal concentrations.

A simulated field study using the optimized parameters acquired from Task 2, to evaluate the application of WTR and Vetiver for soil erosion and metal leaching control was conducted at the SIU's Energy Park located in Carterville, Illinois. An incubation test was setup based on the results of the Vetiver/WTR/Contaminated soil column study performed in our green house facility as reported previously. Based on the level of metal contamination, the seep area soil which is comparatively more contaminated in some relevant metals (Fe, Mn etc) than the Oxidation Pond area soil was decided on for use in this task. The use of the more contaminated soil type for further treatment study was to assume and take care of a worst case scenario of AMD impacted soil. The

treatment mix (rate of WTR, lime and manure application) applied in this relatively larger scale experiment was based on the pH, percent retention of metals in the columns and the plant chlorophyll sufficiency index from the column study in Task 2. Previously reported experimental run 3 which includes the following applications: 50 ton/acre WTR, 15 ton/acre lime and 10 ton/acre of manure, was used in setting up the incubation test for Task 3.

Large quantities of AMD impacted soil were collected from several sampling points within the definition of the seep area at Tab-Simco. The collected soil sample was transported to the Energy Park and was spread for air drying. After days of air-drying, the soil samples were crushed and sieved as shown in figure T3.1. The weighted amount of the AMD impacted soil and the calculated amount of WTR was mixed thoroughly using a mixer (figure T3.2). Calculated amount of water was added to establish soil/WTR incubation at about 80% field capacity for 21 days.



Figure T3. 1 sieving of air dried AMD impacted soil.



Figure T3.2 Preparation for incubation test –mixing of WTR with AMD impacted soil.

Two prepared wooden platforms were used to setup the experiment. One platform (6 ft long x 3 ft wide) contained treated soil with Vetiver whilst the other platform was divided into two from the width (ie. 6 ft x 1.5 ft each). The second platform contained untreated soil with planted Vetiver and Bermuda grass. Figure T3.3 shows the schematic components of the platforms. To prevent leachate's contact and loss through the wood, a plastic material was used to line the wooden platform.

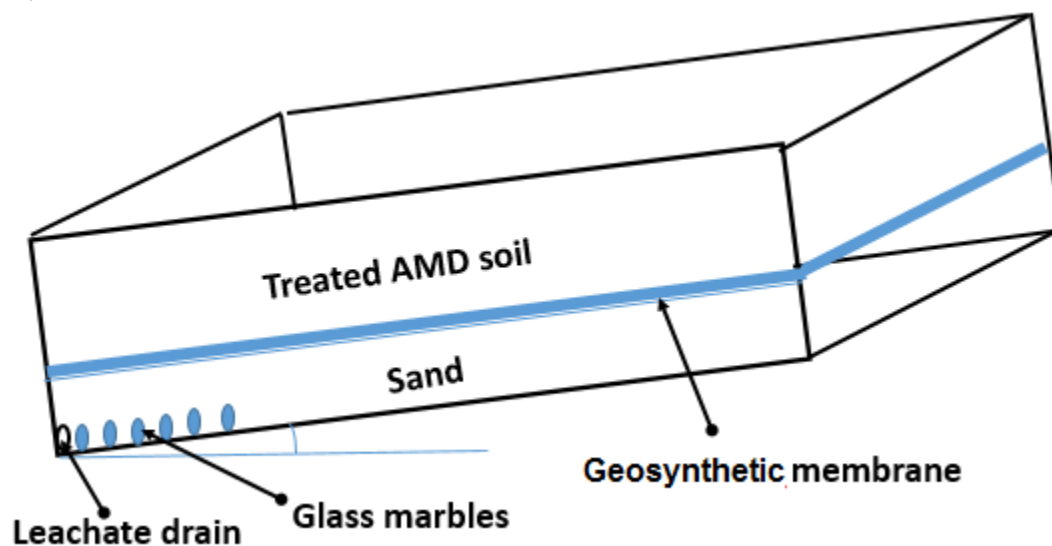


Figure T3.3 Schematic illustration of the wooden platform.

PVC containers were used as sediment check dams into which eroded soil particles from the platforms was measured to study the effects of Vetiver and Bermuda grass on soil erosion under normal environmental conditions. Figures T3.4 to T3.6 show the platforms setup.



Figure T3.4 wooden platform lined with plastic bag and a separating column.



Figure T3.5 Placement of permeable geosystemic material as a divide between AMD soil treatment layer and free draining soil.

The three study platforms were each connected with a container at the lower end to collect leachate after rain events. Leachates are collected after rain events for metal analysis. The amount of sediments that was collected in the sediment dam was filtered, oven dried and weighed. This is to quantify and compare the rate of erosion.



Figure T3.6 Task 3 set up with sediment check containers (dams) at week 2



Figure T3.7 Task 3 set up with sediment check containers (dams) at week 12

3.3.1 Erosion Measurement: After sudden storms, it was observed that that deposits of fine material washed down from the abovementioned sloped area. Soil erosion was assessed by the potential for soil particles to be transported and/or deposited beyond the limits of the prepared soil platform. All plots were placed on similar slopes of <12%. Following rain events, the amount of sediments collected in prepared sediment dams was filtered, dried and weighed for each of the 3 individual sets; Vetiver in treated soil, Vetiver in untreated soil and Bermuda grass in untreated soil.

During the period of the study, recorded monthly precipitation values for Carterville, Illinois was observed (weather.com). The rates of erosion observed in the 3 platforms differed over the study period as a result of the difference in recorded precipitation. The monthly recorded precipitation during the study period were 4.03 inches, 0.84 inch, 4.12 inches and 5.02 inches for May, June, July and August respectively. Due to the low amount of rainfall in June coupled with high temperature (average 86°F), the Vetiver grass was stressed from the hardening of the soil but survived. There was no recorded death of any Vetiver grass during the study. Overall, the erosion rates were comparatively low. The highest erosion rate (0.075 kg/m^2) was recorded from the 3' x 6' platform in July which recorded the highest individual storm. The erosion rates during the study period for each set up ranged from 0.002 to 0.006 kg/m^2 (Bermuda grass in untreated soil), 0.003 to 0.02 kg/m^2 (Vetiver grass in untreated soil), and 0.007 to 0.075 kg/m^2 (Vetiver grass in treated soil). The comparatively higher rate of the Vetiver in treated soil is as a result of the size of the platform. The Bermuda setup recorded the lowest erosion rate mainly because of its characteristic ground covering capacity. The relatively higher erosion rates of the Vetiver setups were because, unlike the Bermuda grass which grew immediately to cover the ground, the Vetiver required time to grow to overlap the 10cm planting interval to form a ground cover. The resistance to sediment

transport and erosion was more pronounced in the treated soil with Vetiver than in the untreated soil. Between July and August, a noticeable decline in the erosion rates in the treated soil even though rain intensity increased was observed. The plot shows the erosion rates for the three set ups and the dates on which the silt dams were emptied, filtered and oven dried to estimate sediment.

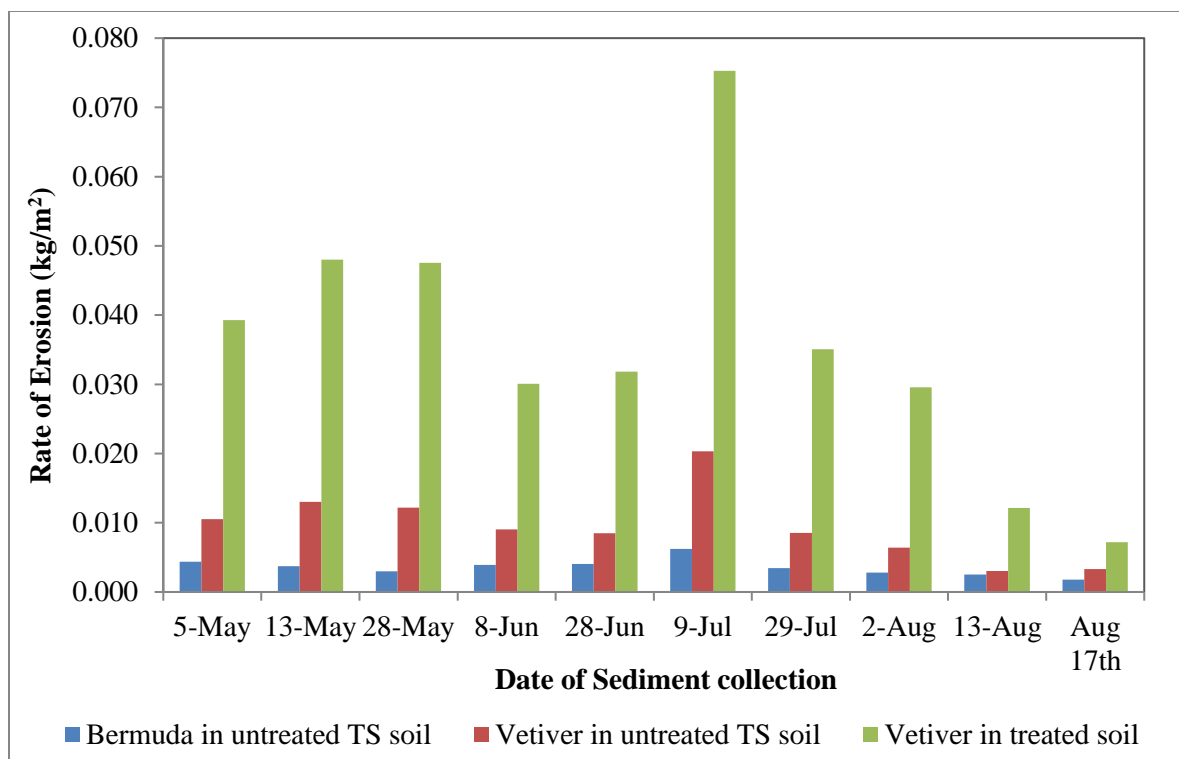


Figure T 3.8: Estimation of the amount of erosion achieved with varying soil conditions through simulated field study

3.3.2 Plant Growth Measurement:

Tissue testing was here again applied to assess the growth condition of the Vetiver grass by estimating the sufficiency index of the grasses growing in the treated and untreated soils. The Minolta chlorophyll meter (model SPAD 502) was used to measure the chlorophyll content of randomly selected Vetiver grasses (N=20). The chlorophyll meter readings from the grass and a reference grass were used to calculate the dimensionless nitrogen sufficiency index. The recorded sufficiency index of the Vetiver grass grown in treated soil was 106.5 and 93.2 for the Vetiver grown in the untreated soil. It can be therefore be inferred that the sufficiency index of 93.2 which is less than 95 suggests a low chlorophyll content in the leaves of the Vetiver grass planted in the untreated soil. The slight yellow coloration observed is evident of low chlorophyll in the Vetiver grass. The sufficiency index of the Vetiver gives an indication of a comparatively better health of the grass in the treated soil.

Vetiver grass samples (N=5) were taken out of the platforms at the end of the study to measure their physical growth by measuring the shoot and root lengths. The measured average change in Vetiver shoot and root from the treated platform was $75 \text{ cm} \pm 7.2$ and 34 ± 3.0 respectively. The Vetiver in the untreated soil recorded an average increase of $50 \text{ cm} \pm 6.3$ in shoot and $20 \text{ cm} \pm 6.7$ in root. It can be seen that there was a significant difference in the growth of the Vetiver grass in

both growth media. The soil amendment with WTR, alkaline agent and manure can fairly be said to have improved the condition of the soil for the grass growth. In the month of June when recorded rainfall was minimum, there were observed exfoliation coupled with some curling of Vetiver grass as well as in the Bermuda. However, upon major rain events in the following months, the grass recovered from the stress.

3.3.3 Leachate Analysis: Due to encountered operational problems, the volume of leachate collected was lower at some points of the experiment. The drainage system designed for the platform malfunctioned, thereby reducing the amount of leachate collected. Five leachate samples were collected from each of three platforms and analyzed for pH, ORP and metal constituent metal concentration. The potential for dilution errors associated with the volume of leachate samples cannot be entirely overruled. Table T3.1 presents a compilation of average parameter values and selected trace metal levels found in the leachate water samples collected during the 4-month study period.

Table T3.1: Average metal concentrations (mg/L) in the leachate samples collected throughout the 4-month simulated field study.

Soil Condition	Study Period	pH	ORP	Fe	Mn	Al	Zn	Cu	As	Pb
Treated soil with Vetiver	< 8 weeks	7.40	209	0.264	0.013	0.082	0.162	0.023	0.009	0.015
	> 8 weeks	7.18	207	0.543	0.012	0.086	0.173	0.026	0.007	0.007
Untreated soil with Bermuda	< 8 weeks	4.11	430	2.45	0.151	3.90	0.611	0.088	0.005	0.005
	> 8 weeks	3.83	460	2.11	0.169	5.10	0.550	0.089	0.005	0.004
Untreated soil with Vetiver	< 8 weeks	4.33	352	1.69	0.139	3.60	0.260	0.077	0.007	0.005
	> 8 weeks	4.23	357	0.903	0.156	4.71	0.345	0.078	0.007	0.005

The study program resulted in a total accumulation of 3,500 ml, 2,060 ml and 1,750 ml of leachate for the treated soil with Vetiver, untreated soil with Bermuda and untreated soil with Vetiver respectively. The low leachate volume could possibly be as a result of the low precipitation and high temperature recorded in June and the usually high precipitation rate resulting in low percolation.

It can be seen from Table T3.1 that the pH of the amended soil remained neutral throughout the study period whilst the untreated soil remained acidic. The lower concentration of major elements in the leachate is because of the reduced mobility as a result of the pH increase. The metal concentration in the untreated soil with planted Vetiver is consistently lower than the untreated soil with Bermuda. This trend can possibly be related to the metal uptake potential of the Vetiver phytoremediation plant. Comparatively, the treated soil shows much soil metal immobilization and pH neutralization than the untreated soil. Though the Bermuda presented a better option for erosion

control from the very beginning, the Vetiver improved its erosion control over time and presented an addition phytoremediation potential.

Percolation of water following rain was reduced, which therefore resulted in low amount of leachate collected for metal concentration analysis. The observed growth of Vetiver resulted in the grass covering the soil. The Vetiver grass growth helped to decrease erosion caused by the flowing water. The growth of Vetiver shoot and root was observed to be significantly different in the WTR treated AMD soil and untreated AMD soil. Nitrogen sufficiency index calculated from measured chlorophyll content was found to be more in the Vetiver grass planted in the WTR amended soil than the untreated soil. Leachate analysis for major trace metal concentration showed a reduced metal concentration in the treated soil with near neutral pH.

3.3.1 Task 3 Findings:

- The major factors that were considered to study soil erosion and metal leaching included rate of precipitation, soil condition and planted grass density. It was observed that in case of high amount of rain in a short period, the water tends to run over the soil surface into the silt dams rather than leach into the soil. The texture of the seep area soil (13.9% clay, 59.4% silt and 26.7% sand) caused significantly dense mass following dry days in June 2016.
- The leachate samples over different time interval were collected for metal concentration analysis. Leachate analysis showed a reduced metal concentration in the WTR treated AMD impacted soil with improved (sustained at neutral) pH.
- There was a better growth of Vetiver grass planted on the WTR treated soil. The Vetiver grass growth helped to decrease erosion caused by the rain water.
- The growth of Vetiver shoot and root was observed to be significantly different in the WTR treated and untreated soil. The growth in root and shoot lengths were higher in the treated soil.
- Nitrogen sufficiency index calculated from measured chlorophyll content was found to be more in the Vetiver grass planted in the amended soil than the untreated soil.
- More research is needed in this area to identify the soil characteristics, initial soil exchangeable metal concentrations, degree of slopes, rainfall intensities, and WTR rate application procedure that help to reduce offsite sediment transport.

3.4 Task 4: Greenhouse Hydroponic Study

The objective of the greenhouse hydroponic study was to investigate applicability of Vetiver Grass (*Vetiveria zizanioides*) and Bermuda grass for the efficient removal of heavy metals from acidic water collected from the Tab-Simco mine site in Carbondale, IL. This part (Task 4) of the study was sub-contracted to the Michigan Technological University (MTU) and all experiments of this task were conducted in a greenhouse facility at Michigan Technological University.

Vetiver grass plugs (Figure T4.1) and grown Bermuda grass (Figure T4.2) were initially nursed prior to the commencement of Task 4. Hydroponic containers were prepared from plastic storage vessels that were painted black to reduce internal algal growth, while the lids were drilled with holes for the placement of plants into the hydroponic media as shown in figure T4.3. Plants were acclimated in hydroponic media (0.5X Hoagland's solution) for 30 days, and were setup at varying plant densities of 4, 3 and 2 plants per container ($n = 3$ for biological replicates) for the 30-day study period with the

start date as day 0. Tab-Simco AMD was used for the passive treatment by the plants and fresh hydroponic media for plant health comparison. A negative control setup was also prepared.



Figure T4.1 Vetiver grass in a nursery.



Figure T4.2 Bermuda grass in a nursery.



Figure T4.3 Sample of a prepared hydroponic container for plant density of 3



Figure T4.4 The complete hydroponic experimental setup in the green house.

To assess plant growth rate in the system, plants were harvested at 0, 5, 15 and 30 days for measurement of biomass, shoot length and root length. On final harvest the plants were stored at -80°C for downstream processing. The Vetiver grass was able to withstand the Tab-Simco AMD for 30 days with only three cases of deceased individuals (two grown in AMD and one grown in media) out of the total 54 Vetiver grass. Two of the deceased Vetiver grass (one in AMD and one in media) showed signs of stunted growth visible in the leaves, after 2 days of the experiment. This could be attributed to shock from the addition of fresh AMD or media solution. In general, both AMD and media grown Vetiver demonstrated some browning on outer tissue layers at the base of the shoots and/or at the end of the shoots as shown in Figure T4.5.

The observed browning of the Vetiver shoot was more significant in a number of Vetiver grown in AMD. Seven (7) out of the twenty-seven (27) AMD grown Vetiver exhibited various degrees of curling of the leaves, and some discoloration in a couple of individuals as depicted in figure T4.5. The curling was observed at day 20 of the experiment. However, 19 of the 27 AMD grown Vetiver grass showed development of fresh root tissues (Figure T4.6). In most cases, Vetiver biomass and root length did not change by more than 2 cm over the course of the experiment. It was observed that with the exception of five individual grasses, shoot lengths increased or remained unchanged for all other individuals. Tables T4.1a and T4.1b presents the changes in Vetiver growth monitoring parameters for all the Vetiver grass setup conditions. On a cross-comparison it can be seen that the AMD grown Vetiver showed fairly uniform response to the as those grown in the media grown. The browning and evidence of curling seen in the leaves suggests that the AMD caused mild toxicity effect on the Vetiver. However, the observed development of fresh root tissues and the net increase in overall shoot length indicates that Vetiver grass can fairly withstand the high heavy metal contamination and low pH of the Tab-Simco AMD.

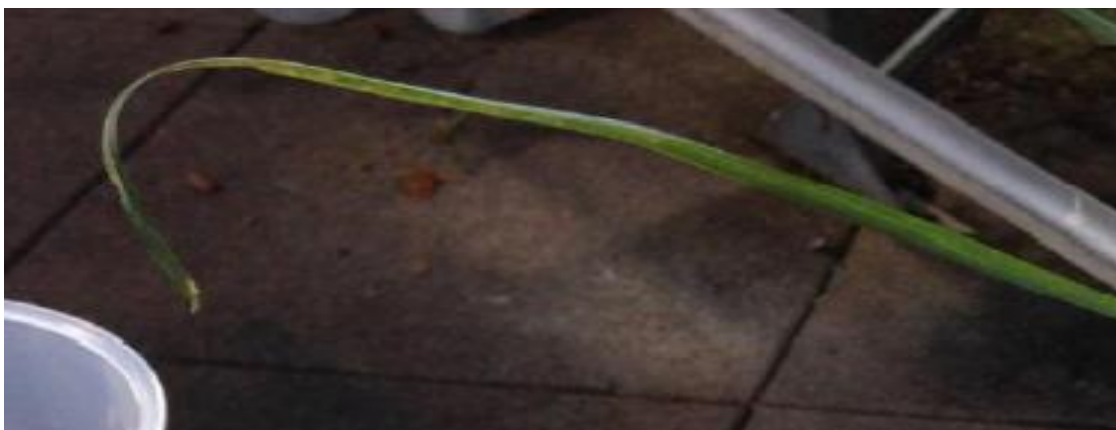


Figure T4.5 Shoot condition of Vetiver grass grown in AMD at day 30.



Figure T4.6 Development of new roots of Vetiver grass grown in AMD at day 30.

Table T4.1a Change in plant biomass, shoot and root length of Vetiver

Container #	Plant Density	Plant ID	Biomass (g)	Shoots (cm)	Roots (cm)
1	4	V01	1.8	27	1.5
		V02	-2.42	0.7	-0.3
		V03	-2.58	-19.9	0.3
		V04	0.81	12.7	7
2	4	V05	0.16	0.7	-0.4
		V06	-0.75	0.4	-0.6
		V07	1.96	31.7	0
		V08	-0.08	12.7	1.6
3	4	V09	-1.45	0.6	-0.6
		V10	-0.85	-0.2	0.2
		V11	0.11	4.3	-0.9
		V12	0.16	12.5	-0.8
4	3	V13	1.2	28.6	-0.9
		V14	-0.13	6.2	-0.4
		V15	2.17	33.3	-0.3
5	3	V16	-3.48	-0.4	-1
		V17	0.1	1.6	0.2
		V18	2.05	0.5	15
6	3	V19	0.37	15.5	-7.8
		V20	-0.03	8.6	-2.9
		V21	-0.13	12.6	-0.4
7	2	V22	0.25	29.7	-1
		V23	-0.13	7.2	5
8	2	V24	0.46	17.8	0.2
		V25	0.64	4.3	-1.4
9	2	V26	-0.85	0.6	0
		V27	0.1	21.1	-1.6

Table T4.1b Change in plant biomass, shoot and root length of Vetiver

Container #	Plant Density	Plant ID	Biomass (g)	Shoots (cm)	Roots (cm)
13	4	V28	-0.31	13.6	-0.6
		V29	0.42	6.9	-5.9
		V30	0.48	5.7	-10
		V31	-0.29	1.1	-1
14	4	V32	0.41	16.5	0.1
		V33	-0.12	1.7	-0.3
		V34	0.99	3.6	-0.4
		V35	-0.22	9.2	-0.1
15	4	V36	0.04	-0.4	-2.8
		V37	0.81	1.1	0.4
		V38	-0.11	1.1	-2.7
		V39	0.39	13.9	0.7
16	3	V40	0.33	8.3	-4.1
		V41	1.37	29.1	-0.7
		V42	-5.55	-0.1	-0.3
17	3	V43	0.41	17.1	-1.6
		V44	0.26	13.5	-0.8
		V45	0.18	6	-0.7
18	3	V46	0.55	13.4	-4.6
		V47	0.04	10.1	-4.7
		V48	0.43	9.8	-1
19	2	V49	0.34	20.1	0.2
		V50	1.14	13.3	-1
20	2	V51	0.19	0	-0.5
		V52	1.3	2.8	0.4
21	2	V53	0.29	18.5	0.5
		V54	0.84	23	-0.3

3.4.1 Water sample analysis: Water samples (n = 3 for technical replicates) and dissolved oxygen measurements were taken at 0, 2, 5, 10, 20 and 30 days. The water samples were analyzed for electrical conductivity (EC), pH, total alkalinity, dissolved sulfate, and dissolved metal ions (Fe, Al, Zn, Ni, Cu and Pb). As it can be seen from figure T4.7 (a-d), the AMD showed a steady increase in pH over the study period, more so for Vetiver grass than with Bermuda grass, as hypothesized.

The average initial pH of the Acid Seep AMD was 2.64 ± 0.103 (average \pm SD for 21 samples). Figure T4.7 (a-d) shows the changes (treated and control) in the planting solution pH of the Vetiver and Bermuda grassing the experimental period.

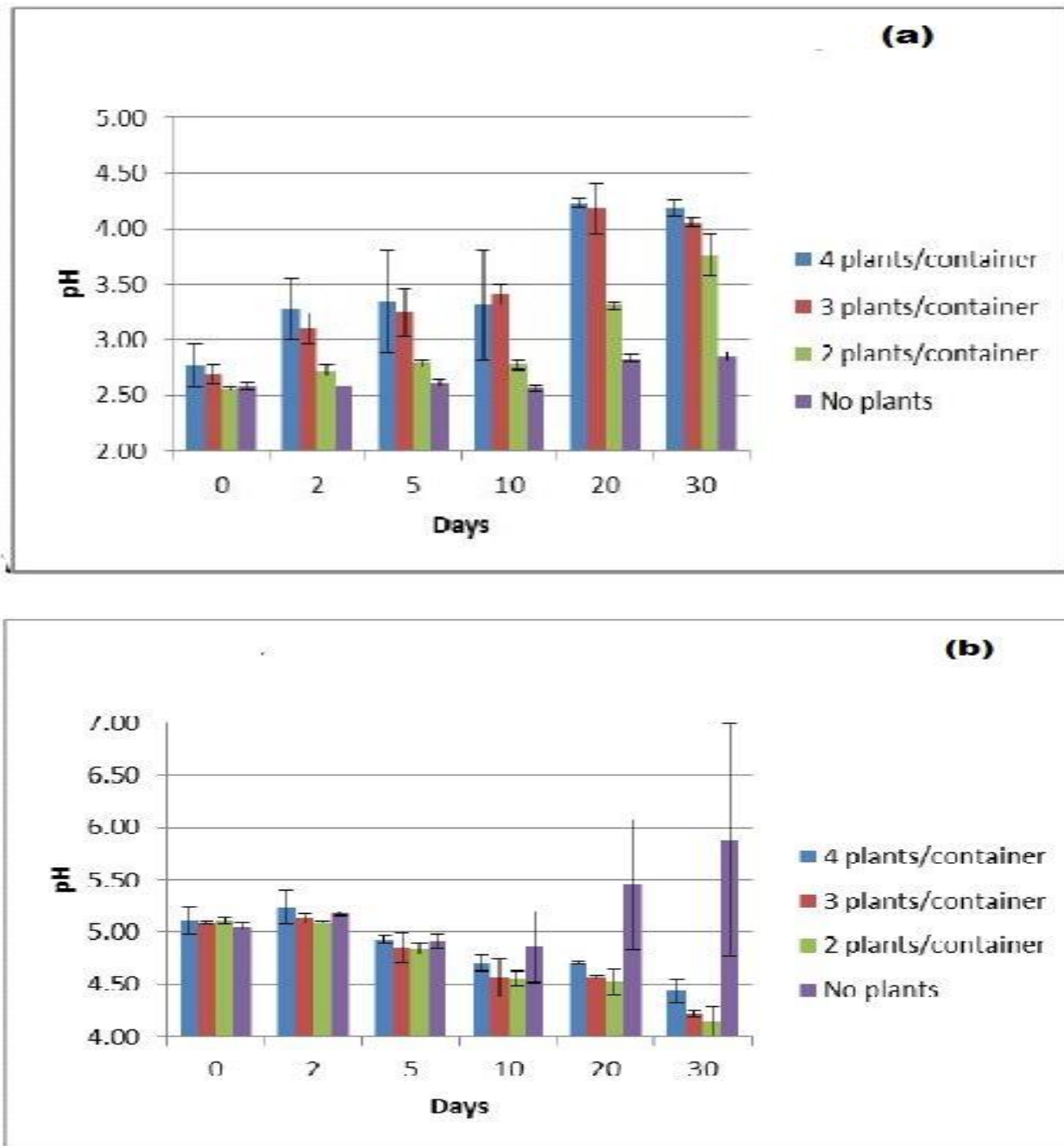


Figure T4.7 Effect of planting densities on solution pH over time. (a) AMD treated with Vetiver (b) control solution with Vetiver.

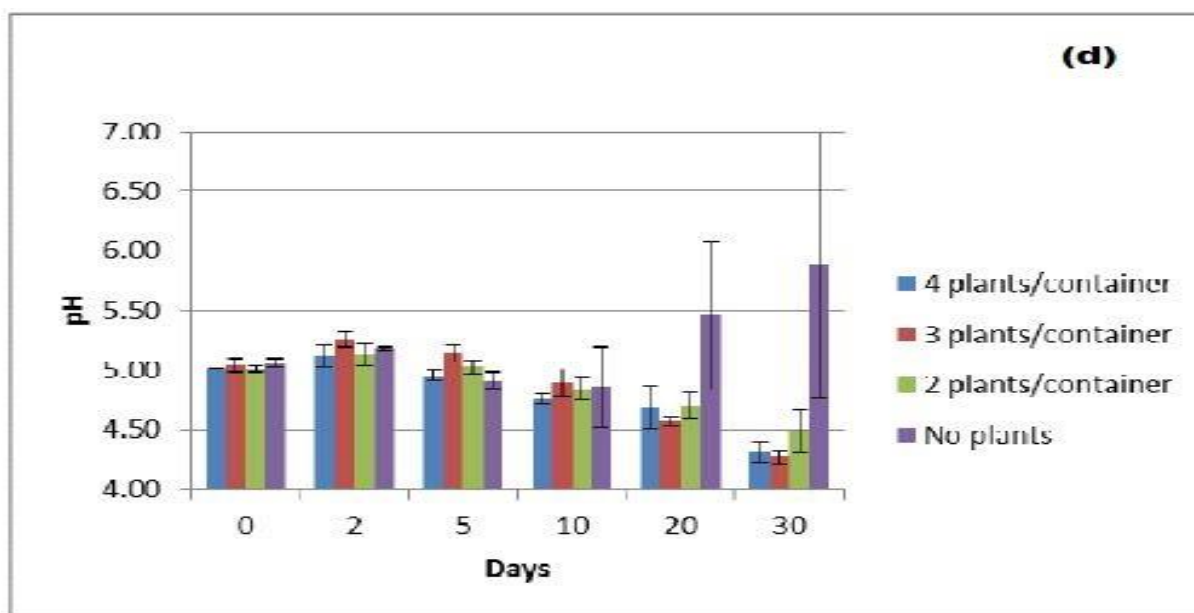
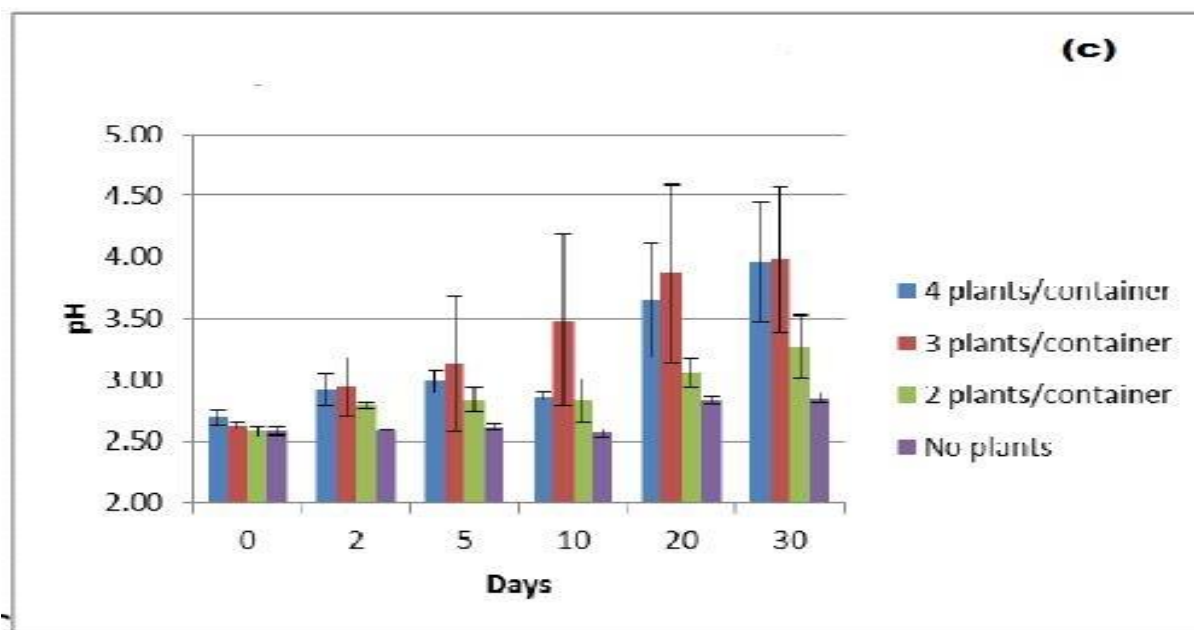


Figure T4.7 Effect of planting densities on solution pH over time (c) AMD treated with Bermuda (d) control solution with Bermuda grass

A single-factor ANOVA identified significant difference ($0.025 < P < 0.01$) across the three planting densities for Vetiver grass at 30 days. Although the difference between densities was not distinctively identified, it would seem that there is less of an increase in pH for the 2 plant density set (3.76 ± 0.186) when compared to 4 and 3 plants (4.19 ± 0.0713 and 4.06 ± 0.0398 , respectively). Total alkalinity was determined for 0 and 30 day AMD samples, though not for media samples because there was no buffering capacity.

Figure T4.8 (a-b) shows significant changes in the starting alkalinity at the end of the 30 days experiment, though this would be expected with a complex system. Starting alkalinity of the AMD across containers was 275 ± 70.7 mg/L CCE and dropped dramatically by 30 days, likely due to plant calcium uptake. Analysis of solution alkalinity indicated that Vetiver grass showed higher decreases in alkalinity across densities than Bermuda grass, which could be explained by the larger biomass of Vetiver.

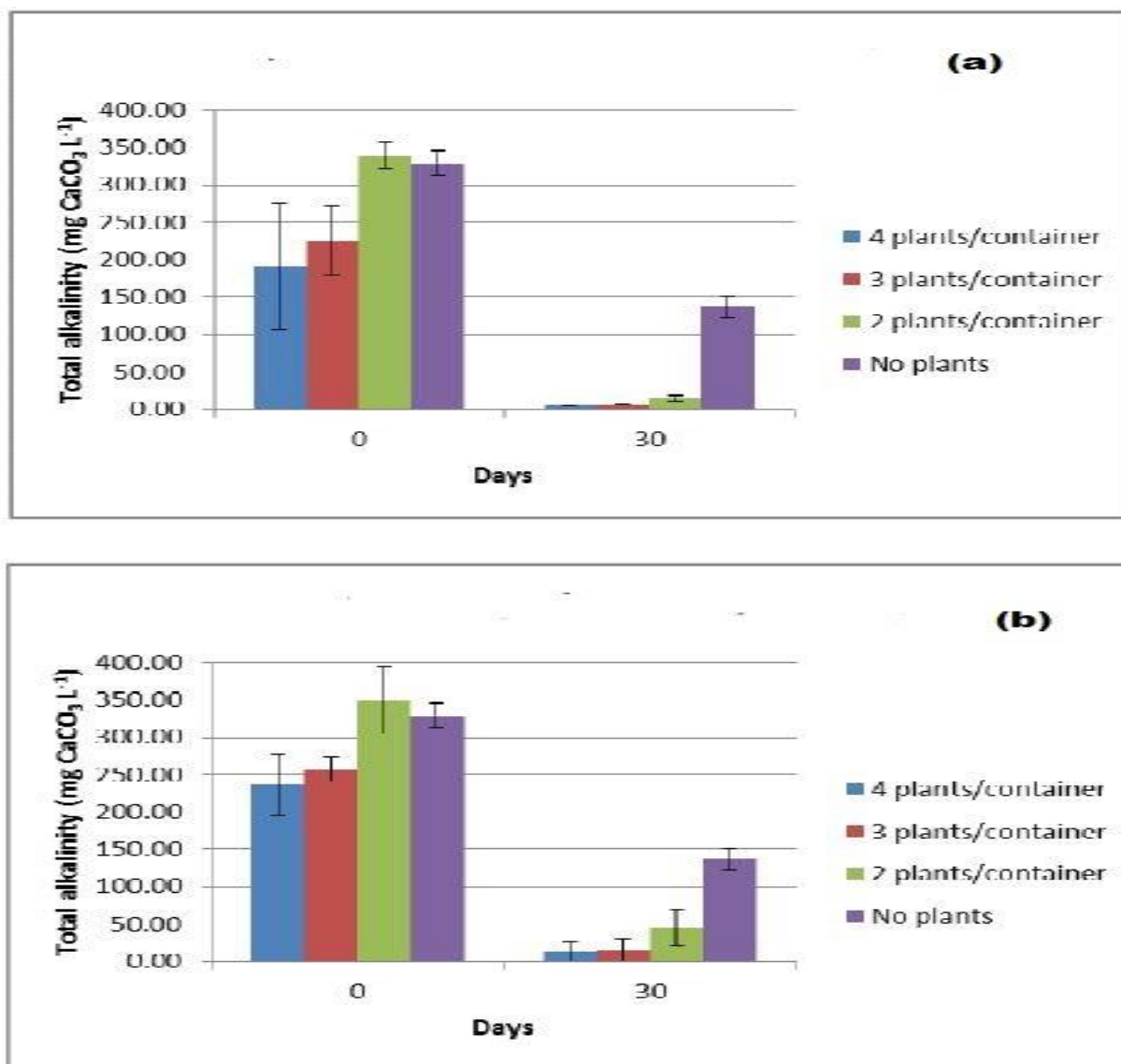


Figure T4.8 Final effect of planting densities on solution total alkalinity (a) AMD treated with Vetiver (b) AMD treated with Bermuda

The AMD also showed decreases in electrical conductivity over the 30 days study period, more so again with the Vetiver grass than with Bermuda grass as shown in Figure T4.9 (a-d). The initial

conductivity of AMD was $3,372 \pm 123.7 \mu\text{S}/\text{cm}$. Single-factor ANOVA showed significant difference ($0.025 < P < 0.01$) across the three densities for Vetiver grass at 30 days. This time the 4 plants ($1,698 \pm 123.3 \mu\text{S}/\text{cm}$) showed the greater difference when compared to the 3 and 2 plants ($2,034 \pm 119.3$ and $1,904 \pm 68.39 \mu\text{S}/\text{cm}$, respectively), likely from removing more metals. A drop was also observed with no plants, though not as drastic as in the presence of the plants. This was attributed to solution loss from the air circulators.

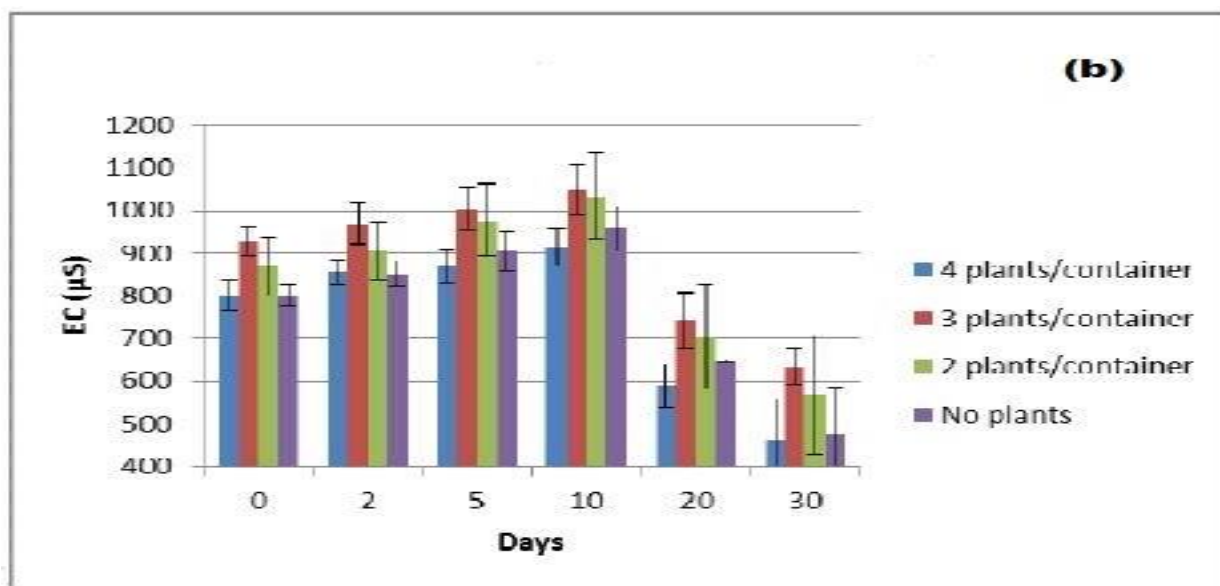
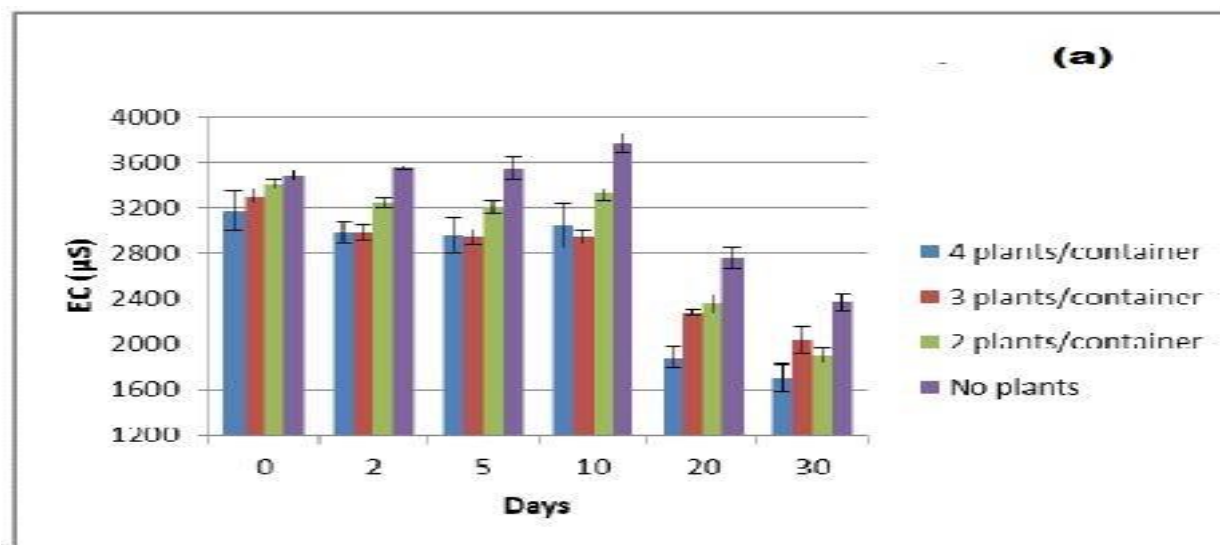


Figure T4.8 Effect of planting densities on solution EC over time. (a) AMD treated with Vetiver (b) control solution with Vetiver

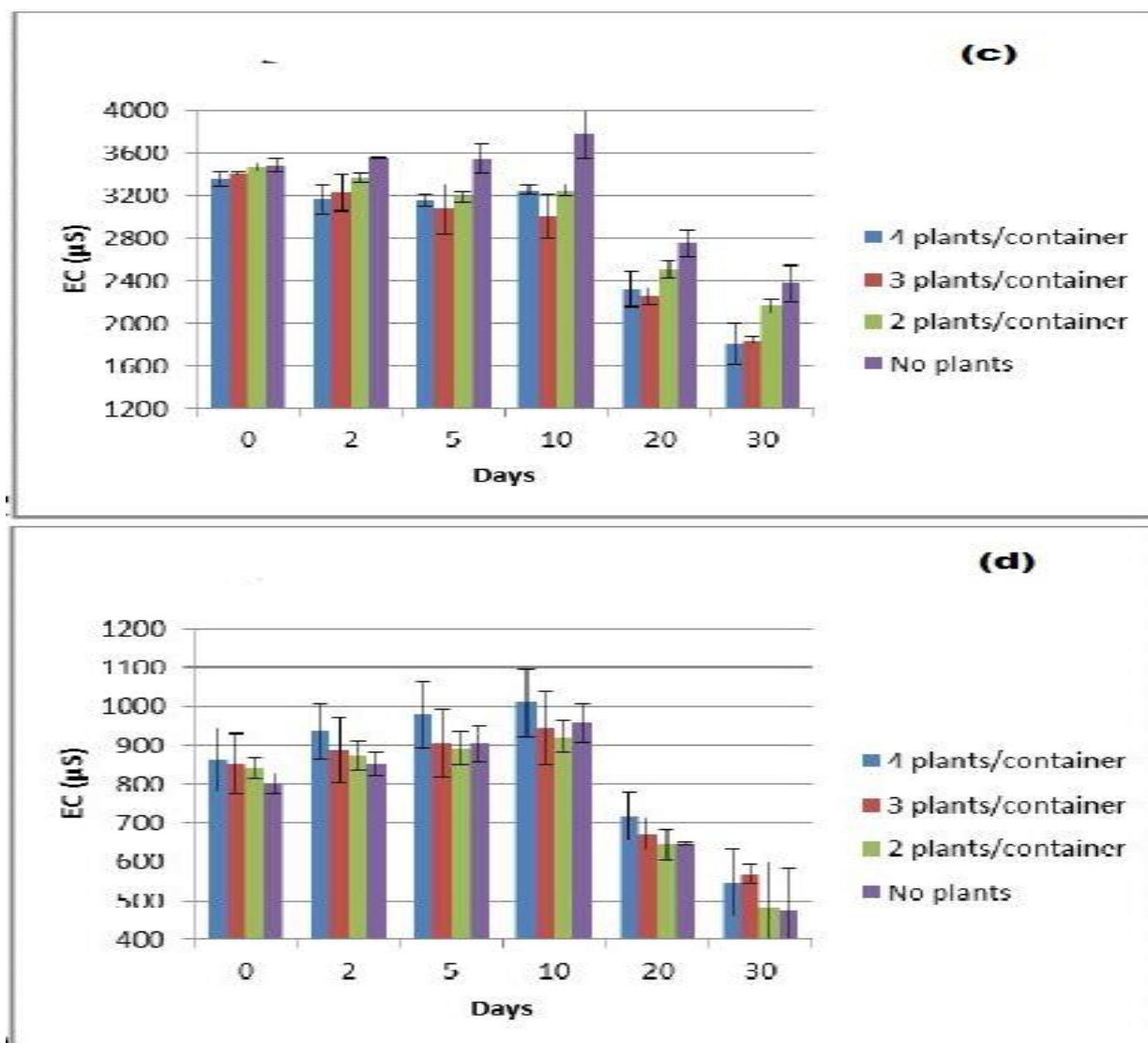


Figure T4.9 Effect of Vetiver grass on sulfate concentrations in AMD over time

The initial sulfate concentration of the collected Tab-Simco AMD was analyzed to be $2,661 \pm 43.60$ mg/L. Figures T4.10 and T4.11 show plant density as a factor of the resulting changes in sulfate concentration over the period of the experiment with Vetiver and Bermuda grass treatments. There were little to no apparent changes in sulfate concentration in the Vetiver solution samples by day 10. Significant sulfate concentration decreases were observed between days 20 and 30 as depicted in figure T4.10. Sulfate removal is more pronounced in the solution with 4 plants, though there was little difference across densities by 30 days (including no plants). This observation was attributed to water loss resulting from the hydroponic air circulators. Similar pattern was observed with the Bermuda grass as shown in figure T4.11 with little to no significant variations across planting densities by 30 days.

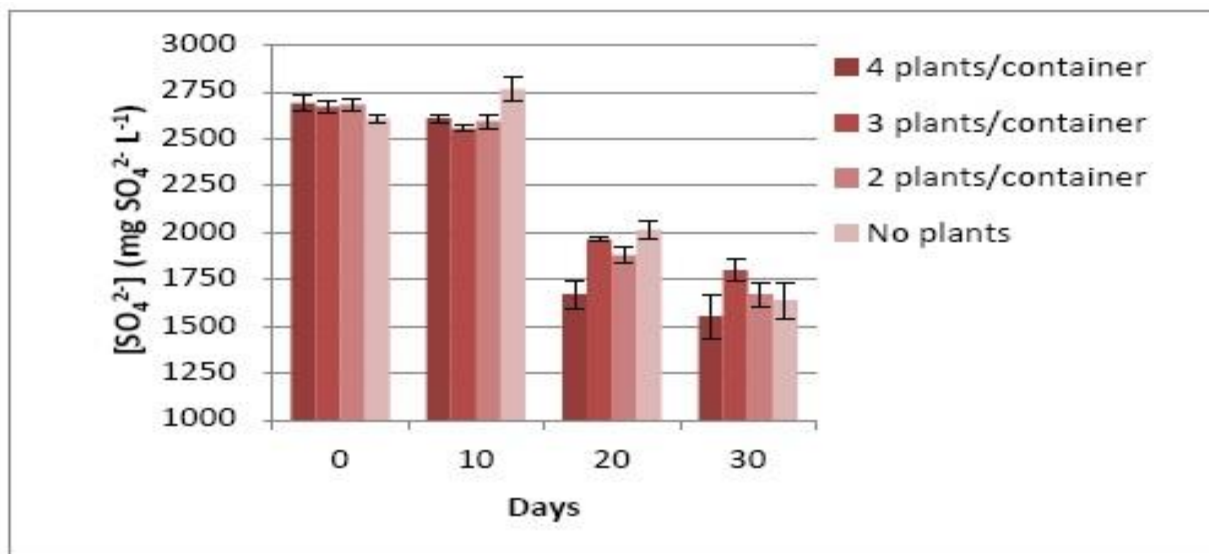


Figure T4.10 Effect of Vetiver grass on sulfate concentrations in AMD over time

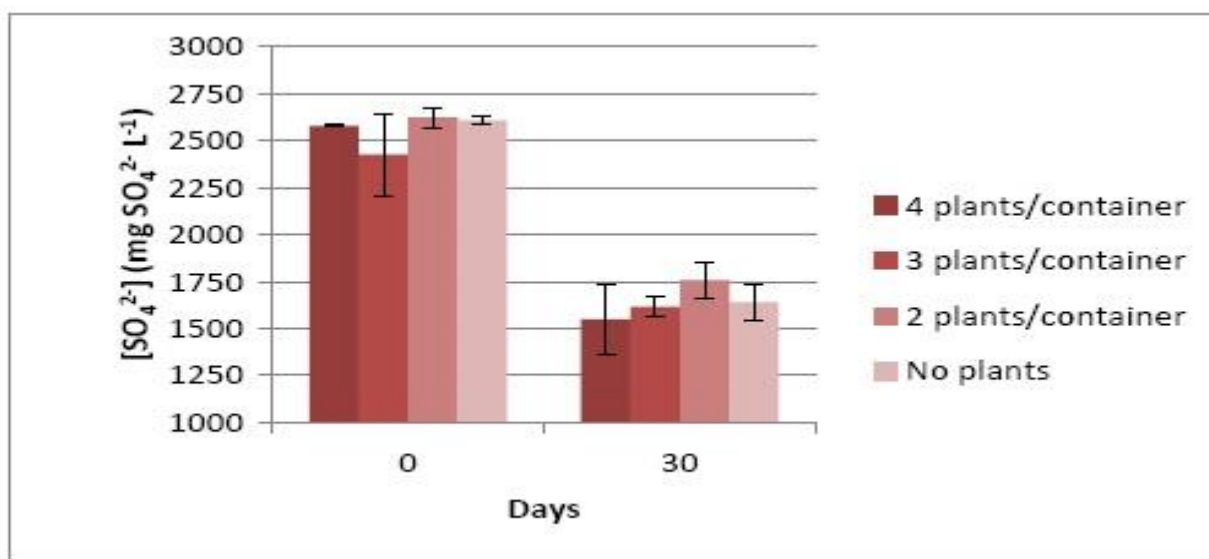


Figure T4.11 Effect of Bermuda grass on sulfate concentrations in AMD over time

The above results give a vivid indication that Vetiver grass is more capable for remediating Tab-Simco AMD than the Bermuda grass as hypothesized. From changes in pH it was shown that the 4 and 3 plants density were more suitable, though the changes in EC and sulfate suggest that the 4 plants are the most suitable. There was no apparent change in dissolved oxygen (data not shown for brevity) and little difference across planting densities for alkalinity. Since the changes in planting density did not reflect to play a role in the drop in alkalinity, it was inferred that higher planting densities are superior.

3.4.2 Vetiver metal uptake analysis: Samples of Vetiver grass were digested following EPA method 3052B for metal uptake and translocation analysis. Heavy metal concentration in the Vetiver tissues was analyzed by ICP-MS and is as shown in Table T4.2. Plant metal content (μg) was calculated using the tissue content for a single plant ($\mu\text{g/g}$) by total tissue biomass of that plant (g) and summing the root and shoot contents. Then the metal content in the Tab-Simco AMD (μg) was calculated using the sample content from a hydroponic container ($\mu\text{g/L}$) by total volume in the hydroponic container (L). The removal efficiency (%) is defined as the ratio of heavy metal uptake to the amount of original heavy metal in the AMD hydroponic container. Table T4.2 shows the percent uptake for individual plants, which suggests that there was fair uptake of Cu, Fe, and Zn, with lesser amounts of Pb, Al, and Ni.

Table T4.2 Percent metal uptake by Vetiver (n=27)

Statistics	Plant metal uptake (%)					
	Fe	Al	Zn	Ni	Cu	Pb
Mean	20.21	2.281	10.05	1.138	37.07	4.638
Median	18.66	1.655	7.681	0.8019	29.37	2.836
Max	46.55	7.182	31.61	8.434	115.1	16.77
Min	4.096	0.6585	1.285	0.3876	13.95	0.3268
SD	10.88	1.777	7.268	1.524	23.34	4.439

Figure T4.12 presents the net metal uptake by plant density per container. It shows high uptake of Cu by Vetiver whereby the 4 and 3 plants density show relatively similar patterns of uptake ($135\pm36.2\%$ and $140\pm41.5\%$, respectively) in comparison with the 2 plant density ($58.5\pm11.4\%$). There were also high amounts of Fe and Zn uptake, though significantly less than the Cu uptake. Fe uptake was the highest in the 4 plant density which showed $74.6\pm53.2\%$ uptake, followed by the 3 plant density with $59.1\pm21.1\%$ uptake, and then the 2 plant density at $48.2\pm6.81\%$ uptake. Zn uptake was moderate in comparison with Cu and Fe, though fairly similar across planting densities at an approximate percent uptake of 30%. The uptake of Pb, Al, and Ni was significantly lower across planting densities ($<10\%$) as with the individual plants, except for the 2 plant density ($22.0\pm8.91\%$ Pb uptake).

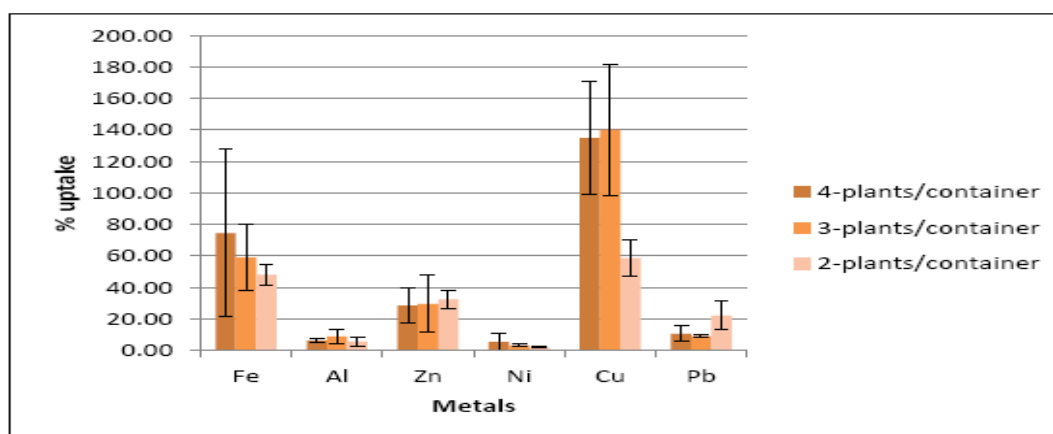


Figure T4.12 Net metal uptake by planting density (mean \pm SD, n = 3)

These results support our previous hypothesis that the observed reduction in electrical conductivity of the AMD solution was a result of metal uptake by the plants. The high Cu uptake (>100%), was likely due to initial accumulation of Cu by the Vetiver grass from watering with local tap water (instead of deionized water) during the nursery stage of the Vetiver grass preparation for the hydroponic study.

Translocation of metal content in the Vetiver tissues is presented in Table T4.3 and the respective percent translocation between the roots and shoots is shown in Figure T4.13. The figure showed that a higher amount of the Fe is accumulated in the roots at $94.5 \pm 7.35\%$. There were also relatively high accumulations of Pb and Al in the roots as well at $83.4 \pm 19.4\%$ and $70.4 \pm 16.2\%$, respectively. The amount of Pb translocated to the shoots ($16.7 \pm 19.4\%$) was similar to the moderate amount (16–33%) suggested from previous studies. Meanwhile, about half of the metal content was translocated with Zn ($45.3 \pm 29.0\%$ root content), Ni ($49.5 \pm 18.9\%$), and Cu ($43.3 \pm 16.9\%$). Despite high standard deviations, the amounts of Ni ($50.5 \pm 18.9\%$ shoot content) and Cu ($56.7 \pm 16.9\%$) translocated to the shoots were higher than in previous studies (16–33%), though the amounts of Zn were relatively similar to the 40% previously documented. This is encouraging since Zn and Cu are both macronutrients with little toxic effect under these circumstances.

Table T4.3 Metal content in Vetiver shoots (S) and roots (R) following 30 days in hydroponic study in AMD (n=27).

Statistics	Metals ($\mu\text{g g}^{-1}$)											
	Fe		Al		Zn		Ni		Cu		Pb	
	S	R	S	R	S	R	S	R	S	R	S	R
Mean	501	21100	429	2310	85.4	198	13.5	18.4	21.1	29.8	0.124	4.46
Median	236	19500	213	1540	52.5	104	5.55	17.0	14.7	31.5	0.0938	2.25
Max	4050	43300	221	7010	359	852	188	40.3	191	58.2	0.377	16.9
Min	15.0	8220	14.2	739	2.95	2.66	0.261	5.70	0.388	11.6	0.0110	0.0935
SD	866	9210	554	169	99.0	197	35.1	7.70	34.8	10.6	0.0883	4.64

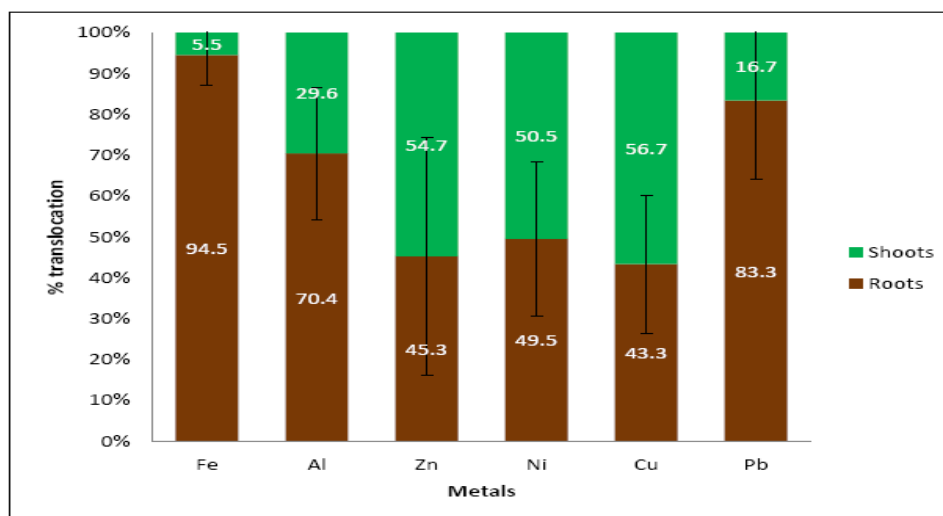


Figure T4.13 Percent metal translocation between Vetiver roots and shoots (n = 27)

3.4.3 Small-scale AMD stress analysis of Vetiver grass: A small-scale hydroponic study was conducted to evaluate the effect of Tab-Simco AMD on Vetiver grass, particularly differential protein expression and metabolism. The study was conducted under the same greenhouse conditions as described for the passive AMD treatment by Vetiver, though Vetiver grass was transferred into 1-liter plastic bottles following acclimation. The AMD from Tab-Simco was used for the treatment and hydroponic media was used for control (n = 3). Plants were harvested and flash frozen in liquid nitrogen at 14 days for a relatively short exposure, and 56 days for relatively longer exposure. Root and shoot tissues were separated, homogenized by mortar and pestle and stored at -80°C for biochemical analysis, aimed to investigate Vetiver's tolerance (stress resistance) to Tab-Simco AMD.

After 14 days in the newly collected Tab-Simco AMD, Vetiver showed signs of toxicity through curling and drying of the shoot extremities, with chlorosis and spotting. The treated plants showed a decrease in biomass by $35.6 \pm 3.11\%$ (n=3) compared with the $101 \pm 9.58\%$ increase in the control set up as shown in figure T4.14. This is indicative of Vetiver growth inhibition by the AMD. Total chlorophyll estimations suggested that the Vetiver in the AMD had similar to higher chlorophyll content (3.42 ± 1.40 mg/g) compared to the Vetiver in the control solution (2.18 ± 0.136) as depicted in Figure T4.15, which suggested that photosynthesis was not affected. Despite these effects, the Vetiver showed signs of acclimation and resistance at the end of the first week. This was evident from the development of fresh roots.

Dissolved oxygen (DO) of the AMD solution with Vetiver was noted to have decreased by day 14 as shown in Figure T4.16; possibly due to an increase in root respiration for metal detoxification. The solution pH was shown to have slightly increased from an initial 2.47 ± 0.00509 to 2.80 ± 0.0418 (Figure T4.17). The pH increment was not as significant as observed in the previously presented study. This was as a result of reduced number of plants (1/container) and study time.

Finally, Vetiver caused a decrease in electrical conductivity of the AMD solution from an initial $3,390 \pm 28.3$ $\mu\text{S}/\text{cm}$ to $2,480 \pm 107$ $\mu\text{S}/\text{cm}$ (figure T4.18) which can be attributed to metal uptake as was observed in Task 4. Observed fluctuations in conductivity readings was much less in the solution with no plants (control) from an initial $3,440 \pm 20.1$ μS to $3,200 \pm 60.5$ μS . Since we did not use air stones in this study, it could be interpreted that metals may have precipitated from the AMD to some extent. The significantly higher conductivity observed in this newly collected AMD would suggest that the metal content is greater than our previously used AMD, which would also explain the increased toxicity observed in the Vetiver.

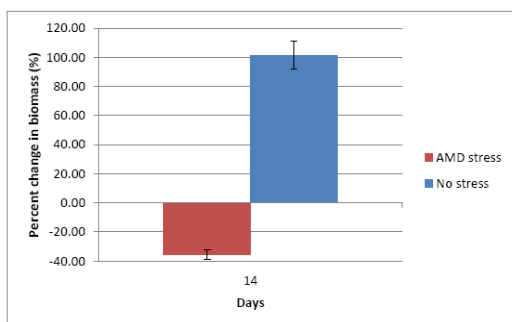


Figure T4.14 %change in fresh Vetiver biomass

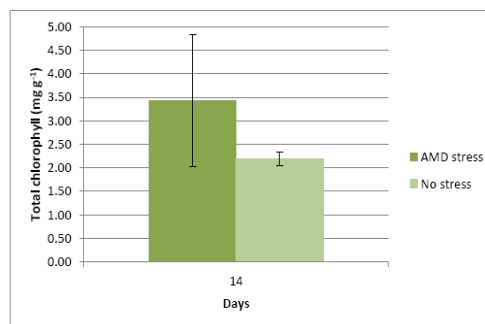


Figure T4.15 total chlorophyll content in Vetiver shoot tissues

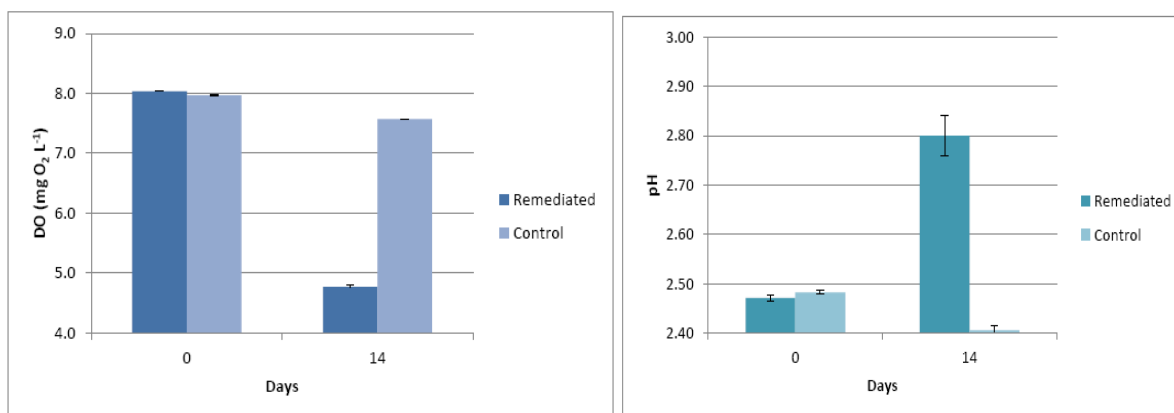


Figure T4.16 Change in DO 14 days with Vetiver Figure T4.17 pH change over the 14-day study period with Vetiver

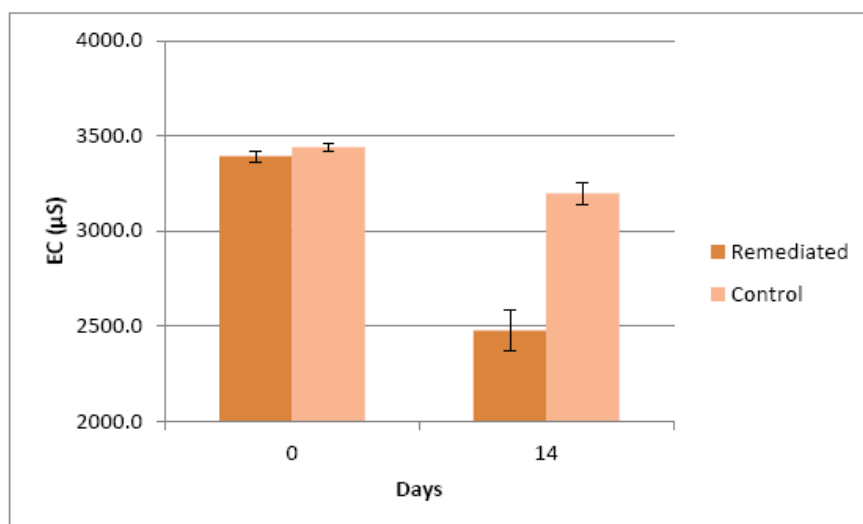


Figure T4.18 Change in EC over the 14-day study period with Vetiver

3.4.4 Task 4 Summary Findings:

- Vetiver plants in AMD water at varying densities (2, 3 and 4 plants per column) was analyzed for growth and metal uptake capacities. In most cases, Vetiver biomass and root length did not change by more than 2 cm over the course of the experiment. It was observed that with the exception of five individual grasses, shoot lengths increased or remained unchanged for all other individuals. Observed browning and curling seen in the leaves suggests that the AMD caused mild toxicity effect on the Vetiver.
- A slight pH increase was observed from initial AMD (2.64) to 3.76, 4.06 and 4.19 for the 2, 3 and 4 plant densities respectively. AMD sulfate content was significantly reduced to 1562.5 mg/L in the 4 plant density container.
- Reductions in soil alkalinity and electrical conductivity were also recorded with the 4 plant densities showing more comparative reductions.

- Analysis of the Vetiver plant at the end of the 30-day study period, indicated the average uptake of the following metals: Fe (20.21%), Al (2.38%), Zn (10.05%), Ni (1.14%), Cu (37.07%) and Pb (4.64%) in support of the observed reduction in EC of the water.
- Translocation of metal content in the Vetiver tissues showed that a higher amount of the Fe is accumulated in the roots at 94.5%. There were also relatively high accumulations of Pb and Al in the roots as well at 83.4% and 70.4% respectively. A moderate amount of Pb (16.7%) was translocated to the shoots. About equal amounts of the Zn, Ni and Cu were translocated between the root and shoot systems. The metal contents of Zn, Ni and Cu translocated were 45.3% root content, 49.5%, and Cu 43.3%.

3.5 Task 5: Greenhouse Hydroponic Study using WTR Plug Filter

3.5.1 Preliminary tests with WTR: A 24-hour batch test results of metal reduction achieved by all three types (Al, Ca, and Fe) of WTR separately and some in combination (1:1 blend) for treating seep AMD (bioreactor inlet). The retention time of 24 hours was selected although different retention times of 17 hours, 24 hours, 48 hours (Dayton et al., 2003; Kim et al., 2002; Yang et al., 2006; Mohan et al., 2002; Razali et al., 2006) have been reported in similar batch studies. A scaled constant WTR dose of 1g to a liter of AMD water was used for these set of tests.

3.5.2 Sorption procedure with Ca-WTR: Sorption studies were performed by the batch technique to obtain rate and equilibrium data. The batch technique was selected because of its simplicity. The effects of various parameters on the rate of adsorption were observed by varying contact time, pH of the solution and adsorbent dosage. The solution volume (V) was kept constant for experiments.

Batch pH studies were conducted to determine the optimum pH at which maximum pollutant metal removal and pH neutralization could be achieved with the Ca-WTR. The effect of pH was observed by studying the adsorption of the Ca-WTR over a pH range of 2.6-8.5. For these experiments, a series of 50-mL test tube was used. Each tube was filled with 25ml of AMD solution at varying initial pH at room temperature (21 ± 2.0 °C). The Adsorbent was added to each solution, and the flasks were agitated intermittently for 12 hrs. The pH was adjusted using dilute nitric acid and sodium hydroxide. The pH measurements were carried out using a two-point calibrated Pasco pH probe with an accuracy of +0.1 and resolution of 0.01.

A known amount of the Ca-WTR adsorbent was reacted with AMD in a 50 ml plastic test tube. The test tubes were agitated intermittently for the time periods ranging from 10mins to 24hrs to ascertain the time for establishment of equilibrium. It was assumed that the applied shaking speed allows all the surface area to come in contact with heavy metal ions over the period of the experiments.

Batch sorption studies were also performed for different adsorbent doses to obtain the data required in the design and operation of a continuous flow column reactor for the treatment of the Tab-Simco acid mine water. Different masses of the Ca-WTR ranging between 0.25g to 1 g and 25 ml of WTR solution were poured into 50 ml plastic bottles. The mixed samples were placed laterally on a

Shaker to agitate at 100 rpm for 12 hours. All experiments were carried out in duplicate and the average value was used for further calculation. After this period, the solutions were filtered using Whatman no. 42 filter paper and the metal concentrations in the samples were determined using Inductive Coupled Plasma Mass Spectrometry (ICP-MS). The amount of metal adsorbed per unit mass of Ca-WTR (q_e , mg/g) was calculated by using equation (5.1).

$$q_e = \frac{(C_o - C_e)}{m} \times V \dots \dots \dots [5.1]$$

Where C_o and C_e are the initial and final (equilibrium) concentrations (M), respectively, of the metal ion in solution; V is the volume (L) of AMD used; and m is the grams (g) of the WTR adsorbent. Percent metal ion removal (%MR) was calculated using the equation (5.2)

$$\% MR = \frac{(C_o - C_e)}{C_o} \times 100 \dots \dots \dots [5.2]$$

3.5.3 Continuous flow test: A laboratory-scale continuous flow system was designed using a plastic tube. Well cut 1-inch diameter, Pyrex PVC pipe (12 inches long) were fitted at the ends with threaded 1 inch male adapters. Both threaded adapters at the ends of the tube was capped. A 1/8" x 1/8" inlet/outlet adapter fitting was installed to the caps and connected with tubing. A set of 1-inch diameter plastic tubes were fabricated in the lab, as shown in Figure T5.1.

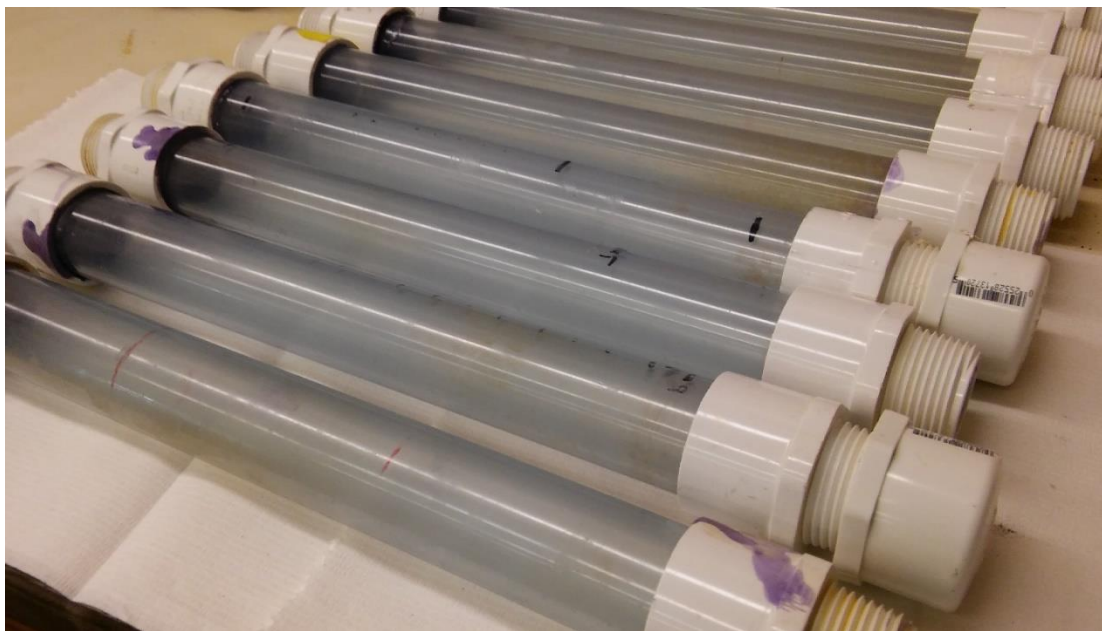


Figure T5.1 Prepared filter columns

Though both Ca and Fe-WTRs showed more metal reduction capabilities than the Al-WTR in the batch tests as discussed earlier, applying either the Ca or Fe-WTR in a downward continuous flow system posed a challenge due to their ultrafine particle size and resulting low hydraulic

conductivity. Several attempts to improve the hydraulic conductivity by combining with Al-WTR or fine non-sorbing uniform size sand were investigated with little success.

Because of the good hydraulic conductivity of the Al-WTR, two (2) continuous flow tests were conducted at flow rates of 15 ml/min (Test 1) and 26.4 ml/min (Test 2) corresponding to a medium and high hydraulic loading of 1.7 m³/m²/hr and 3.0 m³/m²/hr respectively. Both columns were filled with 50g of dried Al-WTR to form a packed bed on top of a supporting layer of cotton to avoid material loss. The AMD solution was then fed into the top of the tube by a peristaltic pump to pass through the sludge bed and the effluent was collected from the bottom of the columns through connected tubing. The experimental set up is as shown in Figure T5.2.

The influent AMD was analyzed for Al, Mn and Fe metal ions. Composite samples of the effluent were collected over time by continuous sampling. By the composite sampling method, a representative average solution characteristics during the compositing period could be attained for analysis. Collected samples were analyzed immediately so there was no need for acid preservation for later analysis. Following USEPA standard reporting procedure, all collected solutions were filtered through a 0.45 µm filter membrane. Dissolved or soluble metals were analyzed by colorimetric procedure using the Hach DR 900 colorimeter methods 8008 for iron, 8012 for aluminum and 8034 for manganese. Metal analysis results are not presented here (for brevity).

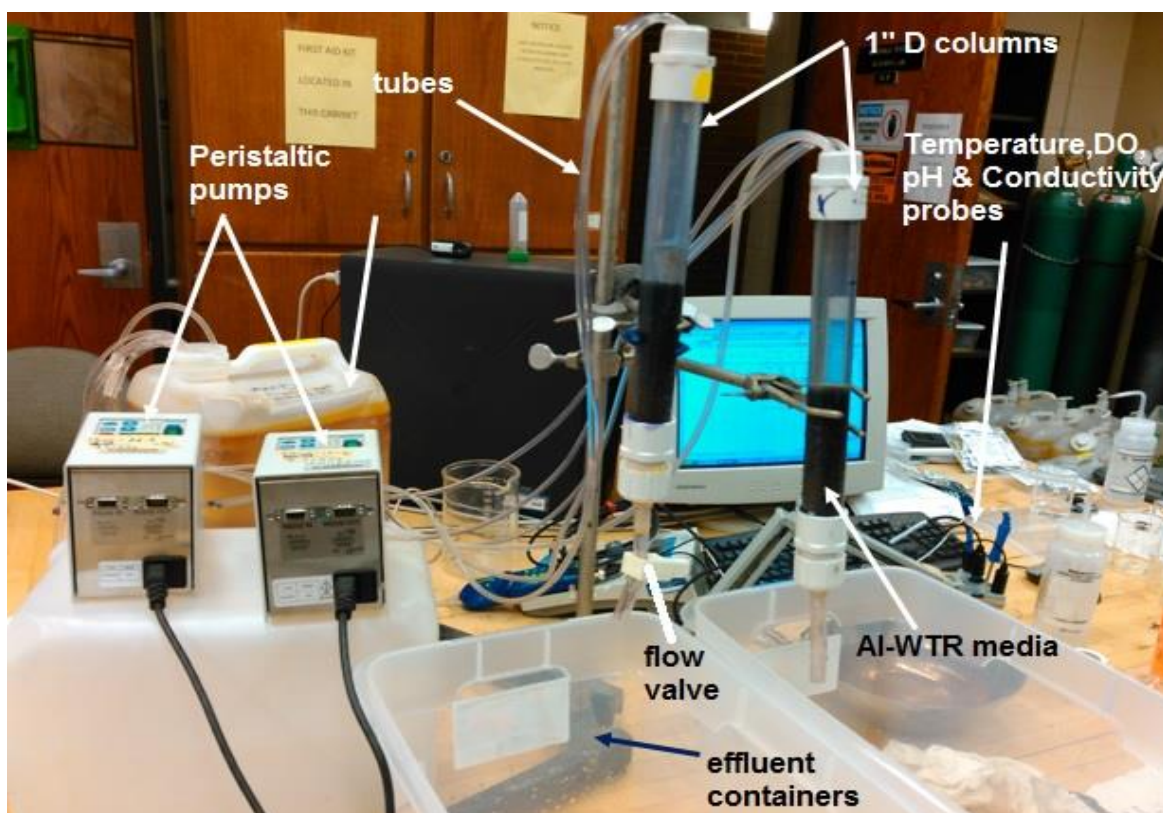


Figure T5.2 Experimental setup of 2 continuous flow tests at varying rates of hydraulic loading.

3.5.4 Ca-WTR filter column flow-through Test: In the previous batch tests, the Ca and Fe-WTRs showed comparatively high capabilities of dissolved metals adsorption and AMD pH

neutralization than the Al. However, the Al-WTR had been used in a continuous flow system with little success. Several experimental trials to use the Ca-WTR in a downward flow mode had been difficult mainly because of the very low hydraulic conductivity of the Ca and Fe-based WTRs in a continuous flow setup. Therefore, a reverse flow approach where the influent AMD was pumped from the bottom of the column filter using a peristaltic pump at an appreciable flow rate was adopted. This system was designed to maintain a fluidized Ca-WTR layer with enhanced media/AMD contact. To avoid WTR material loss, the top cap was packed with glass wool and a spongy (foam) material. Possible leakage from the system resulting from the system pressure build-up was controlled by using a Teflon thread sealant. Two continuous flow columns were set up using two 1-inch diameters, 12 inch (30 cm) and 18 inches (45 cm) long columns, with end caps that contain packing materials to restrict the media material from passing as mentioned earlier. The 12 inch set up is as shown in figure T5.3. Forty-five grams (45 g) of the WTR media material was weighed into both columns. The intent for inclusion of the 45cm column was to study the effect of increased AMD/Ca-WTR contact time due to the increased column length, without compensating for flow rate reduction.

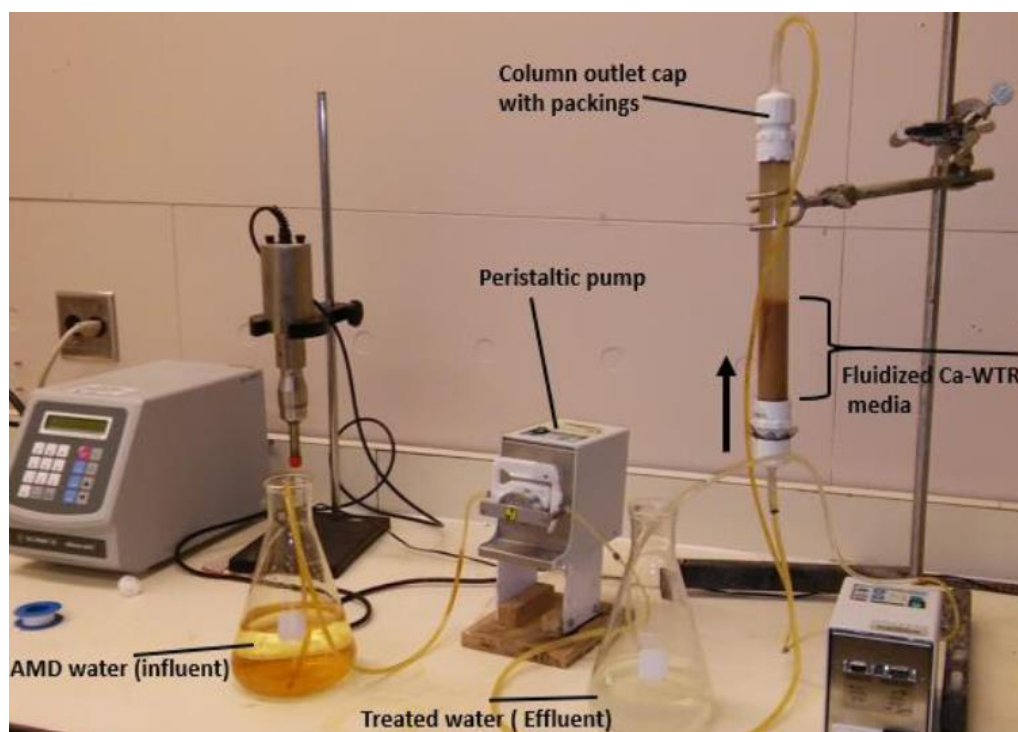


Figure T5.3 Column filter set up

Fresh Tab-Simco AMD was collected for the immediate use in this set of experiments. The collected influent AMD was analyzed for Al, Mn, Fe and SO_4 prior to the experiment. Composite timed samples of the effluent were collected by continuous sampling. Effluent samples were analyzed immediately for pH and redox potential. Dissolved or soluble metals were analyzed by colorimetric procedure using the Hach DR 900 colorimeter methods 8008 for iron, 8012 for aluminum, 8034 for manganese and Sulfaver Method (Method 8051) for sulfate.

3.5.5 Preliminary Tests with WTR: The batch test results are summarized in Table T5.1.

Table T5.1 Batch test sample analysis of Al, Fe and Mn using a Hach DR 900 Colorimeter

Treatment mix	Reduction % (Aluminum)	Reduction % (Iron)	Reduction % (Manganese)
Al-WTR	-71	89.23	57.3
Fe-WTR	98	100.00	31.6
Ca-WTR	100	99.76	87.8
Al:Fe-WTR	30	96.91	46.7
Al:Ca-WTR	96	100.00	26.3

It may be noted that metal concentration analyses conducted in these tests were conducted using a Hach DR 900 colorimeter methods 8008 for iron, 8012 for aluminum, 8034 for manganese. In the few instances where effluent concentrations were below equipment detection limit, it was indicated as 0 ppm, hence the 100% removal. The table shows that the Ca-WTR has the best performance in terms of removal of Fe (99.8%), Al (100%) and Mn (87.8%). The treatment mix of Al: Ca shows good removal efficiencies of 96%, 100% for Al and Fe respectively. However, the same mix yielded the least removal efficiency for Mn (26.3%). The Al-WTR was found to release significant amounts of aluminum into solution, although, it also reduced Fe and Mn appreciably by 89.2% and 57.3% respectively. The Fe-WTR on its own does well in removing Al (98%) and Fe (100%). However, it does not show much efficiency in Mn reduction like most of the other treatment mix evaluated.

3.5.6 Batch adsorption kinetics tests:

3.5.6.1 Adsorption of the heavy metals onto Ca-WTR: Concentrations of heavy metals in the Tab-Simco AMD analyzed by ICP-MS are presented in Table T5.2. Iron concentration in the collected sample constituted the major portion of the total metal ions determined (341.17) followed by aluminum (128.78) and manganese (31.13). The other metals were comparatively lower than the three, with concentrations in the following order; zinc (2.65), nickel (1.35), cobalt (0.36), copper (0.03) while cadmium (0.016) and arsenic (0.002) concentrations were the lowest. After the batch treatment of the AMD with the Ca-WTR, the concentration of aluminum in the treated sample was 0.30 ± 0.03 ; which is 99.8% less compared to untreated AMD. The level of iron was 0.53 ± 0.14 in treated samples which means 99.85% less in treated sample. The percent of Cu, Zn, As and Cd metal ions removed as shown in the table were 84.9%, 95.5%, 80.26 and 86.8% respectively. Removal of Ni and Co were minimal but notably, manganese level reduction was the lowest at 9.6% even though the AMD pH had been increased from 2.64 to a near neutral point of 6.74. In general, the obtained results revealed that the Ca-WTR has good adsorption capacity and is effective for the removal of heavy metals from AMD.

Table T5.2 Mean concentration (mg/L \pm S.D) of metal ions in the treated and untreated Tab-Simco AMD water

Sample	Parameter	Al	Mn	Fe	Co	Ni	Cu	Zn	As	Cd
Untreated	Mean	128.8	31.13	341.2	0.36	1.35	0.03	2.65	0.002	0.016
	\pm S.D	± 3.27	± 0.63	± 8.33	± 0.007	± 0.03	± 0.007	± 0.07	± 0.00	± 0.0
Treated	Mean	0.3	28.12	0.53	0.22	0.95	0.005	0.12	0.0004	0.002
	\pm S.D	± 0.03	± 0.54	± 0.14	± 0.006	± 0.02	± 0.00	± 0.01	± 0.00	± 0.00
% MR		99.77	9.67	99.85	37.93	29.64	84.98	95.56	80.26	86.87

3.5.6.2 Effect of contact time on adsorption: Contact time is one of the most effective factors in batch adsorption process. The effect of contact time on the adsorption of metal was investigated and depicted in figure T5.4 showing the percent metal ion removal with respect to time. Ten (10) contact times were investigated to know the optimum time for metal adsorption. It is apparent that adsorption rate initially increased rapidly (first 2 hours), and the optimal removal efficiency was reached within about 80 mins for the considered metals except manganese. Further increase in contact time did very little to change the equilibrium concentration of aluminum and copper; thus, the adsorption phase reached equilibrium. Iron and zinc adsorption however continued up to the 12th hour before reaching equilibrium.

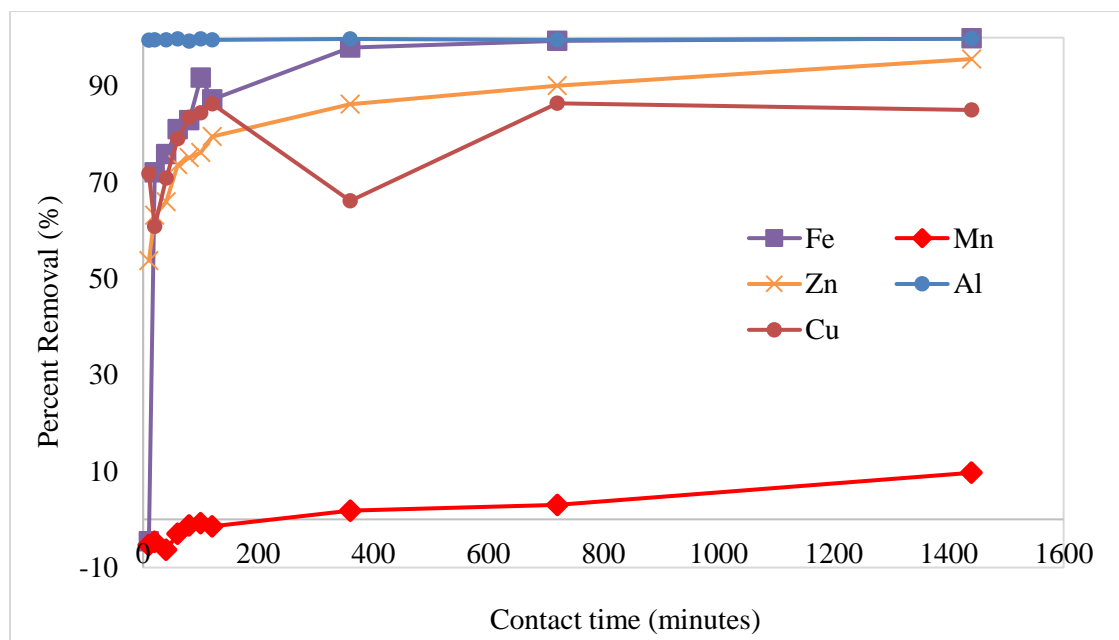


Figure T5.4 The effect of contact time on Ca-WTR adsorption efficiency.

The special case of manganese adsorption onto the Ca-WTR was observed. It is apparent from the figure that manganese reduction only started after 6 hours of contact time. The metal concentration reduction as shown in the above figure shows that manganese was released into solution prior to

the 6th hour. Though it is known that manganese reduction is highly dependent on pH, it can be shown (Figure T5.5) that the pH of the solution reached near neutral after 20 minutes and stabilized (6.51 – 6.74) with little fluctuations in the course of the study. The reduction was however more gradual between 12 and 24 hours contact time.

Fig. T5.6 shows clearly illustrates the variation in adsorption rates between the metal ions in the AMD solution. This can partly be as a result of the difference in initial concentrations of each of the metal ions present and by competitive adsorption in the case of metals of similar initial concentrations. A contact period of 12 hours was selected for all of the equilibrium tests.

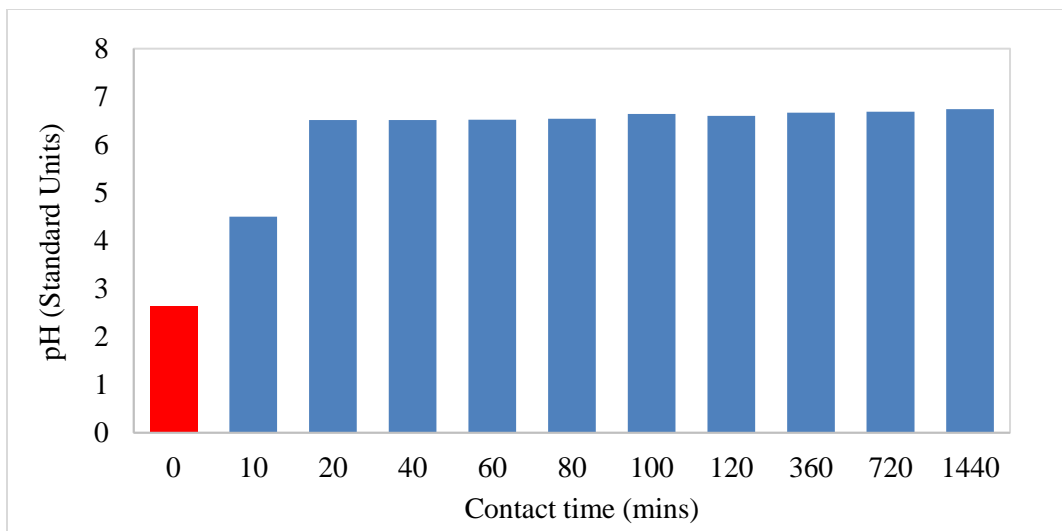


Figure T5.5 pH change over the contact time

3.5.6.3 Effect of adsorbent dose on adsorption: The effects of the amount of adsorbent on the rates of uptake of the metal ions were also studied for Al, Fe, Zn, Cu and Mn in a 12-hour batch test. The uptake of the metal ions increased with increasing amount of adsorbent material as shown in figure T5.6.

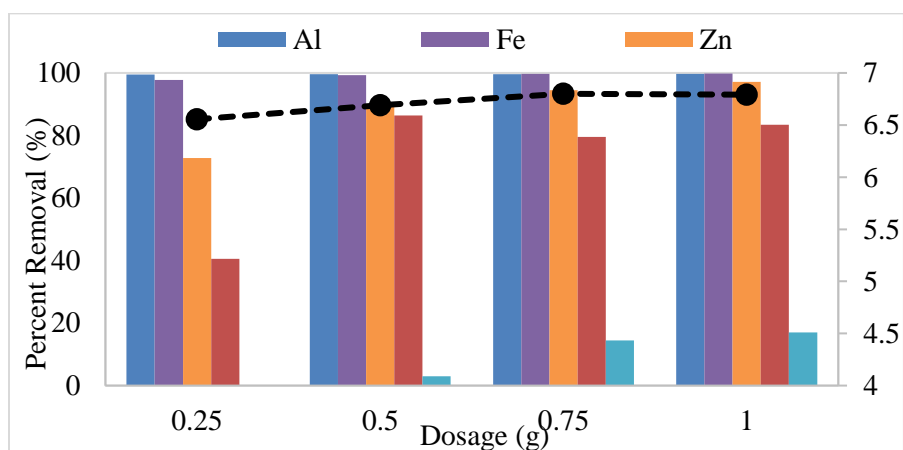


Figure T5.6 The effect of adsorbent dose on Ca-WTR metal adsorption

In the case of aluminum and iron, the adsorption capacity increased slightly when the dose of Ca-WTR was increased from 0.25 to 1 g, which showed additional 2% and 0.2% respectively. Beyond 0.5g dose of the Ca-WTR there was virtually no change in the amount of Al and Fe metals adsorbed. There was however, a significant increase in the amount of zinc (72.7% to 97%) and copper (40.5% to 83.4%) upon the further dosing of Ca-WTR. In the case of manganese, there is a substantial increase in adsorption when the Ca-WTR was increased. The percent removal increased highly from 0.06% at 0.25g to 17.0% at 1g dose of the WTR, though the overall manganese reduction still remained comparatively, the lowest. It is worth noting that, increase in the dosage also resulted in slight increase in the solution pH. A stable pH was however observed between 0.75g and 1g dose. As metals adsorption efficiency was increased with increase in adsorbent dose it was revealed that the adsorption sites remain unsaturated during the adsorption reaction whereas the number of sites available for adsorption site increased by increasing the adsorbent dose. With this results, the amount of Ca-WTR was kept at 0.5g in subsequent adsorption parameter effect studies.

3.5.6.4 Effect of pH on adsorption: The pH of a solution is an important parameter in the physicochemical adsorption process (Kim et al., 2002; Galarneau and Gehr, 1997; Razali et al., 2006; Mohan et al., 2002). During this study, results revealed that the removal of metal ions was strongly dependent on the pH of the solution. The effects of pH on adsorption of metal ions onto Ca-WTR was studied at pH range of 2.64 – 8.5, and the optimum removal capacity of the Ca-WTR was found to be at pH 6.5. Percent manganese metal ions removal sharply increased with the increase in initial pH of the solution. From the results shown in Figure T5.7, the effect of pH on aluminum and iron removal was surprisingly insignificant. The sorption of aluminum was rather a little higher at $\text{pH} \leq 2.6$ than at $\text{pH} = 4.5$ and remained unchanged between pH 6.5 and 8.5. Similar trend was observed for iron. This was partly as a result of observed precipitation of the metals as the pH was adjusted. Studying the effect of varying pH in a multi element solution is difficult because the pH must be less than the pH for precipitation of respective metal ions. For example, at $\text{pH} \geq 4.0$, the removal of iron (II) takes place by sorption as well as precipitation. That is, the OH^- ions from the solution forms various complexes with iron (II). It can be said then that within certain pH ranges, neutralization and sorption process are parallel (Mohan and Chander, 2006). The capability of the Ca-WTR to obtain efficient removal of Al, Fe and Zn (> 80%) at very low solution pH was a positive observation. The optimal pH was observed at pH 6.5 where further increase in metal adsorption recorded was very low. In the isolated case of copper, it was observed that increase in pH caused a drop in adsorption capacity of the Ca-WTR. This might be due to the weakening of electrostatic force of attraction between the oppositely charged adsorbate and adsorbent which ultimately leads to the reduction in sorption capacity (Baral, 2006). The changes in adsorption of the metal ions over a broad pH range of 2.6 - 8.5 are depicted in Figure T5.7.

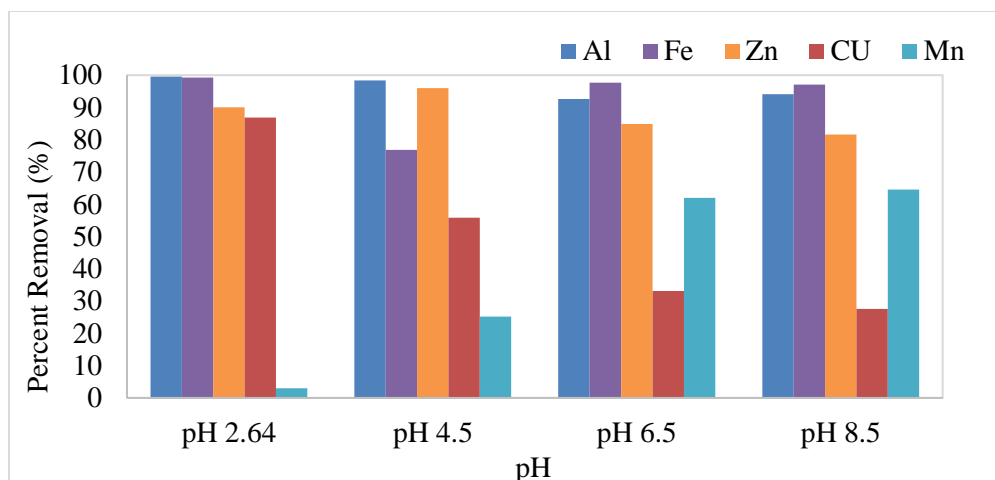


Figure T5.7 pH as a factor of % removal

3.5.7 Continuous flow through test: A major operational problem that was observed during the flow-through experiment at the high hydraulic loading rate (Test 1) was the reduction of the infiltration rate as shown in Figure T5.8. At the beginning of the experiment, the infiltration rate was 26.4 ml/min but after 2 hours (approx. 3.2 liters treated) of continuous flow, the infiltration rate was reduced to 6.6 ml/min (approx. 75% reduction). Just before the end of the experiment, there was further 77% reduction to 1.5 ml/min.

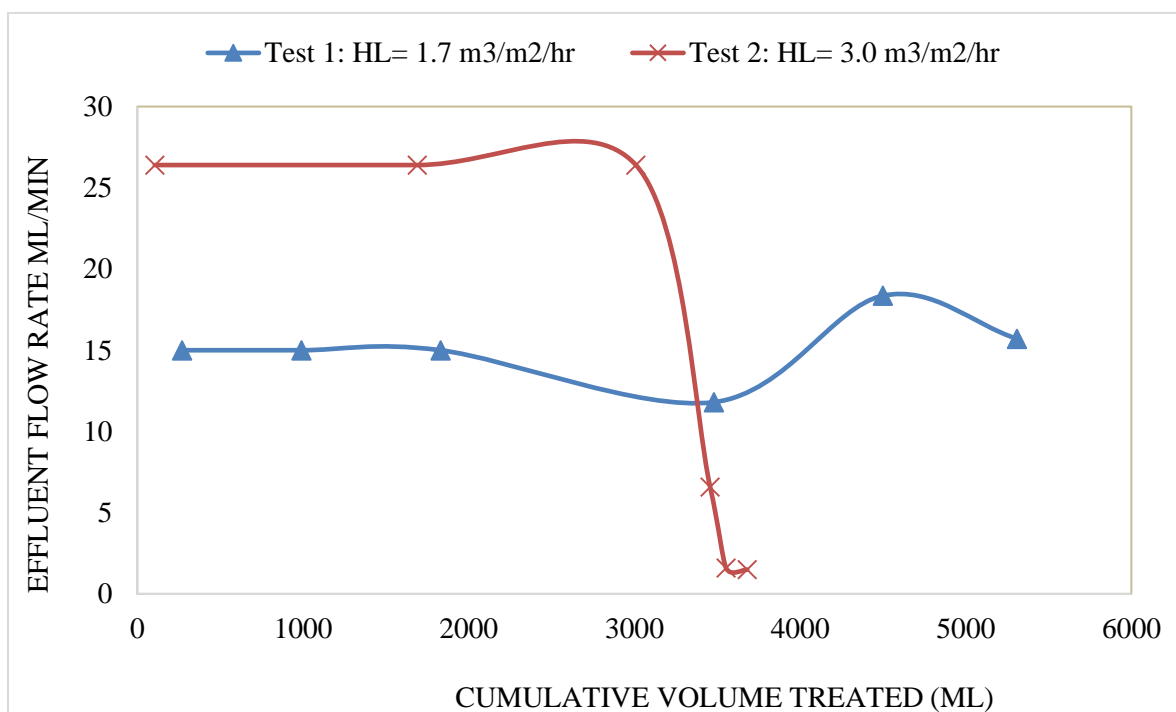


Figure T5.8 The infiltration rate change with respect to volume of accumulated treated water

This observed decrease in conductivity of the filter media can be explained to have occurred from plugging due to the formation of precipitates of the metal ions mostly due to the pH change (Figure T5.9A&B). Furthermore, in an extended saturated condition, the WTR particles have the capacity to swell in size which may cause a reduction in the pore spaces of the medium and inhibit water flow. To this end, further studies to improve the hydraulic conductivity for better performance in a long term run at a higher hydraulic loading was investigated.

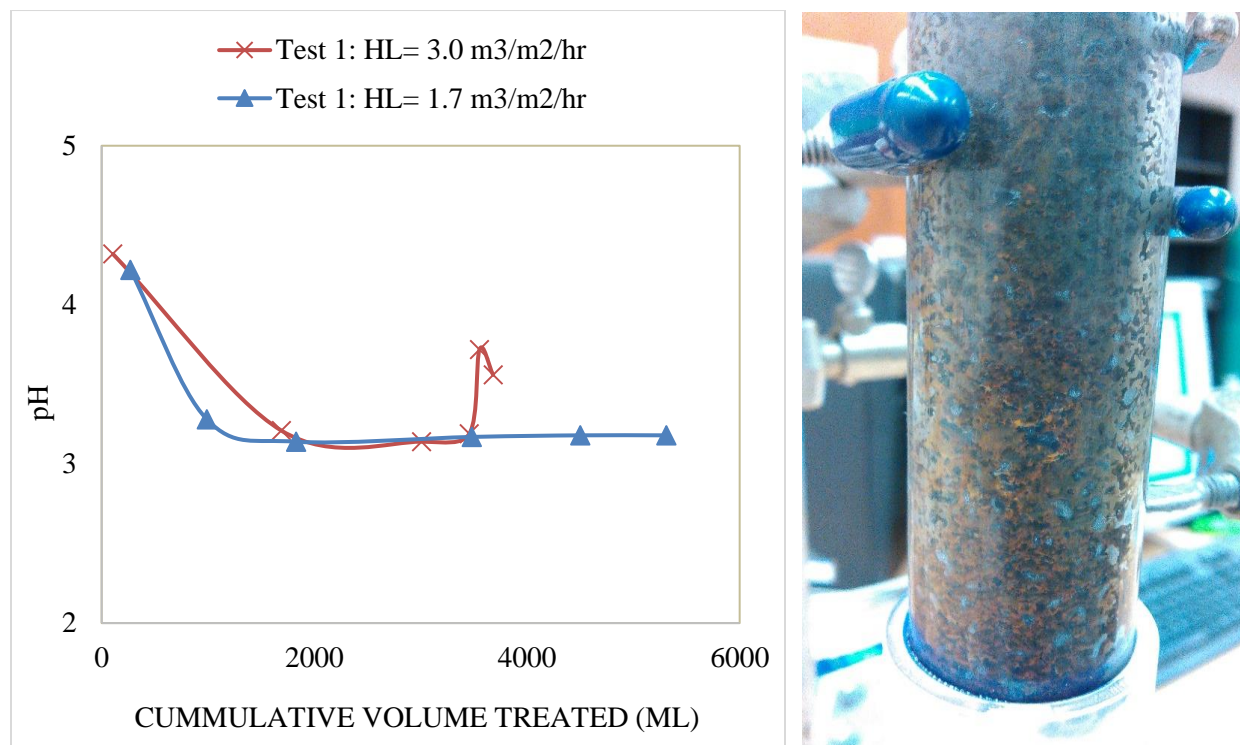


Figure T5.9 (A) The pH change vs. volume of accumulated treated water (B) Precipitation in column

The use of the Al-WTR proved unsuitable for use in a continuous system for treating the low pH acidic water partly because of its relatively low acid neutralizing capability. Under both hydraulic loading conditions, the maximum effluent pH recorded at the first few minutes were within acidic range (<4.5). Owing to the importance of pH in the physicochemical process of adsorption, the Al-WTR could not be used efficiently in the continuous flow column.

3.5.7.1 Ca WTR filter column flow-through Test: Continuous tests were conducted for the two tubes of variable heights in a reverse flow mode. The same operating conditions in terms of hydraulic loading and AMD sample metal composition was used in both set up. AMD was collected from the bioreactor inlet at Tab-Simco site. Analysis of specific constituents that were considered for this set of tests were performed which showed the respective concentrations of the following metals in the AMD water: iron (400 mg/L), manganese (24 mg/L), aluminum (300 mg/L) and sulfate (6,100 mg/L). The influent AMD used had a pH and ORP of 2.41 and 464.7 mv, respectively.

Breakthrough curves were used to quantify the dissolved metal adsorption under continuous loading. The respective metals normalized concentration (ratio of effluent concentration (C_e) to influent concentration (C_o)) was plotted against the column operation time for both columns, as shown in Figures T5.10 – T5.14. The aluminum breakthrough curves in both cases of the columns showed a quick drop in concentration and gradual increase from the beginning till 4hr (30 cm column) and 3 hours (45 cm column) operation respectively. From the 45 cm column effluent, it was observed that, at the 3-hour point, the pH dropped as shown in figure T5.11. This was found to have resulted from loss of fluidization of the media which at this point had begun to settle. The settling of the media at the bottom of the column resulted in the channelization of influent water. Once the reactive surface area of the created channel was used up, the system performance reduced. Figure T5.12 shows the channelization of influent in the media. Redox potential can be seen to increase with time. The 30cm column reached breakthrough concentration of 22 mg/L (at MR=92%) and reached exhaustion in the next few hours. The 45 cm column gradually increased the ratio of C_e/C_o without a specific point for breakthrough before the experiment was stopped. However, when the experiment was stopped after 6 hours operating time, the aluminum percent reduction was 86.6%.

Percent removal of sulfate concentration in the effluent did not show a particular pattern of increment over the period of both experiments. The highest sulfate concentrations were recorded prior to the end of both experiments with the 30cm and 45cm columns recording 31% and 49% removal respectively. This was indication that the Ca-WTR fluidized column presents an efficient aluminum and sulfate immobilization capability irrespective of the extremely high concentrations and hydraulic loadings.

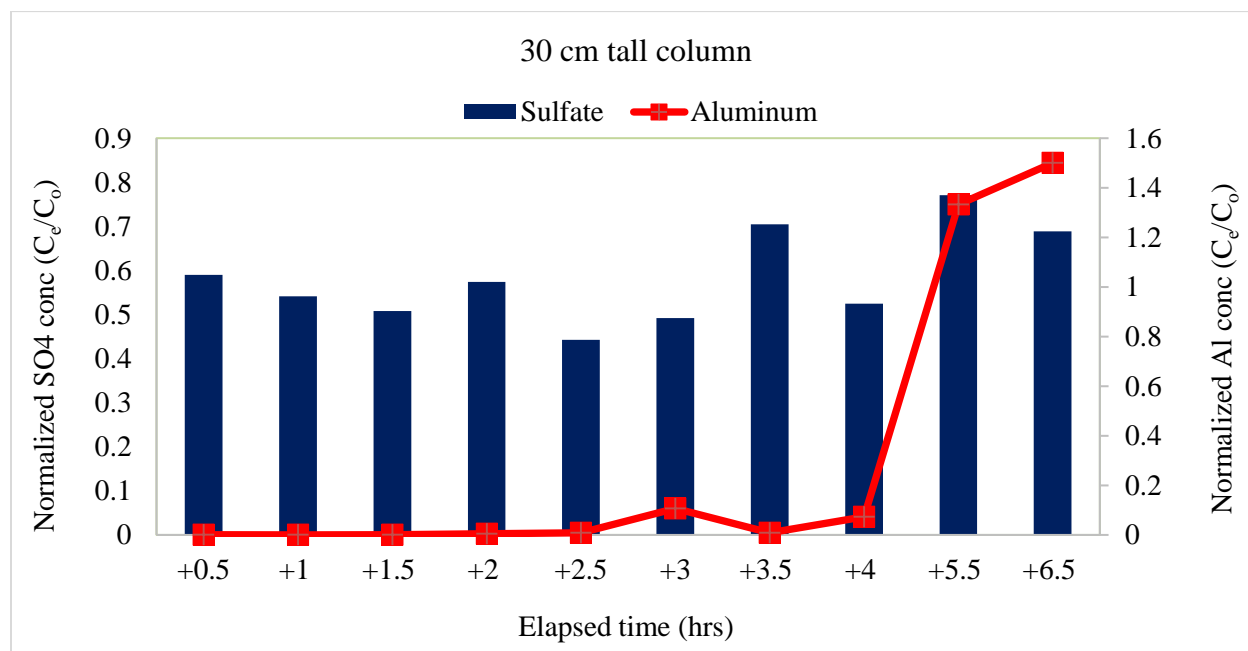


Figure T5.10 Dissolved SO₄ and Al-concentration at different time intervals (30 cm)

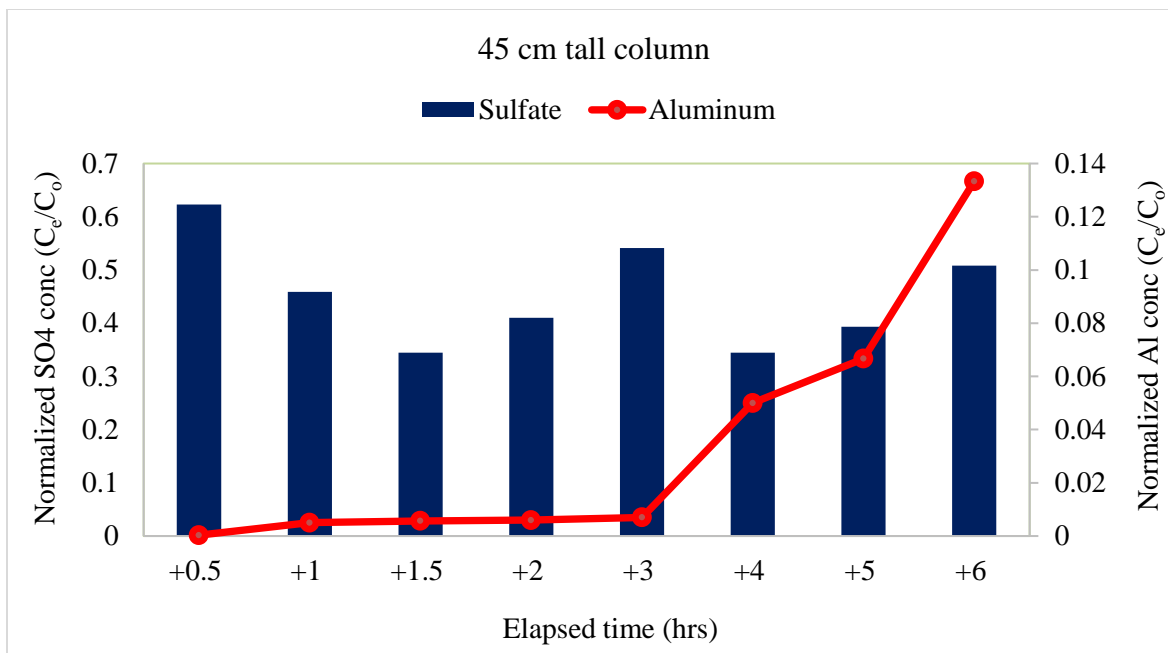


Figure T5.11 Dissolved SO₄ and Al-concentration at different time intervals (45 cm)

Figures T5.13 and T5.14 show the breakthrough curves of iron and manganese for both columns. The plot for the 30cm column clearly shows that iron reached what can be considered breakthrough and sharply showed some increase at an unusually low concentration (0.59 mg/L) after 4 hours of operation but regained adsorption capabilities without reaching exhaustion at the time the experiment was ended. Due to the added AMD/WTR contact time in the 45 cm column, it was able to sustain a low residual iron concentration beyond the 5hours with a gradual increase in C_t/C_0 reaching an order of magnitude lower than that of the 30 cm column at 6hours.

Initially iron removal efficiencies remained over 99% for the first 4 hours of the 30 cm column, but the ratio of C_t/C_0 increased more rapidly to 0.2 compared to the 45cm column at 0.05 when the experiment was ended. This implies that the even the 30 cm long column with shorter retention time could last about 5 times more the operating time before approach of saturation point of iron adsorption. Though both columns did not reach exhaustion, the increases of C_t/C_0 of iron was relatively slow in the longer height column.

Manganese reduction however did not follow a particular pattern in the 30 cm column. However, there was an observed low ratio of C_t/C_0 between the 2 and 4 hour times before a gradual increase to the end of the experiment. At the end of the experiment, the ratio of C_t/C_0 was recorded as 0.70 and 0.75 for the 30 cm and 45 cm columns respectively, corresponding to a lowest removal efficiency of 29 % and 25 %.



Figure T5.12 channelization in filter media

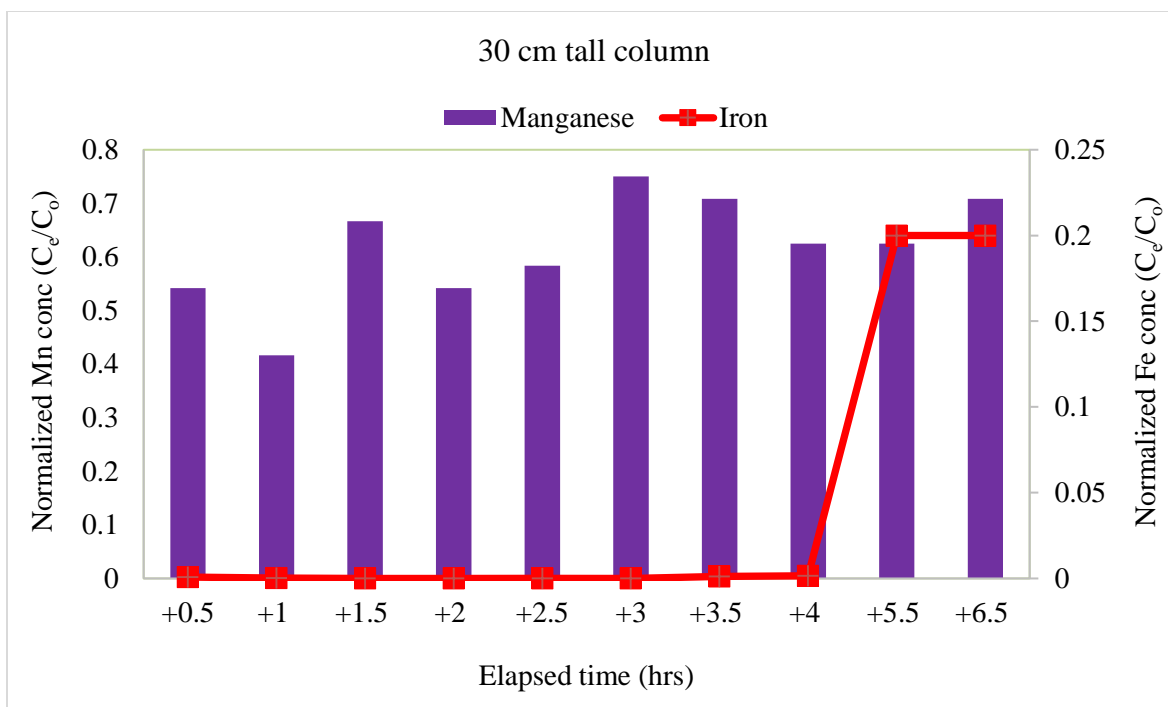


Figure T5.13 Dissolved Mn and Fe-concentration at different time intervals

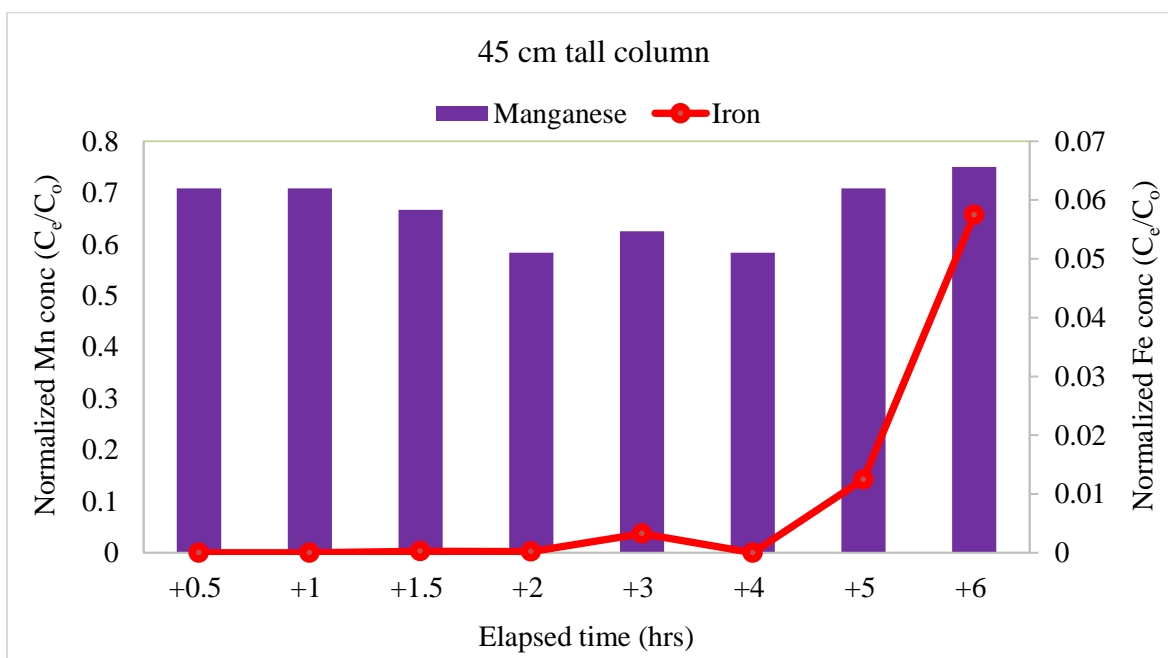


Figure T5.14 Dissolved Mn and Fe-concentration at different time intervals

Figures T5.15 and T5.16 show the pH and ORP pattern for both columns over the entire operating time. The high dependence of adsorption on pH required continuous monitoring of pH changes with respect to the operating time. The pH of the AMD water used was 2.41. In the 30 cm column, the pH gradually decreased with operating time from an initial high of 6.54 to an unusual 2.7, for

which reason the experiment was ended after 6 hours. The effluent pH of the 45 cm column showed a reduction from 6.34 in the first hour of the experiment to 4.2 at 3 hours due to an observed early channelization. The drop in pH was seen to have reduced the adsorption process. The column regained fluidization and increased the effluent pH to a recorded maximum of 6.4 by the end of the experiment.

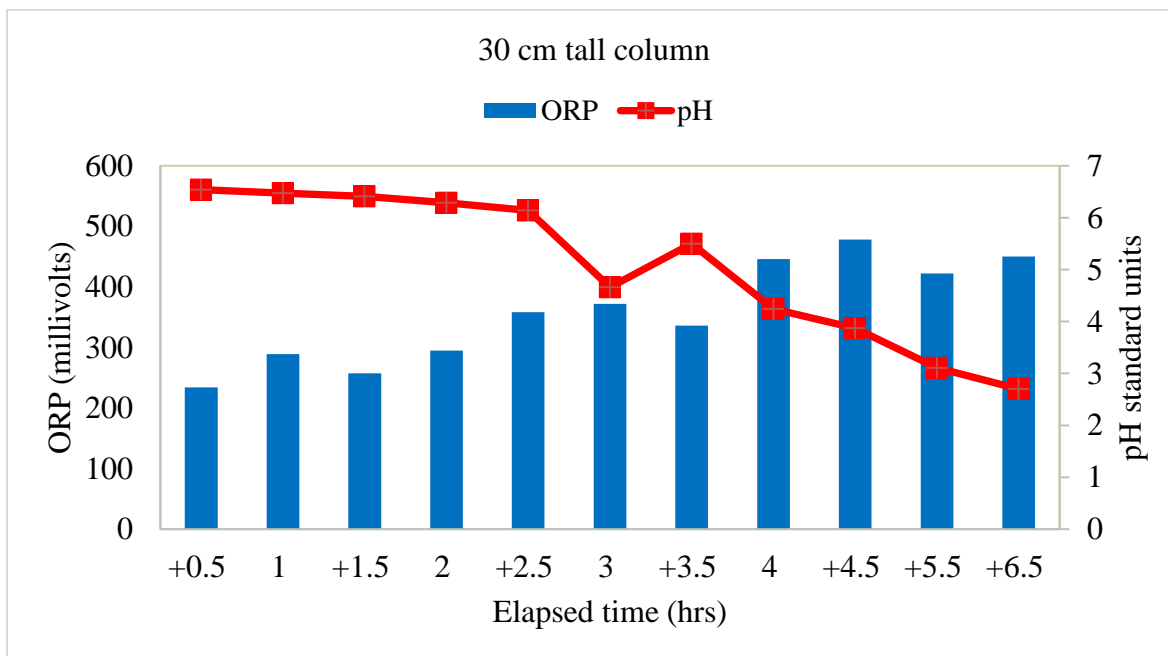


Figure T5.15 Change in pH and ORP as a function of time

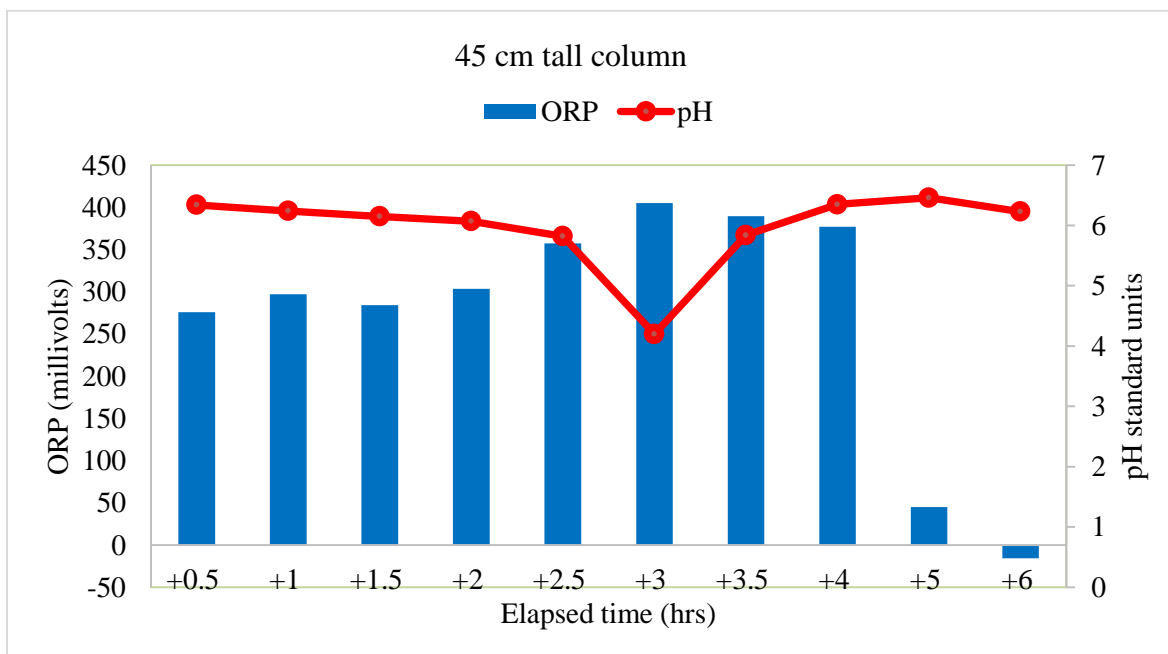


Figure T5.16 Change in pH and ORP at different as a function of time

It can be stated with much confidence that the higher the metal adsorption, the higher will the pH be and vice versa. These results are consistent with the batch results discussed earlier. It can be seen that maximum ORP was reached at the point when pH was lowest, which also establishes an inverse relationship of ORP and pH.

The observations from the 30-cm tall column prompted the research team to look further into ways to maintain fluidization for a longer period to increase the life of the system, hence the development of the 45-cm column. Furthermore, it happened as was expected that with the increased column height there was an increase in pressure at the bottom of the column. This condition was responsible for the end of the experiment at 6 hours since the peristaltic pump could no longer efficiently distribute influent water into the system.

The breakthrough plots for aluminum, sulfate, manganese and iron provides adequate indication about the effective use of Ca-WTR as an adsorption and pH neutralizing material in a fluidized bed column filter for immobilization of heavy metals and sulfates. The presented results of the continuous flow experiments revealed that the Ca-WTR performed extremely well in treating the low pH Tab-Simco's AMD water under unfavorably high hydraulic loading ($1.7 \text{ m}^3/\text{m}^2/\text{hr}$) and extremely high concentrations of dissolved heavy metal ions and sulfates. Most flow through tests are performed with analytical grade AMD with controlled specific constituent metals, however this field level AMD presented a real field case scenario of the AMD. As was observed from the results, the high influent AMD loadings did not significantly decrease removal efficiency rapidly over the study time. As a matter of fact, the longevity of the system with improved contact time was appreciably high. Having successfully executed this part of the objectives of this study under task 5 which was to explore the feasibility of using the most appropriate of the locally acquired dewatered WTR as a potential material for heavy metal adsorption/removal and pH neutralization, it is reasonable to believe that the fluidized column filter can be operated for a much longer time at lower hydraulic loading.

Estimation of some important continuous flow performance evaluation parameters were applied in this study. The calculation of breakthrough capacity, exhaustion capacity and Ca-WTR usage rate were done as per the following considerations. From the presented breakthrough plots, the break points for the respective metals and sulfate were identified as well as the assumed exhaustion point. With the exception of Al (30 cm column), none of the other considered elements reached exhaustion at the time the experiments were stopped. The normalized concentration (C_e/C_o) at the end of the experiment (\approx maximum C_e/C_o) was considered as the comparatively near exhaustion concentration.

The calculated masses of sulfate, Al, Mn and Fe per mass of the Ca-WTR used in the 30-cm column, at breakthrough were 146.91 mg SO_4/g , 14.09 mg Al/g and 0.53 mg Mn/g, 19.19 mg Fe/g and 184.68 mg SO_4/g , 17.2 mg Al/g and 0.7 mg Mn/g, 26.3 mg Fe/g during the entire continuous operation period and at exhaustion for iron metals. A similar computation for the 45cm column showed breakthrough capacities of 226.47 mg SO_4/g , 17.3 mg Al /g and 0.75 mg Mn/g, 26.66 mg Fe/g and 281.2 mg SO_4/g , 21.69 mg Al/g and 0.8 mg Mn /g, 29.5 mg Fe/g at the end of the experiment.

The results of the continuous flow test indicate clearly that the decrease in removal efficiency of aluminum after 4-hour operation is more significant. Removal of sulfate and manganese were comparatively lower than that of other metals. Though the Ca-WTR was able to quickly increase the pH of the solution, the manganese reduction was low. The observed lower reduction could be related to the competition for adsorption site among the metals or preferential adsorption by the Ca-WTR. The very high initial concentration of the metals in the AMD could result in specific adsorption sites becoming exhausted leading to competition between manganese and other metal ions for common surface sites. During the continuous column testing, a close observation was made to estimate Ca-WTR material loss. Material loss was checked by proper packing of filter materials in the cap. It was observed that after 3 hours of operating, some WTR particles started packing at the bottom of the column as a result of the particles gaining weight from the adsorption of the metals. This led to a gradual loss of fluidization and created channeled pathways for influent AMD. The reduced contact decreased the effluent pH in some cases and caused subsequent decrease in removal efficiency and hydraulically active pore volume in the long term.

3.5.8 Task 5 Summary Findings:

- Preliminary batch test to identify the WTR (among the three different types) or any combination of them that gives the maximum metal adsorption capacity showed that the Ca-WTR gives the best performance in terms of removal of Fe (99.8%), Al (100%) and Mn (87.8%) and complete pH neutralization.
- Sorption studies conducted with the Ca-WTR showed 99.8% removal of aluminum and 99.85% of iron in treated sample. The percent of Cu, Zn, As and Cd metal ions removed were 84.9%, 95.5%, 80.26 and 86.8% respectively. Removal of Ni and Co were minimal but notably, manganese level reduction was the lowest at 9.6% even though the AMD pH had been increased from 2.64 to a near neutral point of 6.74.
- It was shown that the effect of contact time in the metal removal efficiency varied. It was apparent that adsorption rate initially increased rapidly (first 2 hours), and the optimal removal efficiency was reached within about 80 min for the considered metals except manganese.
- Metals adsorption efficiency increased with increase in adsorbent dose. It was revealed that the adsorption sites remained unsaturated during the adsorption reaction as the number of sites available for adsorption increased by increasing the adsorbent dose.
- The effect of pH on adsorption of metal ions onto Ca-WTR was studied at pH 2.64 – 8.5, and the maximum metal removal capacity of the Ca-WTR was found to be at pH 6.5. However, except for Mn, most of the other metals did not show significant response to pH changes mainly as a result of observed precipitation of the metals as the pH was adjusted. It was then noted that observation of pH effect should be best conducted at a pH below which the metals still stay in solution and does not precipitate.
- Continuous flow tests conducted for two designed columns of variable heights (to create difference in retention time) in a reverse fluidized flow mode with Ca-WTR as media showed an increase in the performance and longevity between the 30cm and 45 cm long columns.
- The 45 cm column gradually increased the ratio of C_e/C_o for Aluminum and reached breakthrough after 4 hours. When the experiment was stopped after 6 hours operating time, the aluminum percent reduction was 86.6% and 49% for sulfate.

- Iron removal efficiencies remained over 99% in the 45 cm high column. The ratio of C_e/C_0 increased to 0.05 when the experiment was ended.
- Manganese reduction was lowest between 2 and 4 hours of operation. At the end of the experiment, the ratio of C_e/C_0 was recorded as 0.75 for the 45cm columns, corresponding to a comparatively low removal efficiency of 25%.
- The effluent pH showed a reduction from 6.34 in the first hour of the experiment to 4.2 at 3 hours due to an observed early channelization. The pH then again increased and stayed around 6.4.

3.6 Task 6: Simulated Hydroponic Field Study

This study was conducted to evaluate the applicability of the successfully completed greenhouse hydroponic study in an open environment. The task of this treatment system was to demonstrate the hydroponic treatment of AMD in a simulated field study under typical Southern Illinois environmental conditions. For this experimental setup, fresh contaminated acidic water was collected from the Tab-Simco site. The study was conducted with Vetiver and Pokeweed. The inclusion of Pokeweed in this experiment was because of its known hyper-accumulation capabilities of manganese which by far has proven difficult to remediate from the acid mine drainage in continuous flow experiment discuss earlier. A bigger size fluidized column filter was used to treat 18 gallons of AMD. About 8 gallons of the treated water was put in a black plastic open container for separate set up of Vetiver and Pokeweed. Another set of untreated AMD was set up for both plants.

In the preceding greenhouse hydroponic study (task 4), it was found that high plant density was better in taking up dissolved metals. The plants were prepared to float on a floating platform (Styrofoam) as shown in figures T6.1 and T6.2. Vetiver and Pokeweed root and shoot lengths were recorded before starting the experiment. The plants were planted at 10cm interval center to center and supported with cotton at the base. Samples of treated and untreated water before and after the experiment would be sampled for metal analysis by ICP-MS. The grass would be harvested at the end of the experiment for tissue metal analysis.



Figure T6.1 Pokeweed floating platform for field study of AMD hydroponic treatment



Figure T6.2 Vetiver floating platform for field study of AMD hydroponic treatment

The prepared setup was placed in an open environment at the premises of college of engineering at SIUC. To avoid over dilution from rainfall events, a transparent structure was made just to cover the top. Air circulation was not blocked in so doing as shown in Figure T6.3. To maintain the concentration of the water, however, the level of the water is maintained by adding deionized water regularly as the water level drop.



Figure T6.3 Hydroponic set up in an open environment.

In the simulated field hydroponic study setup shown in Figure T6.3, the Vetiver grass as well as Pokeweed was subjected to a fixed volume of untreated AMD sampled from the seep at the Tab-Simco site. The plants were also subjected to AMD treated by a reverse flow column filter using WTR as filter media. The 2-inch diameter column filter increased the pH of the treated water and adsorbed significant amounts of the constituent heavy metal ions. The hydroponic remediation technique functions to further remove dissolved heavy metals from the column effluent. The average performance of the WTR column in removing manganese (observed during preliminary studies) led to the introduction of the manganese hyper-accumulator, Pokeweed. The column filter reduced the dissolved aluminum (from 130 to 0.43 mg/L), iron (327 to 1.75 mg/L), manganese (32 to 28 mg/L) among other metals from the influent Acid Seep. Two separate containers received 8 gallons of treated AMD water that was used to set up the Vetiver and Pokeweed hydroponic study that lasted for 30 days under southern Illinois ambient weather conditions.

3.6.1 Plant growth in hydroponic media: The plants were closely monitored to during the study period to carefully examine their growth pattern. The initial root and shoot lengths of the 20 plants on each raft was measured. The difference between the initial length and final length measured at the end of the experiment was used for growth estimation.



Figure T6.4 Hydroponic set up in an open environment.

The Pokeweed and Vetiver that were grown in the untreated acidic water was observed to show no signs of growth in the first week. Subsequently, the shoot yellowed and dried. At the end of the 30-day experiment, none of Pokeweed/Vetiver survived in the untreated acidic water condition. It was observed, as shown in figure T6.4, that the Vetiver developed new roots. The initial root system of Vetiver measured prior to the experiment did not grow significantly in length. All the Vetiver in the treated water grew to newly developed root length of 11.6 ± 2.7 cm more (0.38 cm/day) on an average. The measured mean change in shoot length was 32.0 ± 11.67 cm.

Analysis of the shoot and root of the Vetiver grass on dry weight (DW) is shown in Figure T6.5. The major elements uptake in the Vetiver grass shown are aluminum, phosphorus, manganese and iron. The low concentration of elements adsorbed is as a result of the lower concentrations of the heavy metals and phosphorus in the column filter effluent. The concentrations of the observed

elements are consistently higher in the roots than in the shoots, indicating very low translocation of the adsorbed metals from the root to the shoot.

The aluminum and iron uptake from the column effluent by the Vetiver are similar and are about twice as high as the manganese uptake. The difference in the uptake capability of Vetiver could be due to selective adsorption and the available concentrations of the metals in solution. The Vetiver hydroponic system, however, reduced the heavy metal concentrations in the column effluent further except for aluminum which unexpectedly increased in concentration. Mn was further reduced by 56.9%, Fe by 44.4% and cobalt reduction by 55.7%. Other considered metals that exhibited varying levels of reduction from the Vetiver hydroponic system included Ni (54.1%), Cu (16.5%), Zn (28.4%) and As (8.6%). The percentage reduction can be seen to be influenced by the initial concentration of the metal in the column treated AMD. The mean Mn, Fe and Al uptake (in mg per Vetiver plant) on DW basis was found to be 0.059, 0.115 and 0.104, respectively.

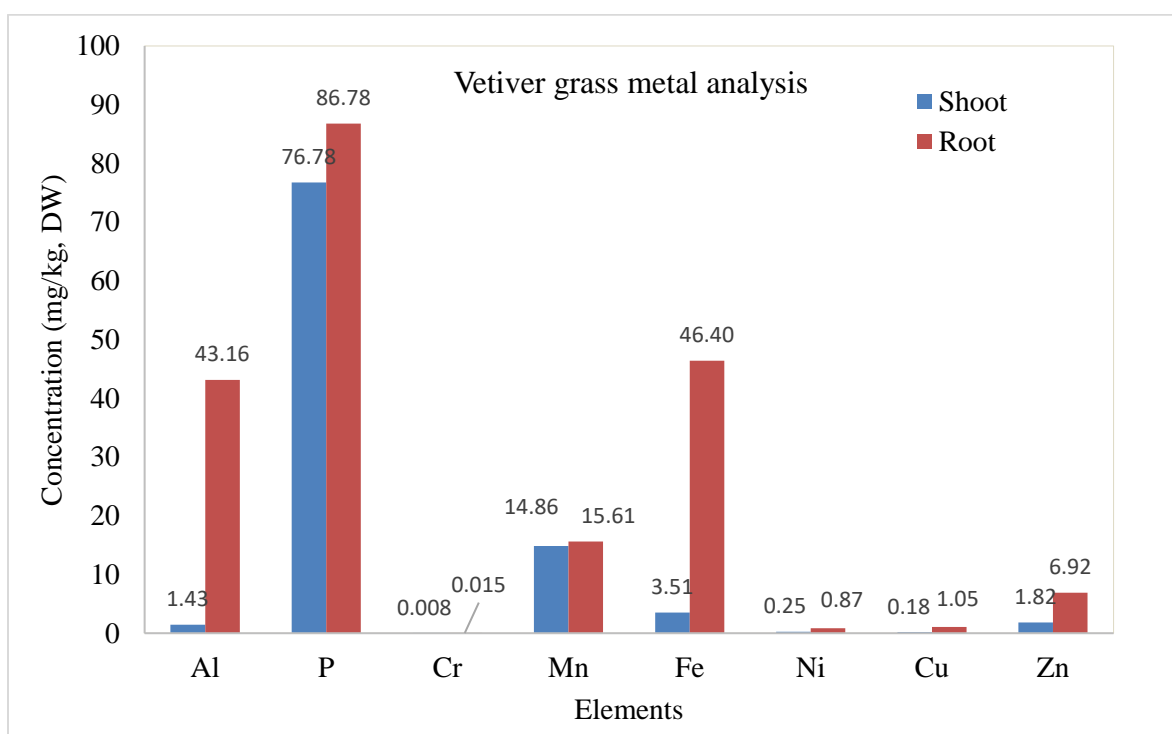


Figure T6.5 Heavy metal and Phosphorous concentration (mg/kg) in the Root and shoot of Vetiver

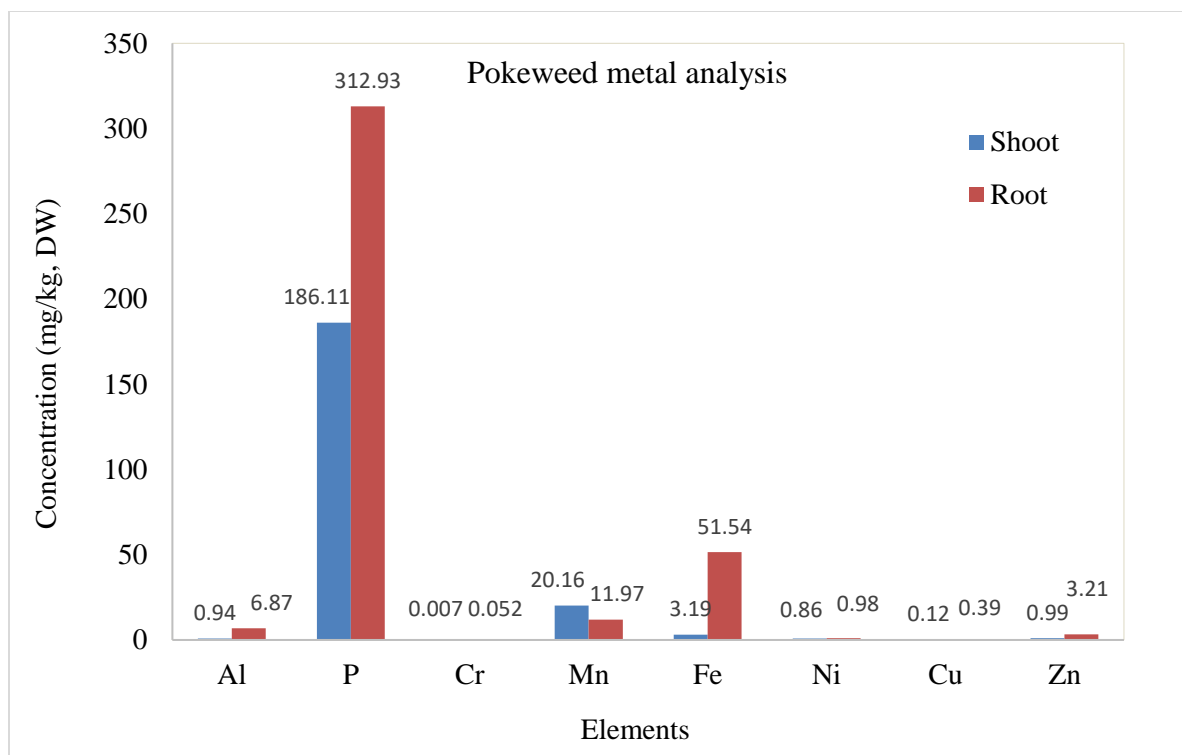


Figure T6.6 Heavy metal and Phosphorous concentration (mg/kg) in Pokeweed

The Pokeweed plant had a difficult time adjusting to the hydroponic condition. The growth of the Pokeweed as mentioned was very little. Analysis of the root and shoot (Figure T6.6) indicate that the Pokeweed contained more P, Mn and Fe than the Vetiver grass. The abysmal and slow growth of the Pokeweed in the hydroponic system resulted in the low Mn uptake than its reported Mn uptake in other studies (Pollard et al., 2009; Dou et al., 2009a; Dou et al., 2009b; Min et al., 2007). The metal and phosphorus translocation in the Pokeweed was less than 1 except for manganese which was 1.6. Though very high Mn translocation has been reported in Pokeweed (pollard et al., 2009), the low translocation and uptake observed in our study was apparently due to the low growth rate experienced by the Pokeweed set used in this study. The adsorption of metals by Pokeweed, however, was significant. The dissolved manganese in the column effluent used was further reduced by 31.8% whilst the concentration of iron was reduced by 48.7%. Significant reduction was also measured in cobalt (26.1%), nickel (24.8%) with the least amount of reduction in copper (3.5%). Figure T6.7 illustrates the change in heavy metal concentration in the column filter/hydroponic AMD treatment. The mean Mn, Fe and Al accumulation (mg) per Pokeweed plant was found to be as low as 0.003, 0.007 and 0.001 respectively.

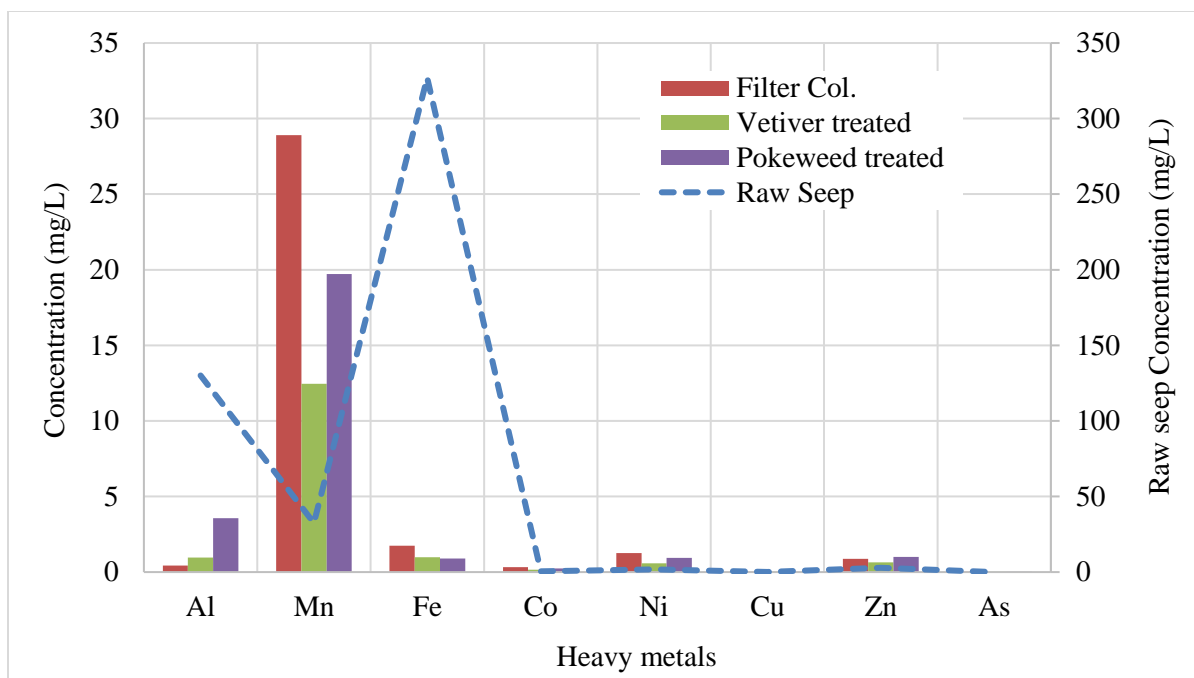


Figure T6.7 Water analysis for Pokeweed and Vetiver heavy metal uptake

From the above results of the hydroponic study, the applicability of phytoremediation for AMD was proven to a reasonable extent. Phytoremediation by a hydroponic system is less expensive to construct and requires little maintenance. Though there are many identified phytoremediation plants, the success of their large scale application for dissolved metal uptake depends on relevant factors which include the composition and level of contamination of the water, climate, and ambient temperature amongst others. The removal efficiency of toxic contaminants in an aquatic system was found to be influenced by plant growth rate and retention time. The higher growth rate of Vetiver aided in its uptake of Mn and other constituent metals than Pokeweed which is specialized for Mn uptake according to the research hypothesis. In the assessment of the performance of Pokeweed as a manganese hyper-accumulator, it was expected to have shoot manganese concentrations greater than 1,000 mg/kg based on the findings of the previous studies (Baker and Brooks, 1989). Though the accumulated manganese in the Pokeweed was low due to its poor growth, it had metal translocation factor greater than 1.

3.6.2 Task 6 Summary Findings:

- The Pokeweed and Vetiver that were grown in the untreated AMD water was observed to show no signs of growth in the first week. At the end of the 30-day experiment, none of Pokeweed/Vetiver survived in the untreated acidic water condition.
- The aluminum and iron uptake from the WTR treated water by the Vetiver are similar and are about twice as high as the manganese uptake. The difference in the uptake capability of Vetiver was apparently due to the selective adsorption and the available concentrations of the metal ions in solution. Mn in the WTR treated water was reduced by 56.9%, Fe by 44.4% and Cobalt by 55.7%. Other considered metals that exhibited varying levels of reduction from the Vetiver hydroponic system included Ni (54.1%), Cu (16.5%), Zn (28.4%) and As (8.6%). The

percentage reduction can be seen to be influenced by the initial concentration of the metal the WTR treated AMD. The mean Mn, Fe and Al uptake (in mg per Vetiver plant) on dry weight basis was found to be 0.059, 0.115 and 0.104, respectively.

- The Pokeweed plant had a difficult time adjusting to the hydroponic condition. However, Pokeweed shoot and root were found to contain more P, Mn and Fe than that of the Vetiver grass. The metal and phosphorus translocation in the Pokeweed was less than 1 except for manganese which was 1.6. Though very high Mn translocation has been reported in Pokeweed in past studies, the low translocation and uptake observed in our study was apparently due to the low growth rate exhibited by the Pokeweed set used in this study. The adsorption of metals by Pokeweed, however, was significant. The dissolved manganese in the WTR treated AMD used was further reduced by 31.8% whilst the concentration of iron was reduced by 48.7%. Significant reduction was also measured in cobalt (26.1%), nickel (24.8%) with the least amount of reduction in copper (3.5%). The mean Mn, Fe and Al accumulation (mg) per Pokeweed plant was found to be as low as 0.003, 0.007 and 0.001 respectively.

4.0 SUMMARY PROJECT FINDINGS

This study examined two green methodologies to treat AMD water and AMD impacted soil obtained from the Tab-Simco abandoned coal mine site. A sulfate-reducing bioreactor (SRB) has been operating at the Tab-Simco site treating a majority of the AMD water. The SRB treated water is collected in the Oxidation Pond before being released to the nearby creeks. To make the findings of this study beneficial not only to the Tab-Simco site, but also for many other old abandoned mine sites, those don't have any AMD remediation system in place, the water and soil samples obtained from the Acid Seep and the Oxidation Pond area were separately examined using the two proposed methodologies as suitable remediation strategies. The first method utilized a tube filter made of fine particles of drinking water treatment residuals (WTR) to treat the AMD water. High porosity, amorphous nature and relatively high pH of WTR particles enable the filtration system to adsorb metal ions and neutralize the AMD water and thus remediate the AMD water. On the other hand, as a soil amendment, WTR particles tend to bind the metal ions effectively and thus reduce metal mobility significantly and remediate the soil. The second approach examined in this study is phytoremediation of AMD water and soil using two different hyper-accumulators, commonly referred as Vetiver and Pokeweed.

Comparative results obtained for the AMD-impacted soils obtained from the Oxidation Pond and Acid Seep of Tab-Simco are summarized in Tables 4.1 and 4.2, respectively. The total metal concentration of the Oxidation Pond area soils for a few metals, such as, Al, Fe and P were relatively high even after being in proximity to Oxidation Pond water, which is treated by the SRB. However, as shown by the respective oxalate extractable, only a small fraction of the total metal remained leachable in acidic conditions. For example, although 3,100 mg/kg of Al is still present in the Oxidation Pond soil, only 45.12 mg/kg is leachable to the surroundings in acidic conditions. The leachability of Al could be further reduced to 15.4 mg/kg and 13.34 mg/kg by using the combination green technologies proposed in this study. This amounts to 65.9% and 70.4% reductions in the Al leaching potential of the Oxidation Pond soil. The reductions are higher with Pb, Ni and Fe but lower for Mn and As.

Table 4.1 Comparative data for Tab-Simco's Oxidation Pond area soil remediation

Oxidation Pond Area Soil (OS) Remediation									
Elements	Al	P	Cr	Mn	Fe	Ni	Cu	As	Pb
Total metal conc. (ppm)	3100	740	9.4	74	53800	8.4	14.59	29.3	22.91
Oxalate Extractable (ppm)									
Oxidation Pond Area Soil	45.12	21.21	0.08	2.49	490.83	0.08	0.18	0.20	0.04
WTR+Vetiver Treated	15.40	12.76	0.04	2.18	217.28	0.02	0.13	0.16	0.00
WTR+Pokeweed Treated	13.34	12.76	0.03	2.29	182.53	0.01	0.08	0.13	0.002
Reduction in % metal leaching									
WTR+Vetiver Treated	65.87	39.86	42.82	12.09	55.73	73.63	32.22	18.94	100.00
WTR+Pokeweed Treated	70.44	39.86	60.79	7.89	62.81	89.02	57.87	34.42	94.49

Table 4.2 lists similar comparative data for the Acid Seep soil, which also provides an opportunity to compare the effectiveness of the existing SRB treatment system as shown in the Oxidation Pond soil with that of the proposed treatment methods. The total metal concentration in the Acid Seep soil was relatively higher than the Oxidation Pond soil. However, as indicated by the oxalate extractable data, only small fractions of the metal concentrations are actually leachable, potentially harming the surrounding areas in direct contact. For example, the Phosphorus content in the AMD seep soil is 1,100 mg/kg, however, only 20.88 mg/kg is leachable.

Table 4.2 Comparative data for Tab-Simco's Acid Seep area soil remediation (oxalate extractable concentrations of soil in mg/kg = ppm)

AMD Seep area soil (SS) Remediation									
Elements	Al	P	Cr	Mn	Fe	Ni	Cu	As	Pb
Total metal conc. (ppm)	6000	1100	15.67	250	65000	24.2	32.67	21.5	14.7
Oxalate Extractable (ppm)									
SS	20.30	20.88	23.15	0.57	415.50	0.01	0.08	0.53	0.01
SRB downstream Soil	45.12	21.21	0.08	2.49	490.83	0.08	0.18	0.20	0.04
WTR+Vetiver Treated	38.52	10.76	0.07	3.39	296.51	0.10	0.25	0.13	0.00
WTR+Pokeweed Treated	41.55	12.09	0.08	3.73	313.66	0.09	0.21	0.14	0.00
Reduction in % metal leaching									
SRB Soil	-122.24	-1.60	99.67	-338.15	-18.13	-440.34	-131.50	61.53	-140.44
WTR+Vetiver Treated	-89.73	48.46	99.70	-497.89	28.64	-601.99	-215.50	75.34	100.00
WTR+Pokeweed Treated	-104.67	42.12	99.68	-557.05	24.51	-571.89	-158.61	73.55	100.00

The current treatment system application does not affect the metal concentration (between the Acid Seep soil and Oxidation Pond area soil) much, however the use of the proposed methods could potentially reduce the P leachability by another 48.46% and 42.14%, respectively. Similarly, better results from the proposed method are also evident for Pb, As and also Fe. Extractable chromium concentration in the Oxidation Pond soil was lower than the concentration in the Acid Seep soil treated by the proposed method. However, in the case of Al, Mn, Ni and Cu, there was observed increase in metal content of the treated soil. The observed increases in the aforementioned metals is as a result of the mineralogy of the Fe precipitates that remove trace metals by adsorption. Ferrihydrite and amorphous ferric hydroxide in the Oxidation Pond (downstream the SRB) are better at absorbing trace metals especially at the higher pH in the Oxidation Pond. Conversely, the high sulfates in Acid Seep (SS) area is indicative of the formation of Fe sulfates which are not as efficient in adsorbing trace metals especially at low pH, therefore, resulting in the selective removal of trace metals and which accumulate in time.

There is marked differences of metal concentration, sulfates, conductivity as well as pH among the Acid Seep water, the Oxidation Pond water of the existing SRB treatment system and the hydroponic treatment effluent of the proposed green technologies presented in this study, as shown in Table 4.3. It is instructive to note that considerable reduction of the dissolved metal concentrations in the Acid Seep water to permissible concentrations for surface water discharge is achievable by both current SRB system and the proposed system. Remarkable pH change from 2.9 to a final 6.7 and 5.24 in the Oxidation Pond and hydroponic effluent was recorded. Though the performance of both systems are very much comparable, it is worth noting that the current SRB system closely outperformed the proposed system for the observed parameters with the exception of sulfate reduction.

Table 8.3 A-B. Comparative elemental concentration (mg/L) of the Acid Seep AMD (influent) and treated AMD by the Tab-Simco's SRB treatment system and the proposed systems.

A.

Sample pt.	Al	Mn	Fe	Co	Ni	Cu	Zn	As
Acid Seep	130	32.4	327	0.426	1.72	0.035	2.88	0.00346
Impoundment	64.1	27.8	158	0.288	0.977	0.017	1.64	0.00217
SRB effluent	BDL	16.5	0.682	0.004	0.008	0.002	BDL	0.00004
Oxidation Pond	BDL	9.92	0.186	0.003	0.008	0.004	BDL	0.00003
Column effluent	0.433	28.9	1.75	0.335	1.26	0.013	0.886	0.00026
Hydroponic effluent	0.958	12.5	0.973	0.148	0.578	0.011	0.635	0.00028

B.

Sample pt.	pH	ORP (mV)	Conductivity (μ S/cm)	DO (mg/L)	Sulfate (mg/L)
Acid Seep	2.9	400.4	4963	15.7	4300
Impoundment	2.54	516.8	3812	6.7	2200
SRB effluent	6.43	-45	3591	3.5	1900
Oxidation Pond	6.7	-58.7	3609	4.8	1050
Column effluent	6.13	299.8	3707	4.5	1650
Hydroponic effluent	5.24	343.5	3048	5.2	1000

The major factors that were considered to investigate soil erosion and metal leaching in the simulated field study conducted in Southern Illinois' ambient environment included rate of precipitation, soil condition and planted grass density. It was observed that in case of high amount of rain in a short period, the water tends to run over the soil surface into the silt dams rather than leach into the soil. The texture of the seep area soil (13.9% clay, 59.4% silt and 26.7% sand) caused significantly dense mass following dry days in June 2016. Leachate analysis showed a reduced metal concentration in the WTR treated AMD impacted soil with improved (sustained at neutral) pH. There was a better growth of Vetiver grass planted on the WTR treated soil than the untreated soil. The Vetiver grass growth helped to decrease erosion caused by the rain water. The growth of Vetiver shoot and root was observed to be significantly different in the WTR treated and untreated soil. The growth in root and shoot lengths were higher in the treated soil. Nitrogen sufficiency index calculated from measured chlorophyll content was found to be more in the Vetiver grass

planted in the amended soil than the untreated soil. More research is recommended to identify the soil characteristics, initial soil exchangeable metal concentrations, degree of slopes, rainfall intensities, and WTR rate application procedure that help to reduce offsite sediment transport.

The simulated field hydroponic study investigated the metal uptake potential of the two grass/plants: Vetiver and Pokeweed. The plants, grown in the untreated AMD water, were deceased by the end of the 30-day study period. The aluminum and iron uptake from the WTR treated water by the Vetiver were found to be similar and were about twice as high as the manganese uptake. The difference in the uptake capability of Vetiver was apparently due to the selective adsorption and the available concentrations of the metal ions in solution. Mn in the WTR treated water was reduced by 56.9%, Fe by 44.4% and Co by 55.7%. Other considered metals that exhibited varying levels of reduction from the Vetiver hydroponic system included Ni (54.1%), Cu (16.5%), Zn (28.4%) and As (8.6%). The percentage reduction can be seen to be influenced by the initial concentration of the metal the WTR treated AMD. The mean Mn, Fe and Al uptake (in mg per Vetiver plant) on dry weight basis was found to be 0.059, 0.115 and 0.104, respectively.

The Pokeweed plant had a difficult time adjusting to the hydroponic condition. However, Pokeweed shoot and root were found to contain more P, Mn and Fe than that of the Vetiver grass. The metal and phosphorus translocation in the Pokeweed was less than 1 except for manganese which was 1.6. Though very high Mn translocation has been reported in Pokeweed in literature, the low translocation and uptake observed in our study was apparently due to the low growth rate exhibited by the Pokeweed set used in this study. The adsorption of metals by Pokeweed, however, was significant. The dissolved manganese in the WTR treated AMD used was further reduced by 31.8% whilst the concentration of iron was reduced by 48.7%. Significant reduction was also measured in cobalt (26.1%), nickel (24.8%) with the least amount of reduction in copper (3.5%). The mean Mn, Fe and Al accumulation (mg per Pokeweed plant) was found to be as low as 0.003, 0.007 and 0.001 respectively.

ACKNOWLEDGEMENTS

We acknowledge the participation of Michigan Technological University (MTU) in this project effort and Dr. Rupali Dutta in particular for her assistance in completing the greenhouse hydroponic study as a part of a subcontract issued from SIU. Dr. Dutta's report forms the Section 3.4 of this project final report. We also express our special gratitude to MS Student, Mr. Rajesh Guru, who assisted the day-to-day project activities. Project participation from the PhD student Mr. Meisam Peiravi and several under graduate student assistants is duly acknowledged.

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APPENDIX

Tab-Simco's Acid Seep Water Analysis in 2015-2016

Sampling Dates	Al*	Mn*	Fe*	Ni*	Cu*	Zn*	As*	pH	ORP	Sulfate (mg/L)
May, 2015	120	97.1	375	0.53	0.002	0.42	0.000	2.80	-	-
Aug, 2015	133		381	10.81	0.341	36.82	0.035	2.60	-	-
Oct, 2015	180	95.0	410	-	-	-	-	2.70	-	-
Jan, 2016	300	24.0	400	-	-	-		2.41	464.7	6100
April, 2016	130	32.4	327	1.72	0.035	2.88	0.003	3.24	543.5	4400
May, 2016	129	31.1	341	1.35	0.030	2.65	0.002	2.64	550	4300

*Metal concentrations are listed as mg/L of water.