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THE ABIOTIC STRESS RESPONSE OF HYDROPONIC VETIVER GRASS (*Chrysopogon zizanioides* L.) TO ACID MINE DRAINAGE AND ITS POTENTIAL FOR ENVIRONMENTAL REMEDIATION

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Recommended Citation

Kiiskila, Jef, "THE ABIOTIC STRESS RESPONSE OF HYDROPONIC VETIVER GRASS (*Chrysopogon zizanioides* L.) TO ACID MINE DRAINAGE AND ITS POTENTIAL FOR ENVIRONMENTAL REMEDIATION", Open Access Dissertation, Michigan Technological University, 2018.
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THE ABIOTIC STRESS RESPONSE OF HYDROPONIC VETIVER GRASS
(*Chrysopogon zizanioides* L.) TO ACID MINE DRAINAGE AND ITS POTENTIAL
FOR ENVIRONMENTAL REMEDIATION

By
Jeffrey D. Kiiskila

A DISSERTATION
Submitted in partial fulfillment of the requirements for the degree of
DOCTOR OF PHILOSOPHY
In Biochemistry and Molecular Biology

MICHIGAN TECHNOLOGICAL UNIVERSITY
2018

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This dissertation has been approved in partial fulfillment of the requirements for the Degree of DOCTOR OF PHILOSOPHY in Biochemistry and Molecular Biology.

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Dedication

This dissertation is dedicated to my parents, David and Laurie Kiiskila, along with my grandmother, Nancy Kiiskila (1935-2017), for their unconditional love and support.

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Author contribution statement

Every chapter was submitted to peer-review journals for publication.

- Chapter 1. “A preliminary study to design a floating treatment wetland for remediating acid mine drainage-impacted water using vetiver grass (*Chrysopogon zizanioides*)” was formatted to the requirements of “Environmental Science and Pollution Research” and was submitted to and published with “Environmental Science and Pollution Research”. The experiment design was prepared by Jeffrey Kiiskila and evaluated by Rupali Datta, Dibyendu Sarkar, Tarun Dam, and Victor Busov. The authors for this publication include Jeffrey Kiiskila, Dibyendu Sarkar, Kailey Feuerstein, and Rupali Datta. The Department of Interior, Office of Surface Mining Reclamation and Enforcement provided necessary funding for the study. Kailey Feuerstein helped with the greenhouse setup, water sample analysis, and acid digestion of plant material. Matthew Adler supplied the AMD from the Tab-Simco site. Abhishek Roy-Chowdhury analyzed the water samples and plant digests for metal content by ICP-MS. Olivia Olsen and Venkataramana Pidatala also helped to carry out the experiment. Jeffrey Kiiskila wrote the full manuscript. The manuscript was reviewed by all authors for approval to submit. The manuscript was peer-reviewed and published.
- Chapter 2. “Remediation of Acid Mine Drainage-Impacted Water by Vetiver Grass: A Multiscale Long-Term Study” was formatted to the requirements of “Ecological Engineering” and was submitted to “Ecological Engineering”. The experiment design was prepared by Jeffrey Kiiskila and evaluated by Rupali Datta, Dibyendu Sarkar, Tarun Dam, and Victor Busov. The authors for this publication include Jeffrey Kiiskila, Dibyendu Sarkar, Saumik Panja, Shivendra Sahi, and Rupali Datta. The Department of Interior, Office of Surface Mining Reclamation and Enforcement provided necessary funding for the study. Emili Heikkinen and Matthew Adler helped collect and transport the AMD from the

Tab-Simco site. Laurie Kiiskila and Dan O’Connell helped transport the AMD for filtration with the plug filter. Abhishek RoyChowdhury provided the plug filter. David Kiiskila designed and constructed the rafts for the FTWs, provided technical assistance in the greenhouse, and transported the AMD for filtration with the plug filter. Saumik Panja analyzed the water samples for sulfate content by IC, and the water samples and plant digests for metal content by ICP-OES. Shivendra Sahi analyzed the plant tissues for microscopic metal dispersal by SEM-EDX. Amy Marcarelli provided suggestions for the statistical analysis. Yi Cui, Mona Fabian, Reginald Mullen, Alexis Rule, Nikki Saari, and Amanda Turcotte also helped to carry out the experiment. Jeffrey Kiiskila wrote the full manuscript. The manuscript was reviewed by all authors for approval to submit. The manuscript was submitted for peer-review and is currently in revision.

- Chapter 3. “Metabolic Response of Vetiver Grass (*Chrysopogon zizanioides*) to Acid Mine Drainage” was formatted to the requirements of “Environmental Science & Technology” and was submitted to “Environmental Science & Technology”. The experiment design was prepared by Jeffrey Kiiskila and evaluated by Rupali Datta, Dibyendu Sarkar, Tarun Dam, and Victor Busov. The authors for this publication include Jeffrey Kiiskila, Kefeng Li, Dibyendu Sarkar, and Rupali Datta. The Department of Interior, Office of Surface Mining Reclamation and Enforcement provided necessary funding for the study. Saumik Panja analyzed the water samples for sulfate content by IC, and the water samples and plant digests for metal content by ICP-OES. Kefeng Li analyzed the plant extracts and water samples for metabolites by LC-MS/MS and helped with the data processing and analysis. Yi Cui, Mona Fabian, Reginald Mullen, Alexis Rule, Nikki Saari, and Amanda Turcotte also helped to carry out the experiment. Jeffrey Kiiskila wrote the full manuscript. The manuscript was reviewed by all authors for approval to submit. The manuscript was submitted for peer-review and is currently in review.

Acknowledgements

I'm extremely grateful for my advisor, Dr. Rupali Datta. She was an ideal advisor who encouraged me to work independently through my graduate studies but made herself available whenever I needed assistance and trusted in my decisions. I'm also thankful for my committee members (Dr. Dibyendu Sarkar, Dr. Tarun Dam, and Dr. Victor Busov) for committing their time and support to evaluate my graduate studies.

I very much appreciate the Department of Interior, Office of Surface Mining Reclamation and Enforcement for funding my research, the Department of Biological Sciences at Michigan Tech for providing me with GTA Fellowships, and the Graduate School for awarding me a Finishing Fellowship. These past few years would have been far more difficult without the funding opportunities that have been made available to me.

I'd like to acknowledge the faculty and staff from Biological Sciences and the BMB program at Michigan Tech for their assistance over the years. In particular, I'd like to thank my fellow graduate students, undergraduate assistants and friends for spending all of their free-time with me, endlessly drinking coffee and listening to loud music while working in the laboratory and greenhouse. And of course, for reminding me not to forget supplies as we moved back and forth between the laboratory and greenhouse... It would not have been possible to get through my studies without your assistance and support.

Last but not least, I'd like to thank my family, friends, and colleagues outside of Michigan Tech for their patience, understanding and support throughout the years. Whether you were analyzing samples for me or just checking to see how things were, it means a lot having you in my life.

List of abbreviations

AAs: Amino Acids

ADP: Adenosine Diphosphate

Al: Aluminum

Al^{3+} : Aluminum Ion

Ag: Silver

AMD: Acid Mine Drainage

AMP: Adenosine Monophosphate

ANOVA: Analysis of Variance

As: Arsenic

AUC: Area Under the Curve

Ba: Barium

BCAAs: Branched Chain Amino Acids

BDL: Below Detection Limit

cAMP: Cyclic Adenosine Monophosphate

Ca: Calcium

CCC: Criterion Continuous Concentration

CDP: Cytidine Diphosphate

Cd: Cadmium

CERCLA: Comprehensive Environmental Resource Compensation and Liability ACT

Chl: Chlorophyll

CO₂: Carbon Dioxide

CMC: Criterion Maximum Concentration

Cr: Chromium

Cr₂O₇²⁻: Dichromate

Cr³⁺: Trivalent Chromium

Cr⁶⁺: Hexavalent Chromium

Cu: Copper

Cu²⁺: Cupric Copper

dAMP: Deoxyadenosine Monophosphate

dCDP: Deoxycytidine Diphosphate

dGDP: Deoxyguanosine Diphosphate

dGMP Deoxyguanosine Monophosphate

DHAP: Dihydroxyacetone Phosphate

dIMP: Deoxyinosine Monophosphate

dITP: Deoxyinosine Triphosphate

DO: Dissolved Oxygen

DSR: Dissimilatory Sulfate Reduction

EC: Conductivity

EDX: Energy Dispersive X-ray

EPA: Environmental Protection Agency

ESI: Electrospray Ionization

FAD: Flavin Adenine Dinucleotide

FAs: Fatty Acids

FDR: False Discovery Rate

FeS₂: Iron Pyrite

Fe: Iron

Fe²⁺: Ferrous Iron

Fe³⁺: Ferric Iron

FMN: Flavin Mononucleotide

FTW: Floating Treatment Wetland

FT: Filtered-Treated

FU: Filtered-Untreated

FWT: Floating Wetland Treatment

F: Final

GSH: Reduced Glutathione

GSSG: Oxidized Glutathione

G-3-P: Glyceraldehyde-3-Phosphate

Hg: Mercury

HSD: Honestly Significant Difference

H₂S: Hydrogen Sulfide

H⁺: Hydrogen Ion

ICP: Inductively Coupled Plasma

IC: Ion Chromatography

IMP: Inosine Monophosphate

ITP: Inosine Triphosphate

I: Initial

k-NN: k-Nearest Neighbors

LC: Liquid Chromatography

MCLG: Maximum Contaminant Level Goal

MCL: Maximum Contaminant Level

metSO: Methionine Sulfoxide

Mn: Manganese

MS: Mass Spectrometry

MS/MS: Tandem Mass Spectrometry

NAD: Nicotinamide Adenine Dinucleotide

NA: Not Applicable

Na⁺: Sodium Ion

Ni: Nickel

NMR: Nuclear Magnetic Resonance

NO₃¹⁻: Nitrate

NPDWR: National Primary Drinking Water Regulations

NR: Not Relevant

NT: Not Tested

OAA: Oxaloacetate

OAs: Organic Acids

OES: Optical Emission Spectrometry

O₂: Oxygen

PC: Phosphatidylcholine

PEP: Phosphoenolpyruvate

PE: Phosphatidylethanolamine

PG: Phosphatidylglycerol

PI: Phosphatidylinositol

PLS-DA: Partial Least Squares-Discriminant Analysis

PO_4^{3-} : Phosphate

P: Phosphate

RCRA: Resource Conservation and Recovery ACT

SEM: Scanning Electron Microscopy

ROL: Radial Oxygen Loss

ROS: Reactive Oxygen Species

R:S: Root-to-Shoot

R: Root

Se: Selenium

SO_4^{2-} : Sulfate

SRB: Sulfate-Reducing Bioreactor

S: Shoot

TCA: Tricarboxylic Acid

TCLP: Toxicity Characteristic Leaching Procedure

TF: Translocation Factor

UDP: Uridine Diphosphate

UMP: Uridine Monophosphate

US: United States

UT: Unfiltered-Treated

UU: Unfiltered-Untreated

Zn: Zinc

3-PG: 3-phosphoglycerate

Abstract

Mining activities can generate acid mine drainage (AMD), an acidic discharge that contains elevated sulfate (SO_4^{2-}), soluble metals, and orange-yellow metal-containing particulates. AMD imposes ecological risks from metal toxicity and physical stress, which may physically spread to nearby water sources or biologically through bioaccumulation. Current methods for AMD treatment, such as chemical or passive biological treatments, are often non-sustainable. Chemical treatments can be expensive and create large amounts of secondary waste, whereas biological treatments like anaerobic wetlands require continuous maintenance through input of organic nutrients. The end goal of this research is to develop a cost-efficient and sustainable floating treatment wetland (FTW) system to remediate AMD-impacted waters from the abandoned Tab-Simco mining site in Southern Illinois. AMD from the site is acidic, having a pH of 2 to 3, with elevated amounts of SO_4^{2-} , Fe, Al, Mn, Zn and lower amounts of various other metals. Vetiver grass (*Chrysopogon zizanioides*) is a non-invasive and fast-growing plant that can grow sustainably under hydroponic conditions. Moreover, vetiver is tolerant of acidic conditions and metals, and is a known hyperaccumulator of Pb and Zn. A short-term (30-day), bench-scale experiment revealed that vetiver was tolerant of Tab-Simco AMD and able to improve water quality by increasing pH and reducing the amounts of SO_4^{2-} and soluble metals. In a follow-up, year-long mesocosm experiment, vetiver plants were positioned on rafts for FTWs and suspended in 378.5-liter containers filled with 170 liters of unfiltered Tab-Simco AMD while additional plants were suspended in AMD that was passed through a plug-filter with recycled materials designed to remove metals and neutralize the water. From the unfiltered AMD, there was high net removal of SO_4^{2-} (28%), Fe (81%) and Pb (81%) with lower removal of Ni (38%), Zn (35%), Mn (27%), Cr (21%), Al (11%) and Cu (8.0%). From the filtered AMD, there was little to no remaining metals, though there was high net removal of SO_4^{2-} (45%). Vetiver accumulated $\text{Fe} > \text{Al} > \text{Mn} > \text{Zn} > \text{Cr} > \text{Ni} > \text{Cu} > \text{Pb}$. The majority of metals were localized on the root surface as Fe plaques, though Mn and Zn

demonstrated greater translocation to the shoots. Moreover, vetiver biomass was deemed non-hazard following metal accumulations through TCLP. An additional year-long microcosm experiment was conducted to ensure complete root coverage by vetiver, which resulted in superior remediation with near complete removal of SO_4^{2-} (91%) and most metals (90-100%) with the exception of Pb (15%) and Cu (0.0%). These remediation experiments demonstrate that vetiver can effectively improve AMD-impacted waters; however, little was known about tolerance mechanisms. Therefore, metabolomic analysis of vetiver was conducted over relatively short-term (7-days) and long-term (56-days) periods of exposure to Tab-Simco AMD. After long-term exposure, vetiver shoots showed dramatic upregulation of amino acid (AA) and glutathione metabolism, along with respiration and photosynthesis pathways, and downregulation of phosphorylated metabolites. There was drastic downregulation of phosphorylated metabolites in the roots, particularly with phospholipids, as well as respiration, glyoxylate and AA metabolism. It was suggested that oxidative stress, resource deprivation (nitrogen and phosphorus), and dehydration were among the main forms of abiotic stress.

Introduction

Industrial activities such as mining operations can result in the formation and exacerbated discharge of acid mine drainage (AMD). AMD is a sulfurous and heterogeneous mixture generated from the oxidation of sulfide minerals such as iron pyrite (FeS_2) in the presence of moisture (RoyChowdhury et al., 2015). Sulfide minerals that are oxidized under hydrolytic conditions will initially release ferrous iron (Fe^{2+}), sulfate (SO_4^{2-}) and hydrogen ions (Eq. 1) that decrease pH (Akcil and Koldas, 2006; RoyChowdhury et al., 2015). Continued oxidation from acidic conditions and microbial activity generates ferric iron (Fe^{3+}) and increases metal mobility (Eq. 2), where Fe^{3+} furthers the process as the primary oxidant (Eq. 3; RoyChowdhury et al., 2015). Characteristics may vary, though AMD general contains an eclectic of transition and post-transition metals that are distinctive to on-site mineralogy (Hogsden and Harding, 2012). A major concern comes from toxicity as metals may be accumulated by living things and contaminate nearby water sources such as streams and groundwater (Oberholster et al., 2010). Moreover, AMD often results with greater soil erosion and damage to waterways (RoyChowdhury et al., 2015).

Ecological concerns with AMD come from bioaccumulation and mortality, along with biomagnification through indigenous species and their consumers. For instance, AMD inflows were reported following a thunderstorm at the Clark Fork River of Southwestern Montana in 1989 where acidity and copper (Cu) increased dramatically within a mere 20 minute period (Jennings et al., 2008). Mortality was reported at greater than 5000 salmonids, which perished as a result of acute Cu toxicity from accumulation in the gills (Jennings et al., 2008). Another interesting case involved the mortalities of Nile crocodiles (*Crocodylus niloticus*) at Lake Loskop of South Africa in 2008, when the

estimated population of about 30 had declined to six crocodiles (Oberholster et al., 2010). These losses were attributed to pancreatitis from fish consumption after a period of die-off that coincided with intermittent inflows of AMD (Oberholster et al., 2010).

In addition to soluble metals, ferric iron from AMD commonly precipitates as orange-yellow particulates composed of metal hydroxides (Eq. 4; Akcil and Koldas, 2006; Hogsden and Harding, 2012; RoyChowdhury et al., 2015). Therefore, the net result from FeS₂ oxidation will often end with ferric oxide precipitation (Eq. 5; Akcil and Koldas, 2006; RoyChowdhury et al., 2015). Metal hydroxides may inhibit photosynthetic activities by reducing light passage; however, they cover surfaces throughout contaminated water bodies and pose a threat by means of physical stress (Hogsden and Harding, 2012). Some sources have suggested that metal hydroxides may even have greater ecological consequences than soluble metals (Niyogi et al., 2002; Bray et al., 2008; Hogsden and Harding, 2012).

Communities of primary producers covering 58 streams from the Rocky Mountains of Colorado were evaluated for effects among varieties of AMD, naturally weathered pyrite, and unaffected streams (Niyogi et al., 2002). It was reported that high zinc (Zn , $> 0.25 \text{ mg L}^{-1}$) and lower pH (< 4) had greater impacts on diversity, whereas biomass and primary production were only negatively affected by physical stress from metal oxide deposition (Niyogi et al., 2002). It was reported that 52% of net primary production and 65% of biomass variations were attributed to aluminum (Al) oxides, particularly when deposition rates were greater than $0.05 \text{ g m}^{-2} \text{ d}^{-1}$ (Niyogi et al., 2002). Similar observations were reported with periphyton communities covering 52 streams from West Coast of the South Island in New Zealand (Bray et al., 2008). Species richness was negatively impacted by acidity, whereas biomass was positively correlated to acidity but negatively correlated with metal oxides (Bray et al., 2008). These field studies have

demonstrated that metal and acid tolerant taxa are not able to withstand physical stresses from particulates within AMD.

AMD presents implications for humans as well from potential contamination of agricultural lands, or ground and well waters that serve as sources of drinking water (Nyamadzawo et al. 2007). The Iron Duke Mine for instance, the only FeS₂ mine in Zimbabwe, contains 5000 m² of waste dumping land (Nyamadzawo et al. 2007). AMD from the waste dump is extremely acidic (pH = 0.52) with elevated levels of SO₄²⁻ ranging from 355 to 425 mg L⁻¹ (Nyamadzawo et al. 2007). Moreover, the waste dump is merely 500 meters from the Yellow Jacket River, which is used for drinking water and irrigation by the nearby town of Glendale and downstream farming communities (Nyamadzawo et al. 2007).

Occurrence of AMD in the US and the Tab-Simco site

Occurrence of AMD in the United States (US) was originally estimated and summarized by the Environmental Protection Agency (EPA) and the US Forest Service. It has been predicted that there are 20,000 to 50,000 mines in the western US that are actively generating AMD and impacting 8000 to 16,000 km of streams (USEPA, 1994; Jennings et al., 2008). It's assumed that some AMD originates from abandoned mining sites in more remote areas; however, a number of known locations are among the more expensive CERCLA (Comprehensive Environmental Resource Compensation and Liability ACT) sites, more commonly known as Superfund sites (Jennings et al., 2008). Included among these Superfund sites are the Butte-Clark Fork River complex in Montana, Bunker Hill in Idaho, and the Iron Mountain Mine in California (Jennings et al., 2008). Additional predictions have suggested that there are more than 7000 km of AMD-impacted streams in the eastern US (USEPA, 1994; Jennings et al., 2008).

Figure 1: Images of the abandoned Tab-Simco mining site near Carbondale, Illinois, including A) an aerial view of the site (Image © 2015 Google Earth, taken on 03-10-2015) and B) a close-up terrestrial view of the SRB.

Table 1: Characteristics of Tab-Simco AMD from the SRB, including pH, sulfate (SO_4^{2-}), and soluble metals (means $\pm$ SD for n = 3 samples). Data © 2016 (RoyChowdhury, 2016).											
pH	Sulfate and Metals (mg L^{-1})										
	SO_4^{2-}	Fe	Al	Mn	Zn	Pb	As	Cu	Ni	Cd	Cr
2.27	2481	137	80	33	11	7	4	4	3	1	1
± 0.2	± 50	± 5	± 15	± 2.4	± 0.9	± 1.2	± 0.01	± 0.05	± 0.25	± 0.01	± 0.04

The abandoned Tab-Simco mining site is a former coal mine found at the Southern Illinois coal basin (Behum et al., 2011; Lefticariu et al., 2017). This site is reportedly among the most heavily AMD-impacted areas in the mid-continental US and extends about 12 hectares (Behum et al., 2011; Lefticariu et al., 2017). The Tab-Simco site is about six miles from Carbondale, Illinois, found near No Name Road at the GPS coordinates of N37°41.200' and W089°10.085' (Figure 1). The most conspicuous sight is a large orange-colored AMD pond originally setup as a passive sulfate-reducing bioreactor (SRB) in 2007 (Behum et al., 2011; Lefticariu et al., 2017). The SRB later failed, now generating AMD with a pH of 2 to 3, excessive SO_4^{2-} , iron (Fe), Al, manganese (Mn), and a variety of other metals (Table 1).

Table 2: National recommended quality criteria for aquatic life in freshwaters and NPDWR for drinking water, including CMCs and CCCs of metals for freshwater (USEPA, 2018b) and MCLGs and MCLs of metals for drinking water (USEPA, 2018a).				
Metal	Freshwater ($\mu\text{g L}^{-1}$)		Drinking Water ($\mu\text{g L}^{-1}$)	
	CMC	CCC	MCLG	MCL
Al	750	87		
As	340	150	0	10
Cd	1.8	0.72	5	5
Cr(III)	570	74	100	100
Cr(VI)	16	11		
Cu			1300	
Fe		1000		
Ni	470	52		
Pb	65	2.5	0	
Zn	120	120		

The majority of metals from the SRB as reported by RoyChowdhury, 2016 are well above recommended concentrations for aquatic freshwater life and National Primary Drinking Water Regulations (NPDWR) for drinking water in the US (Table 2). Excluding Cu and Mn, all metals were above recommended concentrations for aquatic freshwater

life as determined from Criterion Maximum Concentrations (CMCs) and/or Criterion Continuous Concentrations (CCC) for Al, arsenic (As), cadmium (Cd), Chromium (Cr), Fe, nickel (Ni), lead (Pb) and Zn. Furthermore, NPDWR for drinking water as determined from Maximum Contaminant Level Goals (MCLGs) and Maximum Contaminant Levels (MCLs) were far in excess for As, Cd, Cr, Cu and Pb. The SRB releases into an oxidation ditch (also known as the Kill Zone) with a settling wetland pond that eventually exits into the nearby Sycamore Creek (Behum et al., 2011; Lefticariu et al., 2017), demonstrating the need for additional remediation efforts.

Floating Treatment Wetlands and Vetiver Grass

There are varieties of remediation techniques for AMD, ranging from chemical treatments (e.g., liming), to biological methods like bioreactors or wetlands (Akcil and Koldas, 2006; Richter et al., 2016). However, these techniques are often high maintenance and non-sustainable, requiring consistent input of resources such as lime or microbial nutrients (Richter et al., 2016). Floating treatment wetlands (FTWs) offer a cost efficient and sustainable alternative for the treatment of contaminated waters (Marchand et al., 2010; Chen et al., 2016a; Chen et al., 2016b; Richter et al., 2016; Pavlineri et al., 2017). FTWs employ floating structures to support plants, which grow hydroponically and remove contaminants from within the watershed (Pavlineri et al., 2017). As artificial wetlands, FTWs are also commonly known as constructed wetlands or constructed floating wetlands, and sometimes as hydroponic root mats (Richter et al., 2016; Pavlineri et al., 2017). In more recent times, FTWs have been used to treat metalliferous waters such as AMD (Sheoran and Sheoran, 2006; Marchand et al., 2010; Chen et al., 2016a; Chen et al., 2016b; Richter et al., 2016).

The most significant features of plants used for the remediation of AMD-impacted waters would include metal tolerance and removal capabilities, acid tolerance, sustained hydroponic growth, and non-invasive taxa or variants. Plants used in FTWs will frequently remove contaminants from water through direct uptake (phytoextraction) or rhizofiltration (Sheoran and Sheoran, 2006; Marchand et al., 2010; Chen et al., 2016a;

Pardo et al., 2016; Richter et al., 2016; Pavlineri et al., 2017). In hydroponics, plants transport oxygen to their roots to meet requirements for respiration and release excess into the rhizosphere (Sheoran and Sheoran, 2006). In metal abundant waters like AMD, excess oxygen will encourage oxidation and precipitation of metals (Sheoran and Sheoran, 2006; Chen et al., 2016a; Li et al., 2017; Jia et al., 2018). Precipitated metals may then settle or adsorb onto roots as plaques (Marchand et al., 2010; Yang et al., 2014; Li et al., 2017). Metal plaques are amorphous buildups that collect along plant roots surfaces and often consist of metal oxides or hydroxides (Yang et al., 2014; Li et al., 2017). In opposition to uptake, rhizofiltration and plaque formations result with metal removal by means of sequestration along and/or within the roots (Marchand et al., 2010; Yang et al., 2014; Pardo et al., 2016; Li et al., 2017; Jia et al., 2018).

A variety of plants have been investigated for their tolerance to and usefulness at AMD-impacted environments, including wetland plants such as *Phragmites australis* and *Thypha latifolia* (Sheoran and Sheoran, 2006). In one case, four common wetland taxa were investigated for metal removal following natural establishment on submerged pyrite tailings at the Boliden mine of Northern Sweden (Stoltz and Greger, 2002). Out of these, *Salix* (sp.) and *Eriophorum angustifolium* demonstrated greater shoot accumulations of metals, whereas *Carex rostrata* and *P. australis* had greater root accumulations (Stoltz and Greger, 2002). *Salix* (sp.) were found to have elevated Cd and Zn in their shoots, which raises concerns as moose (*Alces alces*) and reindeer (*Rangifer tarandus*) are known consumers (Stoltz and Greger, 2002). Wetland plants like *C. rostrata* and *P. australis* may be effective for metal remediation; however, *P. australis* is a well-known invasive species in the northeastern US (Sheoran and Sheoran, 2006). In another case, six native plants were investigated for remediation potential at a silver (Ag) mining site in the ghost town of Yerranderie of New South Wales, Australia (Archer and Caldwell, 2004). Out of these plants, *Cynodon dactylon*, *Juncus usitatus* and *Lomandra longifolia* were identified as having potential for rhizofiltration and mild acid tolerance (Archer and Caldwell, 2004). However, none of these plants had roots that could reach the depths

required for proper rhizofiltration, nor could they grow where $\text{pH} < 4$ (Archer and Caldwell, 2004).

Vetiver grass (*Chrysopogon zizanioides*) is a large perennial plant that is fast-growing but non-invasive (Figure 2; Andra et al., 2009). Owing to an extensive root system, vetiver was originally used to prevent and reduce soil erosion (Truong et al., 2010; RoyChowdhury et al., 2015). However, vetiver is also tolerant of acidic conditions as well as a variety of metals (Truong et al., 2010), and is a known hyperaccumulator of Pb and Zn from soils (Antiochia et al., 2007). Though commonly used to treat soils and reduce erosion, vetiver can grow sustainably under hydroponic conditions (Roongtanakiat et al., 2007) and effectively remove metals from contaminated water (Roongtanakiat et al., 2007; Andra et al., 2009; Chen et al., 2012; Singh et al., 2015; Pidatala et al., 2016). For instance, vetiver has demonstrated 37% Cu removal from hydroponic media containing 5 mg Cu L^{-1} within 7 days (Chen et al., 2012) and 88% from ink manufacturing wastewaters containing 126 mg Fe L^{-1} and 119 mg Cu L^{-1} over 120 days (Roongtanakiat et al., 2007). At higher loads of metals vetiver had stunted growth, shoot chlorosis, as well as cracking and browning of the roots (Roongtanakiat et al., 2007). Vetiver has demonstrated Pb removal at 73% from hydroponic media with 5 mg Pb L^{-1} in 7 days (Chen et al., 2012) and 78 to 94% of Pb from 5 to 20 mg Pb L^{-1} over 15 days

(Singh et al., 2015). There has also been 77 to 79% removal of Cr by vetiver from 5 to 20 mg Cr L⁻¹ in synthetic wastewaters over 15 days (Singh et al., 2015). In milk factory wastewaters, vetiver has removed 28% Fe, 53% Zn and 34% Mn from 16 mg Fe L⁻¹, 4.1 mg Zn L⁻¹ and 0.49 mg Mn L⁻¹ (Roongtanakiat et al., 2007).

In addition to removal, vetiver is known for storing metals in the roots. From hydroponics, vetiver has demonstrated lower translocation ($\leq 20\%$) of Pb in multiple studies (Roongtanakiat et al., 2007; Andra et al., 2009; Chen et al., 2012; Pidatala et al., 2016) as well as Fe in at least one case (Roongtanakiat et al., 2007). Two studies have suggested lower to moderate translocation of Cu, along with moderate to higher translocation of Zn and Mn (Roongtanakiat et al., 2007; Chen et al., 2012). Meanwhile, Cr translocation in vetiver has been higher in at least one case (Singh et al., 2015). Since vetiver has extensive and self-regenerative roots (Singh et al., 2015) it should be an ideal plant for reaching lower depths to remove and sequester metals from AMD.

Combined Abiotic Stresses and Metabolomics

Systems analyses such as transcriptomics, proteomics, and metabolomics are commonly used to understand the biochemical tolerance mechanisms of plants to abiotic stresses. Single independent factors have held the focus in a vast majority of systems analyses, including those relating to agriculture like drought or temperature (Mittler, R., 2006) and metals such as with vetiver and its tolerance for Pb (Pidatala et al., 2016). Under normal conditions however, plants are not simply exposed to a single stress but rather a combination of two or more stresses (Mittler, 2006; Cui et al., 2009; Sun et al., 2015; Li et al., 2017; Martinez et al., 2018). For instance, agricultural plants often experience drought and heat simultaneously, which creates further complications (Mittler, 2006). Plants open their stomata for transpirational cooling under excessive heat; however, they would not be able to open their stomata when combined with drought, leading to further increase of internal temperature (Mittler, 2006). Furthermore, the upregulation of proline is well-known as a plant response to drought, whereas proline is downregulated when drought is combined with heat (Mittler, 2006).

Metabolomics is invaluable for understanding plant abiotic stress as it generates large amounts of data but does not rely on genetic information (Pidatalla et al., 2016). Combined stresses have revealed shifts in metabolism that don't compare with individual stresses (Mittler, 2006; Sun et al., 2015; Li et al., 2017; Martinez et al., 2018). Leaves of maize (*Zea mays*) for example, have been evaluated for physiological changes following drought, salinity, and a combination of the two stresses (Sun et al., 2015). Nuclear magnetic resonance (NMR)-metabolomics revealed that out of 26 differentially regulated metabolites, only eight were changed among the three stresses while six were unique to the combination of drought and salinity (Sun et al., 2015). Moreover, there was greater correlation between drought and the combined stresses in opposition to their relationships with salinity (Sun et al., 2015). It was also reported that the metabolic response related with the observed decreases in plant biomass, carbon content, and photosynthesis, as well as increased nitrogen content (Sun et al., 2015). There are only a handful of studies that focus on combined stresses, though they have already revealed their importance.

Dissertation Objectives

Presented here is an alternative method for remediation of AMD-impacted waters using FTWs with vetiver grass. The primary focus was to determine tolerance and remediation efficiency of vetiver for AMD-impacted waters originating from the Tab-Simco mining site in Southern Illinois. Tolerance and remediation efficiency of vetiver was determined through various multi-scale greenhouse experiments, whereas tolerance mechanisms were determined through physiological and large-scale assessment of plant metabolism. The end goal of this project is to develop a low-cost and sustainable FTW system with vetiver for deployment at the Tab-Simco site to reduce on-site contamination.

Objective 1 (Chapter 1): Screen vetiver grass for tolerance and treatment of AMD-impacted water from the Tab-Simco site using a short-term (30-day) bench-scale hydroponic experiment in order to optimize parameters for an FTW system.

Aim 1: Determine if vetiver can tolerate the acidic and metalliferous conditions present from the AMD.

Aim 2: Determine the effects of vetiver on the quality of AMD by monitoring changes in pH, conductivity, SO_4^{2-} , and dissolved metals.

Aim 3: Estimate the distribution and translocation of metals between vetiver roots and shoots.

Aim 4: Estimate the optimal planting density of vetiver for effective treatment of AMD.

Objective 2 (Chapter 2): Conduct a longer-term feasibility study for the construction and deployment of vetiver FTWs for the treatment of AMD-impacted water from the Tab-Simco site using large- and small-scale remediation experiments.

Aim 1: Evaluate vetiver for its capabilities at improving the quality of AMD over an extended period using a large-scale mesocosm experiment with vetiver FTWs along with a small-scale microcosm experiment with complete vetiver root coverage and statistical replication.

Aim 2: Estimate metal uptake and distribution throughout vetiver roots and shoots after prolonged AMD exposure.

Aim 3: Determine if vetiver biomass presents any environmental and/or ecological risks for disposal following AMD treatment.

Objective 3 (Chapter 3): Evaluate the physiological and metabolic responses of vetiver to AMD-impacted water from the Tab-Simco site after relatively short-term (7-days) and long-term (56-days) exposure.

Aim 1: Evaluate the physiological responses of vetiver to AMD through changes in growth, photosynthesis, and metal accumulation/translocation.

Aim 2: Evaluate the metabolic responses of vetiver roots and shoots to AMD through targeted, large-scale metabolomics by LC-MS/MS.

Aim 3: Evaluate metabolic changes to AMD as a result of root exudates from vetiver and/or rhizospheric microbial activity by LC-MS/MS.

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Chapter 1. A preliminary study to design a floating treatment wetland for remediating acid mine drainage-impacted water using vetiver grass (*Chrysopogon zizanioides*)^a

Chapter 1 was submitted to and published with *Environmental Science and Pollution Research*.

^a The following chapter is a manuscript published in *Environmental Science and Pollution Research*.

A preliminary study to design a floating treatment wetland for remediating acid mine drainage-impacted water using vetiver grass (*Chrysopogon zizanioides*)

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Abstract

Acid mine drainage (AMD) is extremely acidic, sulfate-rich effluent from abandoned or active mine sites that also contain elevated levels of heavy metals. Untreated AMD can contaminate surface and groundwater and pose severe ecological risk. Both active and passive methods have been developed for AMD treatment consisting of abiotic and biological techniques. Abiotic techniques are expensive and can create large amounts of secondary wastes. Passive biological treatment mainly consists of aerobic or anaerobic constructed wetlands. While aerobic wetlands are economical, they are not effective if the pH of the AMD is < 5 . Anaerobic wetlands use organic-rich substrates to provide carbon source to iron- and sulfate-reducing bacteria. The efficiency of these systems declines overtime and requires continuous maintenance. Our objective is to develop an alternative, low-cost, and sustainable floating wetland treatment (FWT) system for AMD for the abandoned Tab-Simco coal mining site in Illinois using vetiver grass (*Chrysopogon zizanioides*). Tab-Simco AMD is highly acidic, with mean pH value of 2.64, and contains high levels of sulfate and metals. A greenhouse study was performed for a 30-day period in order to screen and optimize the necessary parameters to design a FWT system. Water quality and plant growth parameters were continuously monitored. Results show significant SO_4^{2-} removal, resulting in increased pH, particularly at higher planting densities. Vetiver also helped in metal removal; high amounts of Fe, Zn, and Cu were removed, with relatively lower amounts of Pb, Al, and Ni. Iron plaque formation on the root was observed, which increased metal stabilization in root and lowered root to shoot metal translocation. Vetiver was tolerant of AMD, showing minimal change in biomass and plant growth. Results obtained are encouraging, and a large scale mesocosm study is now in progress, as the next step to develop the vetiver-based system for AMD treatment.

Capsule: Vetiver grass improves water quality parameters in acid mine drainage

Keywords: AMD, Hydroponic, Remediation, Translocation, Uptake, Vetiver

Introduction

Industrial activities, such as mining, can lead to the production of acid mine drainage (AMD), an acidic discharge that stems from the exposure of sulfide minerals to moisture under oxidizing conditions (Das et al. 2009). These minerals undergo hydrolysis and oxidation to produce ferric iron and sulfuric acid which results in an acidic and sulfate-rich discharge that contains various heavy metals and metalloids (Nyamadzawo et al. 2007). AMD develops a characteristic yellow-orange coloration that results from ferric hydroxide precipitation and may prevent photosynthesis by reducing the passage of light into the benthic layer of afflicted water bodies (Das et al. 2009). Furthermore, microbial oxidation from lithotrophic bacteria can lead to increased acidity and reduced oxygen availability (Das et al. 2009). The generation of AMD can be influenced by the surface area of exposed minerals, oxygen saturation, and chemical activity of present metals, along with pH and temperature (Akcil and Koldas 2006).

Earlier estimates from the United States Environmental Protection Agency (USEPA) predicted that 7000 km of eastern US streams and 8000–16,000 km of western US streams were impacted by AMD (USEPA 1994; Jennings et al. 2008). These types of impacted environments often contain erodible soils, damaged waterways, and reduced biodiversity (Akcil and Koldas 2006). Moreover, the largest and most costly of Superfund sites found in the western US are afflicted by AMD, including the Iron Mountain Mine of California, Bunker Hill of Idaho, and the Butte-Clark Fork River complex of Montana (Jennings et al. 2008). These sites could lead to groundwater contamination or bioaccumulation and magnification of metals in plants and their consumers. For instance, AMD discharges into the Clark Fork River following a 1989 thunderstorm led to an increase in acidity and copper (Cu) content over 20 min, resulting in a high loss of fishes from acute Cu toxicity of the gills (Jennings et al. 2008). Furthermore, AMD presents serious implications for its potential to contaminate agricultural lands or in places where well water is the major source of drinking water (Nyamadzawo et al. 2007).

Treatment techniques for AMD often include liming, bioreactors, or wetlands which tend to be high maintenance and non-sustainable from resource requirements, such as lime or organic nutrients to feed microbes (Johnson and Hallberg 2005). Our goal is to develop an alternative, low-cost, and ecologically sustainable floating wetland treatment (FTW) system for AMD and other mine impacted water systems. Floating treatment wetlands comprise floating structures that hold and poise plants over a water body, thereby allowing the plant roots to extend into the water for the uptake of various elements or compounds (Chen et al. 2016; Pavlineri et al. 2017). The upper portion of the plants (stems, leaves, etc.) are then able to grow as normal by means of hydroponics. These FTWs are economical and environmentally friendly and have been used to remove nutrients and organic pollutants from water and improve water quality (Chen et al. 2016). Floating treatment wetland studies have been mostly reported at micro- or mesocosmic scales, with a few field scale studies for the treatment of ponds, lakes, and rivers (Pavlineri et al. 2017). Vetiver grass (*Chrysopogon zizanioides*) is a non-invasive, fast-growing perennial grass that has a large biomass with a massive and complex root system that can grow well in soil as well as water (Truong and Hart 2001). Vetiver can withstand a number of environmental extremes, though the main features for AMD treatment come from its tolerance to acidic conditions and heavy metals (Truong and Hart 2001). Moreover, dense and widespread roots are necessary for adequate performance of FTWs, providing vetiver with an additional advantage (Pavlineri et al. 2017). Given these natural advantages, we wanted to use vetiver to develop a FTW technology for AMD, and test it at the abandoned Tab-Simco coal mining site of the Illinois coal basin. The Tab-Simco site in Southern Illinois is reported among the most contaminated AMD sites of the mid-continental USA (Behum et al. 2011).

There are a number of key components to consider in the development of FTWs for AMD treatment, some of which include design and application of the FTWs, ability and efficiency of plants for the treatment process, and management strategies during and after treatment (Chen et al. 2016; Pavlineri et al. 2017). It has been indicated that FTW can remove metals from AMD through uptake and accumulation, adherence of metals to

the roots, or by rhizospheric transformation of the metals (Das et al. 2009). It was also noted that a number of plants have been reported to significantly increase the pH of AMD (Das et al. 2009). In order to optimize the parameters for our FTW system, a hydroponic experiment was set up under controlled greenhouse conditions with the intention of screening vetiver for the treatment of Tab-Simco AMD. The objectives of the study were (1) to determine the effects of vetiver on water quality (pH, EC, sulfate) of Tab Simco AMD, (2) to estimate the optimal planting density for effective AMD treatment, (3) to determine if vetiver grass would be effective in lowering heavy metals in AMD, and (4) to determine the capability of vetiver to tolerate the extreme low pH and high metal concentrations in AMD.

Materials and methods

Hydroponic AMD phytoremediation experiment

The AMD was collected from an oxidation waterway located at the kill zone of the Tab-Simco site (Behum et al. 2011). Vetiver grasses were grown in potting soil until they achieved usable biomass, were cleaned of soil using tap water, and were then transferred to hydroponic containers with ~ 3.3 L of hydroponic media (0.5X Hoagland's solution) and air circulators for oxygen flow. The plants were allowed to acclimate for 30 days until they were placed in Tab-Simco AMD (control plants were placed in Hoagland's media for comparison) for 30 days at planting densities of four plants (~25 g, 8 g L⁻¹), three plants (~20 g, 6 g L⁻¹), and two plants (~15 g, 4 g L⁻¹) per container with no plant containers for comparison (n = 3 for biological replicates). Water samples (AMD and media) were collected in triplicate at 0, 2, 5, 10, 20, and 30 days while dissolved oxygen (DO) was measured directly with a waterproof DO Pen Meter (Sper Scientific). The water volume of the hydroponic containers was maintained by the addition of deionized water around days 15 and 30. The plants were measured for root and shoot length, along with fresh biomass at 0, 5, 15, and 30 days. On the final day, the plants were harvested for uptake and translocation analysis.

Water chemistry and plant analysis

Water samples were analyzed for pH with an Accumet™ pH Meter (Fisher Scientific™) and EC with an ECTest® MultiRange Conductivity Tester (Oakton). Total alkalinity was determined by the APHA method 2320B for potentiometric titration of low alkalinity in a complex system (APHA 1992). The remaining water samples were filtered using Whatman 40 ashless circles to remove visible solids for downstream analyses. For dissolved SO_4^{2-} analysis, the filtrates were secondarily filtered using 0.25- μm pore size nylon syringe filters (Cole-Parmer®), and SO_4^{2-} concentrations were determined by ion chromatography (IC) with a Dionex™ IC System-900 (Thermo Scientific™). Vetiver roots and shoots were separated, incubated at 100 °C until they were completely dried (24–48 h), and measured for total dry biomass. Tissue subsamples (0.25 g) were then digested following EPA method 3052B using an ETHOS 900 microwave and filtered through Whatman 1 circles for metal analysis. Water filtrates and plant digests were filtered using 0.45- μm pore size syringe filters to determine dissolved metal content (Fe, Al, and suspected RCRA metals) by inductively coupled plasma-mass spectrometry (ICP-MS, Thermo Scientific™ X series). Analysis of variance (ANOVA) and Tukey's honest significant difference (HSD) comparisons were carried out using JMP® Pro 11 software in order to detect statistical variations across planting density and its comparison to no treatment (four-, three-, and two-plant densities in comparison with no plants).

Metal content in AMD and plant tissue, plant metal uptake, and translocation were calculated as

1. *tissue metal content* = *metal concentration in tissue* ($\mu\text{g g}^{-1}$) $\times$ *tissue mass* (g)
2. *inflow metal content* = *metal concentration in water* ($\mu\text{g L}^{-1}$) $\times$ *water volume* (L)
3. *percent uptake* = $\frac{\text{shoot metal content } (\mu\text{g}) + \text{root metal content } (\mu\text{g})}{\text{inflow metal content } (\mu\text{g})} \times 100\%$
4. *percent translocation* = $\frac{\text{tissue content } (\mu\text{g})}{\text{plant content } (\mu\text{g})} \times 100\%$

$$5. \quad TF = \frac{\text{shoot concentration } (\mu\text{g g}^{-1})}{\text{root concentration } (\mu\text{g g}^{-1})}$$

Results and discussion

Effect of plants on water chemistry

As expected, the AMD had very low pH (2.64) and elevated amounts of SO_4^{2-} with high EC content (Table 3). The metals of concern included Fe, Al, Zn, Ni, Cu, and Pb. A single factor ANOVA was performed for each chemical property on each day of the study period to detect statistical significance across planting densities. Multiple comparisons through Tukey's HSD test were used when the F distribution showed a probability (p) value less than 0.05. No additional statistical analyses were performed if the F distribution p value was greater than 0.05. Single-factor ANOVA showed that there were no statistically significant differences in these chemical properties across planting densities with the exception of EC and Al content, which would suggest that the AMD was relatively homogeneous for each planting density at the beginning of the study. There was a steady increase in pH across vetiver-planting densities over the 30-day period (Figure 3) from the initial 2.64 ($n = 21$ for 0 day AMD samples). Significant difference was detected as early as 2 days across densities ($p = 0.0022$ for $p > F$), though this became more evident by days 20 and 30 ($p < 0.0001$ for $p > F$). It is apparent that the four- and three-plant densities had a more significant increase by the final day (4.19 and 4.06, respectively, for $n = 3$) as opposed to the two-plant density (3.76). Tukey's HSD revealed that pH at each density of vetiver was significantly different compared to no plants ($p < 0.0001$). The four- and three-plant densities showed no significant difference in pH, though there was significance when the four and three plants were compared with the two plants ($p = 0.0045$ and $p = 0.0346$, respectively). This shows that higher planting densities allow for more significant changes in the pH of AMD.

Table 3: Characteristics of the Tab-Simco AMD ($n = 21$ samples) and hydroponic media prior to plant treatment. Content is represented by arithmetic mean, median, maximum, and minimum measurements, as well as SD for pH, EC, dissolved SO_4^{2-} , and total dissolved metals.

AMD characteristics									
Stats	pH	EC (μS)	$\text{mg SO}_4^{2-} \text{ L}^{-1}$	Metals ($\mu\text{g L}^{-1}$)					
				Fe	Al	Zn	Ni	Cu	Pb
Mean	2.64	3370	2610	12000	11300	385	388	21.8	10.5
Med	2.62	3410	2640	11900	11200	376	388	21.3	9.09
Max	2.99	3540	2730	17100	19600	496	500	33.5	21.5
Min	2.54	3000	2180	6620	8020	350	368	13.7	5.82
SD	0.103	123	114	2240	2560	32.5	17.4	6.02	3.89
Media characteristics									
Stats	pH	EC (μS)	$\text{mg SO}_4^{2-} \text{ L}^{-1}$	Metals ($\mu\text{g L}^{-1}$)					
				Fe	Al	Zn	Ni	Cu	Pb
Mean	5.06	851	87.0	332	40.8	21.6	3.43	19.4	0.900
Med	5.05	836	85.5	338	48.0	20.7	3.15	19.2	0.500
Max	5.26	961	119	405	67.8	38.7	6.63	25.3	3.16
Min	4.98	764	67.6	239	1.60	13.3	2.57	14.4	0.214
SD	0.0629	61.4	15.0	45.2	20.7	6.00	0.927	3.51	0.928

A number of sources have indicated that acidity is among the most important of factors to consider for the treatment of AMD due to the direct and indirect effects that it has on dissolved metals and conductivity (Akcil and Koldas 2006; Johnson and Hallberg 2005; Miguel et al. 2013). Lower pH values (2.0–4.0) are known to influence Fe oxidation, particularly through accelerated microbial activity. It has been noted that the rate of oxidation is independent of pH when the values are below 4.0, which would be nearing the optimal range for acidophilic microbes (Akcil and Koldas 2006; Johnson and Hallberg 2005). The pH readings of the Tab-Simco AMD in this study were above 4.0 following higher density vetiver treatments, demonstrating the positive impact of vetiver on water quality. Previous reports have shown that plants can help increase pH of AMD effluents. For example, two AMD contaminated Andean wetlands of Peru were utilized in a study to determine metal content and bioaccumulation factors of *Calamagrostis ligulata* and *Juncus imbricatus* (Miguel et al. 2013). Huancapetí and Mesapata wetlands were monitored over dry and rainy seasons (August 2008 and March 2009, respectively). Measurements from the Huancapetí wetland showed variations from acidic inflows to outflows nearing neutral, having pH changes from 3.65 to 5.78 in the dry season and 3.58 to 5.35 in the rainy season (Miguel et al. 2013). Meanwhile, the Mesapata wetland

showed changes from acidic inflows to neutral outflows, from 4.80 to 6.70 in the dry season and 3.99 to 6.67 in the rainy season (Miguel et al. 2013).

Although plants have been reported to help increase pH in AMD, the underlying mechanism is still not fully understood. Various possibilities for pH increase, including the release of organic acids, (Niu et al. 2007; Javed et al. 2013), inorganic ions, or CO₂ generation through respiration (Javed et al. 2013) have been proposed. In a study using nutrient media and soils, *Eriophorum angustifolium* was exposed to mixtures of As, Cu,

Pb and Zn, and Cd. The root mucilage of these plants showed higher pH values of 7.86 ($n = 3$) in comparison with non-treated roots at 7.11 (Javed et al. 2013). Secretions of organic acids with higher amounts of formic acid, oxalic acid, and succinic acid along with cationic nutrients by plants or rhizospheric microorganisms were believed to have led to the higher pH (Javed et al. 2013). These increases in pH have been thought to reduce metal availability by inducing precipitation, which would provide the plants with a mechanism for tolerance.

There was little change in SO_4^{2-} content among plant densities at 10 days (Figure 4), though Tukey's HSD revealed differences among the four-, three-, and two-plant densities in comparison with no plants ($p = 0.0055$, 0.0008 , and 0.0031 , respectively). At this time, the treatments with three plants were showing the most change at $2560 \text{ mg SO}_4^{2-} \text{ L}^{-1}$ ($n = 3$) in comparison with no plants at $2770 \text{ mg SO}_4^{2-} \text{ L}^{-1}$. Interestingly, the SO_4^{2-} content of the no plant containers was increasing from the initial $2610 \text{ mg SO}_4^{2-} \text{ L}^{-1}$, which was likely due to water evaporation. There were significant decreases in SO_4^{2-} across densities by 20 days. However, after 20 days, the SO_4^{2-} level in no plant containers also declined, which is likely due to water loss from the air circulators, along with some precipitation on the inner surfaces of the hydroponic containers, which was

observed in the no plant containers. White, flakey powder sometimes accumulated at the base of the plant shoots that may have come from volatilization of sulfates. There may also have been some transitions of sulfate into alternative states, perhaps in the form of sulfur oxides or sulfide minerals. The greatest change in SO_4^{2-} was observed at 20 days between four plants and no plants at $1670 \text{ mg SO}_4^{2-} \text{ L}^{-1}$ and $2020 \text{ mg SO}_4^{2-} \text{ L}^{-1}$, respectively ($p < 0.0001$). Overall, the data indicate that there was SO_4^{2-} removal by 20 days in the presence of the plants, more so at the highest plant density investigated here.

It is common for plants to show an increased sulfur usage and amino acid synthesis (cysteine in particular) when under metal stress (Andra et al. 2009; Villiers et al. 2011). Metals are complexed in plants with small peptides such as phytochelatins or metallothioneins (Andra et al. 2009). The thiol groups of cysteine found on these chelators are utilized to bind with metals, which can then be transported into the vacuole to reduce intracellular damage. In order for plants to synthesize these thiol-containing chelators, it is important for them to have an increased SO_4^{2-} uptake. In a hydroponic study, maize (*Zea mays*) was investigated for the effects of cadmium (Cd) stress on SO_4^{2-} uptake (Nocito et al. 2006). Upregulation of a SO_4^{2-} transporter (ZmST1;1-encoded) was reported. These transporters were found to be expressed in root epidermal tissues and around the vascular bundles (Nocito et al. 2006). The reduction of sulfate content during our remediation experiment is promising, though the potential transformation of sulfate to other species needs to be further investigated.

In addition to a decrease in SO_4^{2-} content, vetiver also reduced electrical conductivity of the AMD, as well as the Fe content. The EC readings showed some decrease in the presence of vetiver at day 10, with an even greater decrease at days 20 and 30 (Figure 3). At 2 days, there were significant changes in EC between each plant density with the exception of four and three plants, which were relatively similar (2980 and $2983 \text{ }\mu\text{S}$, respectively). The four- and three-plant densities both showed similar high differences to no plants ($p < 0.0001$). Less variation was seen at 5 and 10 days, though significant changes were again seen at 20 and 30 days. The most significant changes were observed between the four-plant density and no plants at both 20 days ($p < 0.0001$) and

30 days ($p = 0.0002$). There was no significant difference between three and two plants at 20 days (2280 and 2350 μS , respectively), though each showed difference with four plants and no plants (1880 and 2760 μS , respectively). With the exception of four plants and no plants, the most significant changes at 30 days were between two plants and no plants ($p = 0.0018$). There were no significant differences in EC between four plants and two plants by the end of the study. In addition, there were no significant changes in metal contents across plant densities with the exception of Fe, though each density showed significant decreases by comparison with the 666 $\mu\text{g Fe L}^{-1}$ in the no plant containers ($p < 0.0001$). This shows that there was removal of Fe in the presence of the plants with little dependence on plant density. Therefore, decreases in conductivity were likely a direct result of SO_4^{2-} and Fe removal.

These findings clearly show that vetiver grass improves the quality of Tab-Simco AMD, particularly through the apparent reduction in SO_4^{2-} and Fe removal as evident from their reduced concentrations and decreased conductivity, along with pH increase. Moreover, it would seem that the higher plant densities (four and three plants) have a greater effect on certain chemical properties, particularly with pH, EC, and SO_4^{2-} . It was expected that a higher number (i.e., increased biomass) would generate more rapid changes in water chemistry and therefore reduce stress on the group as a whole. Dissolved oxygen (DO) measurements (not shown) did not present any significant changes in containers with and without plants because of air circulation.

Vetiver growth, metal uptake, and translocation

Vetiver grass showed minimal signs of toxicity from the Tab-Simco AMD by the end of the 30-day study period (Figure 7). Browning at the outer layer of the basal shoot tissues along with some curling and discoloration of the shoots was observed, but both treated and control plants showed signs of browning. Also, some curling and discoloration of the shoots was observed in less than a third of the treated vetiver 20 days into the study. There were signs of Fe plaque buildup on the root tissues early on. Interestingly, fresh roots developed over the 30-day study period in vetiver plants growing in AMD as well

as in media. There was little change in biomass as shown in the fold change of fresh biomass over the study period (Figure 5). Plants grown in AMD had slightly lower fold change for four and two plants (0.96 and 1.0, respectively) when compared to those grown in media at four and two plants (1.0 and 1.1, respectively). Vetiver grown in AMD at three plants showed slightly higher fold change (1.0) when compared to those grown in media at three plants (0.97). Overall, vetiver was tolerant of AMD, and there was no significant impact on biomass and general plant growth as compared to those grown in media.

Metal contents in vetiver tissues grown in AMD and hydroponic media were measured (**Error! Reference source not found.**). The percent metal uptake for individual plants suggests that there was moderate removal of Cu, Fe, and Zn, with lesser removal of Pb, Al, and Ni (**Error! Reference source not found.**). Meanwhile, net metal uptake based on plant density was determined and shown as net uptake per hydroponic container (Figure 6). Data indicate that there was large uptake of Cu by vetiver, where the four and three-plant densities showed relatively similar patterns of uptake (135 and 140%, respectively) in comparison to the two-plant density (58.5%). Moreover, the only statistical variation across densities was detected with Cu uptake ($p = 0.0377$ for $p > F$),

where the three and two plants were the only densities to show statistical significance ($p = 0.0494$). Vetiver took up high amounts of Fe and Zn; however, they were considerably less than Cu uptake. Fe removal was highest at the four-plant density which showed 74.6% uptake, followed by the three-plant density with 59.1% uptake, and then the two-plant density at 48.2% uptake. This was expected from our earlier observations that Fe content in the hydroponic containers was significantly decreased in the presence of plants. Zn uptake fairly similar across planting densities at 30.1% uptake ($n = 9$ hydroponic containers). Meanwhile, the uptake of Pb, Al, and Ni was significantly lower across plant densities ($< 10\%$) as with the individual plants, except for the two-plant density (22% Pb uptake).

Table 4: Metal content, TF, and uptake by AMD grown Vetiver grass after 30 days ($n = 27$ plants) is represented using arithmetic mean, median, maximum, and minimum measurements, as well as SD for Fe, Al, Zn, Ni, Cu, and Pb. A) Tissue-specific content is shown for Vetiver shoots (S) and roots (R) per dry plant mass ($\mu\text{g g}^{-1}$), along with B) TF for metals (shoots/roots), and C) percent uptake (%) of metals by Vetiver from the Tab-Simco AMD.

	A) Metal content of AMD grown Vetiver tissues											
	Fe		Al		Zn		Ni		Cu		Pb	
Tissue	S	R	S	R	S	R	S	R	S	R	S	R
Mean	500	21000	430	2300	85	200	14	18	21	30	0.12	4.5
Med	240	20000	210	1500	53	100	5.6	17	15	32	0.094	2.3
Max	4100	43000	220	7000	360	850	190	40	190	58	0.38	17
Min	15.0	8200	14	740	3.0	2.7	0.26	5.7	0.39	12	0.011	0.094
SD	850	9000	540	1700	97	190	35	7.6	34	10	0.087	4.6
	B) TF of AMD grown Vetiver grass											
	Fe		Al		Zn		Ni		Cu		Pb	
Mean	0.025		0.20		1.2		0.73		0.84		0.11	
Med	0.013		0.14		0.49		0.37		0.47		0.039	
Max	0.18		1.1		5.1		9.0		9.0		1.3	
Min	0.00082		0.0085		0.0035		0.014		0.012		0.00097	
SD	0.040		0.21		1.5		1.6		1.6		0.25	
	C) Metal uptake (%) by AMD grown Vetiver grass											
	Fe		Al		Zn		Ni		Fe		Pb	
Mean	20		2.3		10		1.1		37		4.6	
Med	19		1.7		7.7		0.80		29		2.8	
Max	47		7.2		32		8.4		120		17	
Min	4.1		0.66		1.3		0.39		14		0.33	
SD	11		1.7		7.1		1.5		23		4.4

Figure 6: Net metal uptake from AMD following plant treatment with Vetiver and percent translocation of metals between tissues for Fe, Al, Zn, Ni, Cu, and Pb after 30 days. A) Net metal uptake (mean $\pm$ SD, $n = 3$ biological replicates) is shown across planting densities (4, 3, and 2 plants) while B) percent translocation (mean $\pm$ SD, $n = 27$ plants) is shown irrespective of planting density.

Figure 7: Images of a hydroponic container with Vetiver grass after 30 days of treatment at 3 plants per container. A) Vetiver are shown at the end of the treatment process, propped in the hydroponic container. Vetiver is also shown placed on top B) following growth in AMD to reveal iron plaque buildup along the root surface and C) following growth in hydroponic media for a controlled comparison.

Vetiver has displayed similar affinities for Cu, Fe, and Zn in other hydroponic studies. Vetiver removed 87.5% Cu from ink manufacturing wastewaters with initial contents of 118.9 mg Cu L⁻¹ (Roongtanakiat et al. 2007). Vetiver also removed Fe (27.3%) and Zn (52.7%) from milk factory wastewaters with 16.15 mg Fe L⁻¹ and 4.09 mg Zn L⁻¹ (Roongtanakiat et al. 2007). In our study, the removal percentages were less for Fe, but higher for Zn. Disparities in metal removal could be explained by the accumulation of iron plaques on plant roots (Figure 7). Fe plaque formation comes from the oxidation of ferrous iron (Fe²⁺) to ferric iron (Fe³⁺), which then precipitate as metal oxyhydroxides that commonly collect on the root surface of aquatic or wetland plants (Liu et al. 2004, 2006; Yang et al. 2014). These plaques have been characterized as amorphous or crystalline and often appear orange in coloration, which were observed in our study. Formation of Fe plaques is thought to be triggered by radial oxygen loss from plant roots and subsequent oxidation of the rhizosphere (Liu et al. 2004, 2006; Yang et al. 2014). Vetiver showed lower amounts of Pb removal in this study in comparison with previous studies. Chen et al. (2012) reported that in a 7-day hydroponic study, vetiver was able to remove 72.9% of Pb at an initial concentration of 5 mg Pb L⁻¹ with tissue accumulations of 378.8 mg Pb kg⁻¹ (Chen et al. 2012). Andra et al. (2009) demonstrated impressive capabilities for Pb accumulation in vetiver at 400 and 1200 mg Pb L⁻¹, showed. In their 7-day hydroponic study, uptake was lower at 400 mg Pb L⁻¹ with 13,240 mg Pb kg⁻¹ accumulating in the roots and 1700 mg Pb kg⁻¹ in the shoots. At 1200 mg Pb L⁻¹, vetiver tissues had a concentration of 19,800 mg Pb kg⁻¹ in the roots and 3350 mg Pb kg⁻¹ in the shoots (Andra et al. 2009).

Metal translocation between vetiver shoot and root is shown in Figure 6. It was observed that Fe had the lowest amount of translocation as 94.5% was present in the roots. This was expected from the formation of Fe plaques on the roots. There was also relatively low translocation of Pb and Al as much higher amounts of these metals were present in the roots (83.4 and 70.4%, respectively). Meanwhile, higher amounts of Ni, Zn, and Cu were present in the shoots (50.5, 54.7, and 56.7%, respectively). In the study by Roongtanakiat et al. 2007, vetiver showed similar tissue exchange patterns for Fe

(5.45%). However, Zn and Cu exchanges were also much lower at 18.8 and 16.6%, respectively. The translocation factor (TF) for Pb in our study was lower on average, though with a high deviation (0.11 ± 0.25) when compared to reported 0.13 and 0.17 TFs for vetiver treated at 400 and 1200 mg Pb L⁻¹, respectively (Andra et al. 2009). Earlier studies from Australia and China have characterized the translocation tendencies for metals in soil-grown vetiver, including Cu, Ni, Pb, and Zn (Truong 1999; Truong and Hart 2001). Truong (1999) reported that 16–33% of Cu, Pb, and Ni were allocated to the shoots, with higher amounts of Zn at 40%. Our data suggested that vetiver has moderate to high affinity for Cu, Fe, and Zn when grown hydroponically in metal-rich AMD.

Earlier studies (cited above) show low translocation of Fe and Pb across vetiver tissues, though in our study, we found higher standard deviations for each metal with the exception of Fe (**Error! Reference source not found.**). This is most likely due to the formation of Fe plaques, which have been shown to influence the amount of metal uptake by plants (Yang et al. 2014). For instance, P deficiency in rice (*Oryza sativa*) with the added presence of Fe and As has been shown to increase root-induced oxygenations which leads to the formation of Fe plaques over 24 h (Liu et al. 2004). It was reported that P deficiency increased As content in the roots but decreased translocation to the shoots, suggesting that Fe plaques have affinity for As. This was further confirmed in natural soils contaminated with 15 mg As kg⁻¹, where plaque contents were as high as 800 mg As kg⁻¹ (Liu et al. 2006). In a hydroponic study, various plants were tested to determine metal tolerance (e.g., Zn, Fe, and Mn) in relation to plaque formation, and it was found that Fe plaque formation increased Zn accumulation on the root surface, particularly in plants with higher radial oxygen losses (Yang et al. 2014). It was also revealed that metal contents were much higher at the plaque surfaces near the rhizosphere, as opposed to being nearer the root surface. In another study using metal contaminated agricultural soil, a positive correlation was reported between Fe plaque formation and Cd, Pb deposition on root surfaces (Cheng et al. 2014). These studies suggest that Fe plaque formation on plant roots enhances metal sequestration in the roots via stabilization within the rhizosphere and by accumulation at the root surface.

Meanwhile, these accumulations would reduce metal translocation to the plant shoots, thereby decreasing toxicity to plants, as well as potential grazing animals.

Conclusions

This 30-day study demonstrated the potential of vetiver grass in passive treatment of acid mine drainage impacted water. Our primary objective was to determine if vetiver could improve the quality of AMD and to estimate the optimal plant density for follow-up large-scale floating treatment wetland studies. Significant decreases in electrical conductivity, SO_4^{2-} , and Fe content in Tab-Simco AMD following plant treatment demonstrated that vetiver can not only survive in highly acidic water, it is actually capable of treating the water. Vetiver treatment resulted in considerable SO_4^{2-} removal from AMD with a corresponding increase in pH. Vetiver also removed metals, more so at higher plant densities. Vetiver removed significant amounts of Fe, Zn, and Cu from the AMD, but not Al, Ni, and Pb. Metal translocation from root to shoot was very low for Fe and relatively low for Al and Pb, while Cu, Ni, and Zn were translocated more. The accumulation of Fe in the root tissues was due to plaque formation. Metal stabilization in the plaques likely resulted in lower root to shoot translocation for Fe, Al, and Pb. Further studies need to be performed to determine the impact of Fe plaque formation on uptake of various metals. Taking into account the significant decrease in EC and SO_4^{2-} , metal accumulation in the shoots, and formation of Fe plaques along the roots, it is clear that phytoremediation by vetiver is a possible solution to the AMD problem. Vetiver at higher plant densities (three to four plants per 3.3 L, roughly 6 and 8 g L⁻¹) were more efficient, which would suggest that a FTW with vetiver density of 6 g L⁻¹ or higher would be effective for treating Tab-Simco AMD and other mining-impacted water bodies with such extreme chemical attributes. Based on the results of this hydroponic experiment, a long-term, large-scale mesocosm study is currently in progress to help develop a FTW technology for AMD treatment.

Acknowledgements

The Department of Interior, Office of Surface Mining Reclamation and Enforcement Grant No. S12AC20001 is acknowledged for funding this study. We thank Matthew Adler for supplying us with Tab-Simco AMD and Abhishek Roy-Chowdhury for analysis using the ICP-MS, along with Olivia Olsen and Venkataramana R. Pidatala for their assistance in the greenhouse and laboratory.

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Chapter 2. Remediation of Acid Mine Drainage-Impacted Water by Vetiver Grass: A Multiscale Long-Term Study^a

Chapter 2 was prepared for submission to *Ecological Engineering*.

^a The following chapter is a manuscript submitted to *Ecological Engineering*, and is under revision

Remediation of Acid Mine Drainage-Impacted Water by Vetiver Grass: A Multiscale Long-Term Study

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Abstract

Acid mine drainage (AMD) is an acidic discharge from mining sites that contains elevated levels of metals and sulfate (SO_4^{2-}). AMD can contaminate surface and groundwater, inflicting health and environmental dangers through metal toxicity and physical stress. Current methods for AMD treatment, including chemical or passive biological treatments, are often non-sustainable owing to expense, require continuous maintenance, or are unsuitable for prolonged treatment. Our ultimate goal is to develop a cost efficient and sustainable floating treatment wetland system using vetiver grass (*Chrysopogon zizanioides*) for AMD treatment. We performed year-long large- and small-scale hydroponic studies to determine the effectiveness of vetiver for the treatment of AMD-impacted waters. AMD was used either directly, or after filtration using a plug-filter system prepared with recycled material, which retained a majority of the metals. The AMD-impacted water collected from the Tab-Simco mine site in southern Illinois contained elevated sulfate and a variety of metals with an acidic pH ranging from 2 to 3. For the large-scale mesocosmic study, vetiver plants were placed on rafts and suspended in 100-gallon containers. Water quality was monitored every 28 days and at the end of the experiment by collecting water samples for chemical analysis; plant health observations were simultaneously recorded. Metal analysis of plant tissues was done after the final harvest. From the large-scale experiment, there was higher net removal of Fe (81%) and Pb (81%) with lower removal of Ni (38%), Zn (35%), SO_4^{2-} (28%), Mn (27%), Cr (21%), Al (11%) and Cu (8.0%). The most accumulated elements in vetiver were Fe ($17,300 \mu\text{g g}^{-1}$), Al ($3405 \mu\text{g g}^{-1}$), Mn ($1080 \mu\text{g g}^{-1}$), Zn ($113 \mu\text{g g}^{-1}$) and Cr ($62.3 \mu\text{g g}^{-1}$) with lesser Ni ($41.2 \mu\text{g g}^{-1}$), Cu ($24.6 \mu\text{g g}^{-1}$) and Pb ($5.37 \mu\text{g g}^{-1}$). Metals were mainly localized on the root surface as Fe plaques, whereas Mn and Zn showed greater translocation from root to shoot. Furthermore, TCLP of used vetiver tissue showed that biomass was not hazardous waste as a result of metal accumulation. From the small-scale experiment, a near complete removal of SO_4^{2-} (91%) and metals (90-100%) with the exception of Pb (15%) and Cu (0.0%) was recorded. These experiments demonstrate that vetiver can effectively remediate AMD-impacted waters over an extended period of time.

Keywords: Acid mine drainage, phytoremediation, metal plaques, metal removal, translocation, vetiver grass

1. Introduction

Acid mine drainage (AMD) is a metal-rich, acidic discharge often generated when sulfide minerals come in contact with atmospheric oxygen and moisture (Akcil and Koldas, 2006; RoyChowdhury et al., 2015). Under oxidative and hydrolytic conditions, minerals often release ferrous iron (Fe^{2+}), sulfate (SO_4^{2-}) and hydrogen ions (H^+), thereby decreasing the pH (Simate and Ndlovu, 2014). Lower pH leads to increased mobility of metals and highly erodible soils (RoyChowdhury et al., 2015). Soluble metals present obvious health and environmental risks from toxicity, which can spread to adjacent sources of water such as streams, rivers or groundwater (Das et al., 2009). Continued oxidation of minerals under acidic conditions leads to the production of ferric iron (Fe^{3+}), which forms orange-yellow precipitates, commonly in the form of metal hydroxides (Hogsden and Harding, 2012). Metal hydroxides can result in photosynthetic inhibition by reducing light passage into the benthic layer of contaminated waters (Das et al., 2009; Hogsden and Harding, 2012), and decreased biodiversity by covering surfaces and overwhelming organisms through physical stress (Hogsden and Harding, 2012). In addition, bioaccumulation of metals by algae presents severe risks to consumers by means of biomagnification (Oberholster et al., 2010).

It was originally estimated by the United States Environmental Protection Agency (USEPA) that AMD is being generated actively by 20,000–50,000 mines in the western US, with impacts on 8000–16,000 km of streams (USEPA, 1994). AMD is assumed to originate mainly from abandoned mining sites of relatively small scale and in remote regions; however, there are a number of larger sites that have contributed extensively (Jennings et al., 2008). For instance, AMD from the Tar Creek Superfund site in Oklahoma resulted from post-abandonment flooding and oxidation of underground support pillars containing sulfide minerals (Gutiérrez et al., 2016). In addition to the west, AMD has impacted more than 7000 km of eastern US streams (USEPA, 1994). The abandoned Tab-Simco mining site is a former coal mine located in Southern Illinois. The Tab-Simco site spans about 12 hectares and is one of the most highly contaminated sites with AMD in the mid-continental US (Behum et al., 2011; Lefticariu et al., 2017). AMD

from the Tab-Simco site typically has a pH of 2 to 3 and contains elevated amounts of SO_4^{2-} and iron (Fe), along with various other metals (Kiiskila et al., 2017).

Constructed floating wetlands, or floating treatment wetlands (FTWs), present a cost effective and environmentally friendly method for improving quality of contaminated waters such as AMD (Marchand et al., 2010; Chen et al., 2016a; Pavlineri et al., 2017). FTWs consist of a floating structure that supports plants that grow hydroponically. The roots are allowed to spread below the water surface while the shoots grow above the water (Pavlineri et al., 2017). Vetiver grass, *Chrysopogon zizanioides*, is a noninvasive perennial grass that is fast-growing with a large biomass and extensive root system (Truong et al., 2010). Even though it is not an aquatic plant, vetiver is able to grow sustainably under hydroponic settings (Roongtanakiat et al., 2007). Furthermore, vetiver is tolerant of acidic conditions (RoyChowdhury et al., 2015; Kiiskila et al., 2017) and can withstand as well as accumulate a variety of metals (Andra et al., 2009; Kiiskila et al., 2017).

There are a number of mechanisms by which plants can remediate metal impacted waters; with AMD this is primarily achieved through direct uptake (phytoextraction) and through the formation of precipitates by rhizofiltration (Das et al., 2009; Marchand et al., 2010; Pavlineri et al., 2017). Precipitates mostly collect on the plant roots as metal plaques and are usually composed of metal sulfides, oxides, hydroxides, and/or oxyhydroxides (Yang et al., 2014; Li et al., 2017). Moreover, plants such as vetiver have self-regenerating and dense roots that provide additional surface area for plaque formation and metal sequestration (Richter et al., 2016; Kiiskila et al., 2017).

A short-term (30-day), bench-scale hydroponic experiment showed that vetiver developed mild symptoms of toxicity to Tab-Simco AMD, but effectively improved water quality by reducing SO_4^{2-} and metal content while increasing pH (Kiiskila et al., 2017). Greater improvements in water quality were observed at higher planting densities of 6 and 8 g L⁻¹ in comparison to 4 g L⁻¹ (Kiiskila et al., 2017). Based on the initial short-term results, a follow-up feasibility study was conducted to evaluate vetiver over a longer

period of time. In this paper, we report two long-term hydroponic experiments; a scaled up study to represent a controlled mesocosm for remediation, and a small-scale study to ensure complete root coverage of the AMD with statistical replication. Our objectives were to (1) assess metal uptake and distribution in vetiver tissues following prolonged exposure to AMD, and (2) examine disposal criteria for the used vetiver biomass by testing if the biomass presents any environmental/ecological risk following AMD treatment.

In a separate study, a sorbent filter was prepared using water treatment residuals (WTRs) to remove metals from the AMD at the Tab-Simco site (RoyChowdhury et al., 2018). WTRs are sediment waste byproducts derived from colloid removal during drinking water treatment (Castaldi et al., 2015), and have high removal capacities for metals (Soleimanifar et al., 2016). While the objective of this paper is to evaluate a vetiver FTW, AMD filtered using the WTR filter was included as a variable, to check for the potential of using vetiver alone or a combination of vetiver and the sorbent filter for the optimum AMD cleanup.

2. Materials and methods

2.1. AMD collection and filtration

AMD was collected at the Tab-Simco site (GPS coordinates = N37°41.200' and W089°10.085') from the sulfate-reducing bioreactor (SRB) installed earlier at the site. This SRB later failed and contained AMD with very low pH and high metal concentration (Behum et al., 2011; RoyChowdhury et al., 2015; Lefticariu et al., 2017). Prior to the phytoremediation experiments described here, ~350 liters of AMD was collected and filtered through a gravity operated plug filter. The filter media was prepared from a mixture of WTRs and sand described by RoyChowdhury et al., 2018 as filter media 1. The experiment included (1) unfiltered Tab-Simco AMD without vetiver or unfiltered-untreated (UU) water, (2) sorbent filtered AMD without vetiver or filtered-untreated (FU) water, (3) unfiltered Tab-Simco AMD with vetiver treatment (UT) and (4)

sorbent-filtered AMD with vetiver treatment (FT). The phytoremediation experiments were conducted and monitored as follows.

2.2. Large-scale phytoremediation experiment

Hydroponic containers measuring 48 inches L x 22 inches W x 25 inches H (capacity 100 gallons or 378.5 liters) were filled to 14 to 15 inches with 170 liters of either UU or FU waters. Vetiver plants were acclimated and maintained in hydroponic media (0.5X Hoagland's solution) for up to four months prior to the experiment, and were then transferred to rafts assembled to support the plants. The rafts were constructed using plywood surfaces measuring 38 inches by 16 inches (L x W), which were attached to rounded rectangular pontoons created from 2 inch PVC piping (Figure 8A). The plywood surfaces had 28 holes drilled and fitted with 2 inch Hydrofarm cups containing rock wool to support the plants; holes were aligned in rows of 3's and 2's, which ran the length of the rafts (Figure 8B). The plants were initially deployed at a planting density of 28 plants at 6 g L^{-1} (983 grams) for both UT and FT waters ($n = 1$ treatment with one raft per water type) for the first 168 days (Figure 8C). Plants in the UT water were replaced after 168 days with fresh plants at a higher planting density of 28 plants at 16 g L^{-1} (2,640 grams) and the treatment was extended until 364 days. Hydroponic containers without plants (UU and FU waters) were used for controlled comparisons ($n = 1$ comparison per water type). Black plastic sheets were used to prevent light passage into the water for each of the hydroponic containers. Every 28 days, dissolved oxygen (DO) measurements were performed using a waterproof DO Pen Meter (Sper Scientific), water loss from plant uptake and evaporation was monitored and replenished using deionized water, and the water contents were thoroughly mixed and sampled in technical replicates ($n = 3$) from each water source for chemical analysis. At the end of the experiment, the plants were collected, measured for fresh biomass, and then stored for further analysis.

2.3. Small-scale phytoremediation experiment

Smaller hydroponic containers with a volume of 4 liters were used for small-scale experiment. Vetiver grass was acclimated and maintained in hydroponic media as described in section 2.2, and then transferred into 3.5 liters of UT water at a planting density of 4 plants at $73 \pm 2 \text{ g L}^{-1}$ (254 ± 7 grams) with $n = 3$ containers for experimental replicates. Hydroponic containers without plants (UU water) were used as a controlled comparison ($n = 3$ containers). Water loss was supplemented using deionized water and samples were collected as described in section 2.2.

Figure 8: Greenhouse mesocosmic study of AMD remediation by floating vetiver rafts. The raft structure including the PVC frame and plywood surface separated (A) and attached (B). A raft with vetiver floating in a hydroponic container filled with Tab-Simco AMD (C).

2.4. Water sample characterization, metal removal, and toxicity screening of plant matter

Water samples were measured for conductivity and pH, and then filtered before SO_4^{2-} and metal analysis as described in Kiiskila et al., 2017. SO_4^{2-} was measured by ion chromatography using a Dionex™ CD25A Conductivity Detector (Thermo Scientific™). Metals were determined by inductively coupled plasma-optical emission spectrometry (ICP-OES) using an Agilent 5100 ICP-OES System. Plants were separated into roots and shoots, shredded and incubated at 80°C until dried (72-96 hours), and measured for dry biomass. Subsamples (0.5 grams) were digested following EPA method 3050B for ICP-OES analysis. Remaining tissues were mixed into root and shoot batches (n = 3 batch replicates) and subjected to toxicity characteristic leaching procedure (TCLP) following EPA method 1311. TCLP extracts were digested following EPA method 3005A for ICP-OES analysis. Samples were screened for Fe, Aluminum (Al), manganese (Mn), zinc (Zn), nickel (Ni), copper (Cu), and Resource Conservation and Recovery Act (RCRA) metals. Metal contents in water and vetiver tissues were used to calculate the following:

1. *tissue metal content* (μg) =
 $\text{metal concentration of tissue } (\mu\text{g g}^{-1}) \times \text{tissue mass (g)}$
2. *plant metal content* (μg) =
 $\text{tissue metal content (shoots)} + \text{tissue metal content (roots)}$
3. *water metal content* (μg) =
 $\text{metal concentration in water } (\mu\text{g L}^{-1}) \times \text{water volume (L)}$
4. *total metal removal* = $\frac{\text{initial metal content } (\mu\text{g}) - \text{final metal content } (\mu\text{g})}{\text{initial metal content } (\mu\text{g})} \times 100\%$
5. *metal removal by vetiver* (%) =
 $\text{total metal removal from UT or FT waters}(\%) - \text{total metal removal from UU or FU waters}(\%)$
6. *estimated metal uptake* (%) =
 $\frac{\text{shoot metal content } (\mu\text{g}) + \text{root metal content}(\mu\text{g})}{\text{water metal content } (\mu\text{g})} \times 100\%$
7. *metal distribution* (%) = $\frac{\text{tissue metal content } (\mu\text{g})}{\text{plant metal content } (\mu\text{g})}$

$$8. \text{ Translocation Factor (TF) } = \frac{\text{shoot metal concentration } (\mu\text{g g}^{-1})}{\text{root metal concentration } (\mu\text{g g}^{-1})}$$

2.5. Metal translocation and distribution analysis

Translocation and distribution of metals throughout vetiver roots and shoots was determined from ICP-OES and scanning electron microscopy-energy dispersive X-ray (SEM-EDX) analyses. Fresh root and shoot samples were collected from AMD grown vetiver at the end of the large-scale experiment. The samples were freeze fractured with liquid nitrogen and dehydrated for 24 hours, after which they were mounted on carbon tape that was attached to Al ends. Samples were viewed in a JEOL 5400 LV SEM with an attached KEVEX electron detector. They were viewed at 20 kV in low vacuum mode and the metals were detected with a Sigma EDX spectrometer.

2.6. Statistical analysis

Statistical analyses were conducted using JMP[®] Pro 13 software. Data was evaluated for normal distribution using the Shapiro-Wilk W goodness-of-fit test ($p < 0.05$), where non-normal data was standardized by Johnson S_U Normalization. Two-factor analysis of variance (ANOVA) was used to detect overall statistical significance ($p < 0.05$), followed by mean comparisons among water types at each sampling date. From the large-scale experiment, conductivity and SO₄²⁻ were evaluated among water types (UU, UT, FU and FT) by Tukey's Honestly Significant Difference test ($p < 0.05$), whereas pH and metals from the UU and UT waters were evaluated by Students two-sample t-test ($p < 0.05$). From the small-scale experiment, all characteristics were evaluated between water types (UU and UT) by Students two-sample t-test.

3. Results and Discussion

3.1. Large-scale phytoremediation experiment

The UU water had low pH and high conductivity, SO_4^{2-} and soluble metals (Table 5), along with an abundance of orange-yellow precipitates. Following filtration with the plug filter, the FU water was completely neutralized with extremely diminished metal content but the conductivity and SO_4^{2-} levels remained high (Table 5).

Table 5: Physicochemical characteristics of AMD-impacted water from Tab-Simco mines used during the large-scale experiment. Characteristics of the unfiltered-untreated (UU) and filtered-untreated (FU) waters before plant treatment (n = 6 samples), along with the unfiltered-treated (UT) and filtered-treated (FT) waters following plant treatment (n = 3 samples), including means $\pm$ standard deviations for pH, conductivity (EC) in mS cm^{-1} , sulfate (SO_4^{2-}) in mg L^{-1} , and soluble metals.

	pH	EC	SO_4^{2-}	Fe	Al	Metals ($\mu\text{g L}^{-1}$)					
						Mn	Zn	Ni	Cu	Pb	Cr
UU	2.5 ± 0.024	3.8 ± 0.021	500 ± 19	81000 ± 1200	70000 ± 1100	22000 ± 270	1400 ± 44	700 ± 41	184 ± 44	80 ± 34	10 ± 0
UT	3.5 ± 0.012	1.8 ± 0.0045	220 ± 9.6	510 ± 49	59000 ± 3400	15000 ± 870	840 ± 53	420 ± 21	94 ± 0.51	13 ± 15	37 ± 5.8
FU	7.5 ± 0.085	3.0 ± 0.035	460 ± 15	65 ± 12	77 ± 33	4500 ± 590	110 ± 0	200 ± 17	92 ± 1.3	100 ± 68	8.3 ± 4.1
FT	7.3 ± 0.049	1.5 ± 0.016	160 ± 8.9	60 ± 17	63 ± 23	3.3 ± 5.8	93 ± 12	0 ± 0	86 ± 0.15	27 ± 12	0 ± 0

Figure 9: Changes in dissolved oxygen (DO) from the large-scale experiment, presented as singular probe measurements (n = 1) for the unfiltered-untreated (UU), unfiltered-treated (UT), filtered-untreated (FU), and filtered-treated (FT) waters.

3.1.1. Effectiveness of the vetiver rafts and plant health

The hydroponic containers from the large-scale experiment had a 1,056 in² (0.68 m²) surface area (Figure 8C) or a mesocosm size between 0.51 and 5 m² (Marchand et al., 2010), where the rafts (608 in²) provided 58% coverage (Figure 8B). DO fluctuated between 6.8-8.3 mg O₂ L⁻¹ in UU water and 7.4-8.4 mg O₂ L⁻¹ in FU water (Figure 9). DO decreased from 7.9 mg O₂ L⁻¹ in the UU water to 6.9 mg O₂ L⁻¹ in the UT water by 28 days, but were similar from 56 to 168 days. Vetiver shoots were severely dried and plant biomass decreased significantly (-47%) by 168 days. The dried vetiver from UT water were exchanged with fresh plants (plant turnover), resulting in a decrease in DO from 7.6 to 5.0 mg O₂ L⁻¹ by 196 days. DO remained steady until 224 days and steadily dropped to 1.4 mg O₂ L⁻¹ by the end. Turnover plants appeared healthy with dark green shoots, minimal browning, and prominent plaque buildup along the root surface (Figure 10A). DO demonstrated correlation with apparent plant health.

DO in the FT water dropped from 8.1 to 4.8 mg O₂ L⁻¹ by 28 days, remained steady until 112 days and gradually decreased to 1.9 mg O₂ L⁻¹ at 224 days. DO suddenly increased to 5.2 mg O₂ L⁻¹ by 252 days, decreased to 2.9 mg O₂ L⁻¹ by 308 days, and remained steady until the end (3.4 mg O₂ L⁻¹). FT plants had normal green shoots, which became pale green between 224 and 252 days, although was quality continued to improve. FT plants had black patches around the roots and prominent fresh root growth by 280 days. This would indicate that vetiver had entered into a decay phase from nutrient deprivation; however, the FT plants gained more collective biomass (63%) than the turnover UT plants (46%).

Figure 10: Images of vetiver grass from the large-scale experiment or hydroponic media. A) vetiver grass with Fe plaque buildup, B) SEM images of root surface and C) root cross section with Fe plaques, D) energy dispersive X-ray spectrum of root surface Fe plaques, and E) SEM image of root cross section without Fe plaques.

3.1.2. Effects of vetiver rafts on pH, conductivity, and SO_4^{2-} removal

Since pH and metals were no longer an issue with the filtered waters (FU and FT), only conductivity and SO_4^{2-} were evaluated using all four water types simultaneously (UU, UT, FU and FT). These experiments present a repeated measure design but do not meet the assumptions; therefore, two-factor ANOVAs were used to detect statistical variance, which was significant for pH, conductivity and SO_4^{2-} ($p < 0.001$), showing that plant treatment was time dependent and resulted in significant changes for all effects tests.

Throughout the large-scale experiment, pH of the UU water fluctuated from 2.34 to 2.48. The pH of UT water increased gradually to 2.54 at 168 days (Figure 11A) in comparison to 2.41 in the UU water ($p < 0.001$). Following the turnover, pH of the UT increased linearly from 2.84 at 196 days to 3.59 at 308 days ($p < 0.001$ at each time point). The pH of FU water increased from 7.58 to 8.33 by 28 days and oscillated between 8.05 and 8.14 thereafter (not shown). Meanwhile, the FT water increased from 7.45 to 7.95 by 28 days and gradually decreased, ending around 7.27.

Plant treatments (UT and FT) resulted with linear decreases in conductivity throughout the experiment (Figure 11B). For instance, decreases occurred between the UU (3.72 mS cm^{-1}) and UT (3.59 mS cm^{-1}) waters at 28 days ($p < 0.001$), which continually decreased by 56 (3.51 mS cm^{-1}) and 84 days (3.42 mS cm^{-1}) with treatment ($p < 0.001$ for all comparisons). The UT and FU waters held similar conductivity at 112 days and again from 196 to 252 days, showing no significant difference. However, UT water (2.30 mS cm^{-1}) decreased below FU water (2.84 mS cm^{-1}) at 280 days ($p < 0.001$), remaining significantly different. Conductivity was significantly reduced in UT (1.77 mS cm^{-1}) and FT (1.50 mS cm^{-1}) waters by the end, regardless of water type (Table 5).

SO_4^{2-} was initially similar among water types, showing that filtration had minimal effect. UT and FT waters showed linear declines in SO_4^{2-} while UU and FU waters showed minor variable decline (Figure 11C). For instance, $481 \text{ mg SO}_4^{2-} \text{ L}^{-1}$ in UU water at 84 days decreased to $417 \text{ mg SO}_4^{2-} \text{ L}^{-1}$ in UT water ($p = 0.013$). With the exception of

UT and FU waters, there was variation among water types at the turnover ($p < 0.001$). There was no significant difference between untreated waters (UU and FU) from 252 to 364 days, nor was there any difference between treated waters (UT and FT). However, all comparisons between untreated and treated waters had $p \leq 0.026$ at 252 days, and $p < 0.001$ at 364 days, demonstrating that plant treatments were significant regardless of water type. SO_4^{2-} removal was estimated at 54% from UT water and 27% from UU water, with 28% net removal by vetiver. Meanwhile, SO_4^{2-} removal was 65% from FT water and 20% from FU water, with 45% net removal by vetiver.

3.1.3. Metal removal by vetiver rafts and toxicity characterization

During the large-scale experiment, plant treatment demonstrated significant decreases in Fe, Al, Mn, Zn and Ni ($p \leq 0.029$), but little to no change in Cu, Pb and Cr. There were minimal changes until the plant turnover (168 days), though $79,800 \mu\text{g Fe L}^{-1}$ in UU water decreased to $72,300 \mu\text{g Fe L}^{-1}$ in UT water ($p = 0.029$; Figure 11D). Fe plummeted to $3,730 \mu\text{g Fe L}^{-1}$ by 252 days ($p < 0.001$) and $507 \mu\text{g Fe L}^{-1}$ by the end ($p < 0.001$). There was 99% Fe removal from UT water and 18% from UU water, with 81% net removal by vetiver (Figure 12A). Uptake estimates for Fe (31%) and Pb (23%) could indicate state-of-matter changes within the water (**Error! Reference source not found.B**). There was 81% Pb removal from UT water but none from UU water. Meanwhile, there was $\leq 51\%$ removal of remaining metals (Figure 12A).

Mn, Zn and Ni decreased linearly after the plant turnover but not as significantly as Fe. For instance, $21,300 \mu\text{g Mn L}^{-1}$ in UU water decreased to $19,300 \mu\text{g Mn L}^{-1}$ in UT water by 252 days ($p = 0.0013$), and continued to $15,000 \mu\text{g Mn L}^{-1}$ by the end ($p < 0.001$). There was 31% Mn removal from UT water and 4% from UU water, with 27% net removal by vetiver (Figure 12A). Similarly, there was 35% net Zn removal and 38% net Ni removal. Estimates suggest that there was greater uptake of Zn (30%) and Mn (18%) by vetiver, while Ni (11%) was excluded (**Error! Reference source not found.B**). Net removal of Cr (21%), Al (11%) and Cu (8.0%) were minor, though uptake estimates for Cr ($>100\%$) suggest that the majority was precipitated.

Figure 11: Effect of vetiver on AMD-impacted waters throughout the large-scale experiment, including one treatment (or control) per water type using singular rafts containing 28 plants with no experimental replicates. Numerical values are presented as means for $n = 3$ technical sampling replicates from each water type. A) Changes in pH between unfiltered-untreated (UU) and unfiltered-treated (UT) water. B) Changes in conductivity among UU, UT, filtered-untreated (FU) and filtered-treated (FT) waters. C) Changes in sulfate among UU, UT, FU and FT waters. D) Changes in soluble iron between UU and UT waters.

Figure 12: Metal removal from AMD-impacted waters and tissue distribution after the large-scale experiment. A) Removal efficiency from the unfiltered-treated waters is partitioned into removal by vetiver and removal by external factors, and B) distribution is partitioned into roots and shoots as means $\pm$ standard deviations for $n = 28$ plants.

Table 6: Metal characterization of vetiver from the large-scale experiment, including means $\pm$ standard deviation for vetiver from unfiltered-treated (UT, n = 28 plants) and filtered-treated (FT, n = 3 plants) waters. A) Tissue content for vetiver shoots (S) and roots (R) per dry plant mass, B) estimated metal uptake by vetiver, C) TFs, and D) TCLP characterization of vetiver S and R per liter of extract (n = 3 batch replicates). BDL = below detection limit; NR = not relevant.

A) Metal content ($\mu\text{g g}^{-1}$) of UT and FT grown vetiver tissues								
Metals	Fe		Al		Mn		Zn	
Tissue	S	R	S	R	S	R	S	R
UT	388 ± 234	16900 ± 5040	487 ± 221	2920 ± 825	499 ± 72.5	581 ± 104	50.8 ± 13.1	62.7 ± 24.3
FT	39.9 ± 14.2	406 ± 128	101 ± 45.0	1020 ± 347	103 ± 17.1	1360 ± 121	35.9 ± 2.17	42.4 ± 1.23
Metals	Ni		Cu		Pb		Cr	
Tissue	S	R	S	R	S	R	S	R
UT	4.16 ± 1.22	37.0 ± 6.56	8.10 ± 3.22	16.5 ± 7.36	1.63 ± 1.86	3.73 ± 2.39	2.91 ± 0.994	59.3 ± 22.7
FT	1.66 ± 0.468	57.6 ± 8.61	4.65 ± 0.928	11.3 ± 2.49	10.6 ± 1.70	10.9 ± 2.14	0.331 ± 0.469	10.9 ± 2.93
B) Estimated uptake (%) by UT and FT grown vetiver								
Metals	Fe	Al	Mn	Zn	Ni	Cu	Pb	Cr
UT	31	10	18	30	11	38	23	≥ 100
FT	≥ 100	≥ 100	10	52	7.8	9.4	12	30
C) TFs (shoot/root) of UT and FT grown vetiver								
Metals	Fe	Al	Mn	Zn	Ni	Cu	Pb	Cr
UT	0.025 ± 0.017	0.18 ± 0.085	0.88 ± 0.19	0.93 ± 0.42	0.12 ± 0.038	0.58 ± 0.35	0.67 ± 0.97	0.056 ± 0.030
FT	0.11 ± 0.055	0.11 ± 0.073	0.077 ± 0.018	0.85 ± 0.077	0.031 ± 0.012	0.46 ± 0.21	1.1 ± 0.41	0.042 ± 0.059

D) TCLP extracts (mg L ⁻¹) of UT grown vetiver tissues, along with EPA limits for RCRA metals (USEPA, 2009).									
Metals		Fe		Al		Mn		Zn	
Tissue	S	R	S	R	S	R	S	R	
UT	2.0	6.7	12	46	23	19	2.6	1.6	
Limit	NR		NR		NR		NR		
Metals		Ni		Cu		Pb		Cr	
Tissue	S	R	S	R	S	R	S	R	
UT	0.22	0.93	0.38	0.41	0.11	0.11	0.020	0.070	
Limit	NR		NR		5.0		5.0		
Metals		Ba		Se		Cd		As	
Tissue	S	R	S	R	S	R	S	R	
UT	0.17	0.16	0.026	0.018	0.0037	0.026	0	BDL	
Limit	100		1.0		1.0		5.0		
Metals		Ag		Hg					
Tissue	S	R	S	R					
UT	0	BDL	BDL	BDL					
Limit	5.00		0.200

Figure 13: Effect of vetiver on AMD-impacted waters throughout the small-scale experiment, including three treatment (or control) per water type using containers with 4 plants and three experimental replicates. Numerical values are presented as means $\pm$ standard deviations for $n = 3$ experimental replicates from each container for both water types. Changes were observed between the unfiltered-untreated (UU) and unfiltered-treated (UT) waters for A) pH, B) conductivity, C) sulfate, and D) soluble iron.

Figure 14: Metal removal from AMD-impacted waters and tissue distribution after the small-scale experiment. A) Removal efficiency from the unfiltered-treated waters is partitioned into removal by vetiver and removal by external factors, and B) distribution is partitioned into roots and shoots as means $\pm$ standard deviations for $n = 12$ plants.

Table 7: Metal characterization of vetiver from the small-scale experiment, including means $\pm$ standard deviation for vetiver from unfiltered-treated (UT, n = 12 plants) water. A) Tissue content for vetiver shoots (S) and roots (R) per dry plant mass and B) TFs.															
A) Metal content ($\mu\text{g g}^{-1}$) of UT grown vetiver tissues															
Fe		Al		Mn		Zn		Ni		Cu		Pb		Cr	
S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
201	14800	672	10100	369	545	36.1	91.5	3.07	23.7	5.96	31.1	2.51	8.63	2.82	114
± 118	± 2270	± 331	± 3680	± 68.9	± 135	± 6.64	± 17.0	± 1.18	± 6.86	± 4.54	± 7.68	± 1.53	± 3.49	± 1.56	± 29.8
B) TFs (shoot/root) of UT grown vetiver															
Fe		Al		Mn		Zn		Ni		Cu		Pb		Cr	
0.014		0.075		0.73		0.41		0.15		0.22		0.33		0.027	
0.0085		0.043		0.27		0.11		0.10		0.24		0.20		0.019	

The UT plants accumulated 17,300 $\mu\text{g Fe g}^{-1}$, 3410 $\mu\text{g Al g}^{-1}$, 1080 $\mu\text{g Mn g}^{-1}$, 113 $\mu\text{g Zn g}^{-1}$, 62.2 $\mu\text{g Cr g}^{-1}$, 41 $\mu\text{g Ni g}^{-1}$, 25 $\mu\text{g Cu g}^{-1}$, and 5.37 $\mu\text{g Pb g}^{-1}$. TCLP revealed greater release of Fe, Al, Mn and Zn, with <1 ppm of remaining metals (**Error! Reference source not found.D**). There were greater releases of Al (45.5 mg L^{-1}) and Fe (6.74 mg L^{-1}) from the roots and Zn (2.59 mg L^{-1}) from the shoots, with relatively equal releases of Mn from roots and shoots (19.0 and 22.6 mg L^{-1} , respectively). It's apparent that Fe and Cr had less mobility as opposed to other metals. RCRA metals (Cr and Pb) were well below EPA permissible limits (5 ppm), hence the biomass generated is non-hazardous. Metals were negligible in FT water and plants, especially Fe, Al, Zn and Cu. The plug filter demonstrated high affinity for all but Pb and Cr, which were virtually unchanged. However, vetiver removed the remaining majority of Pb (80%) and the entirety of Cr (100%).

3.1.4. Metal distribution and translocation throughout vetiver

After the large-scale experiment, UT vetiver accumulated greater Fe (90%) and Cr (79%) in the roots (Figure 12B), with TFs of 0.025 and 0.056, respectively (**Error! Reference source not found.C**). To a lesser extent, the majority of Ni (65%, TF = 0.12), Al (57%, TF = 0.18), Pb (39%, TF = 0.67), and Cu (32%, TF = 0.58) were in the roots. Meanwhile, there was greater translocation of Zn (21%, TF = 0.93) and Mn (20%, TF = 0.88) to the shoots. In comparison, FT vetiver had less Fe (65%, TF = 0.11) and Pb (16%, TF = 1.1) in the roots, but more Ni (86% TF = 0.031) and Mn (71%, TF = 0.077). FT vetiver showed similar patterns for Cr, Al, Cu and Zn.

SEM images of vetiver shoots showed similar metal profiles to hydroponic plants without exposure to AMD; however, UT vetiver revealed metal buildups located on clover-shaped pockets near the stomata mainly characterized as Pb. Similar shoot buildups were present on hydroponic vetiver but were not as abundant. As expected, roots showed prominent amorphous and irregularly spaced buildup along their surface from plaques (Figure 10B). External buildup was primarily characterized as Fe, lesser Al

and Pb, and low levels of Zn, Ni, and Cu. Cross sections revealed buildup at the cortex side of the endodermis/pericycle, around the vascular tissues, and within the pith (Figure 10C). Metals were primarily characterized as Pb with lower levels of other metals (Fe, Al, Zn, Ni and Cu). There was an additional element within the pith of cross sections at 4 kV, which is a match to Ca (Ijaz et al., 2017; Lynch and Miotti, 2017). Therefore, the majority of Fe and Al are sequestered externally on the roots, whereas Pb is more equally distributed, and Ca is mainly found within the stele.

3.2. Small-scale phytoremediation experiment

3.2.1. Effects of vetiver on pH, conductivity, and SO_4^{2-} removal

During the small-scale experiment, pH of the UU water fluctuated (2.29 to 2.41) while the UT water increased in a rapid linear fashion from 2.40 to a high of 5.01 by 364 days with $p < 0.001$ for all comparisons (Figure 13A). Conductivity dropped from 3.98 to 1.87 mS cm^{-1} in 28 days, and continued dropping exponentially to 0.0324 mS cm^{-1} by 196 days, and then gradually decreased to 0.00637 mS cm^{-1} by the end (Figure 13B). Despite initial measurements, t-tests revealed significant variation in conductivity at each day ($p < 0.001$). Meanwhile, SO_4^{2-} dropped exponentially from 400 to 191 $\text{mg SO}_4^{2-} \text{ L}^{-1}$ by 28 days ($p = 0.022$) and 59.5 $\text{mg SO}_4^{2-} \text{ L}^{-1}$ by 84 days ($p < 0.001$; Figure 13C). There was 100% SO_4^{2-} removal from UT water and 9.0% from UU water, with 91% net removal by vetiver.

3.2.2. Metal removal by vetiver and translocation

During the small-scale experiment there were significant decreases in Fe, Al, Mn, Zn and Ni ($p < 0.001$), with no change in Cu, Pb or Cr. There were rapid decreases from 70,600 $\mu\text{g Fe L}^{-1}$ in UU waters to 33,900 $\mu\text{g Fe L}^{-1}$ in UT waters ($p = 0.015$) by 28 days (Figure 13D). This continued to 807 $\mu\text{g Fe L}^{-1}$ by 84 days ($p < 0.001$), gradually reaching an all-time-low of 50 $\mu\text{g Fe L}^{-1}$ by 140 days ($p < 0.001$). There was 100% Fe removal, with 90% from vetiver (Figure 14A). Cubic or linear decreases were observed in most

metals with 100% removal of Cr, 93% Mn, 91% Al and Ni, and 90% Zn. However, there was only 15% Pb removal and no Cu removal (Figure 12A).

Meanwhile, metal accumulation mirrored that of the large-scale experiment, including 15,000 $\mu\text{g Fe g}^{-1}$, 10,800 $\mu\text{g Al g}^{-1}$, 914 $\mu\text{g Mn g}^{-1}$, 128 $\mu\text{g Zn g}^{-1}$, 117 $\mu\text{g Cr g}^{-1}$, 37.1 $\mu\text{g Cu g}^{-1}$, 26.8 $\mu\text{g Ni g}^{-1}$, and 11.1 $\mu\text{g Pb g}^{-1}$. Vetiver showed higher accumulations of Fe (97%) and Cr (94%) in their roots (Figure 14B), with TFs of 0.014 and 0.027, respectively (Table 7B). They also showed higher Al (85%), Ni (74%), Cu (69%) and Pb (58%) in the roots. Meanwhile, there was even Zn (50%, TF = 0.41), and lesser Mn (37%, TF = 0.73) in the roots.

4. Discussion

4.1. Effectiveness of the vetiver rafts and plant health

In previously reported studies plant coverage varies, ranging from 20% to 100%, leading to reduced light penetration and greater removal efficiency (Chen et al., 2016a; Pavlineri et al., 2017). Appearance and growth of the UT plants from the large-scale experiment and accompanying water characteristics made it clear that the initial planting density (6 g L^{-1}) was insufficient for remediation. This was mitigated by increasing the planting density to 16 g L^{-1} . Decreased DO is common with higher plant coverage (Pavlineri et al., 2017); however, plant health had a greater influence. Reduced light passage inhibits photosynthetic activity and reduced algal growth (Chen et al., 2016a; Pavlineri et al., 2017). Therefore, it was assumed that the majority of microbes were non-photosynthetic, where reduced DO would relate to microbial and root respiration. Increased respiration would be necessary for root allocation of resources, which would provide vetiver with a mechanism to bypass nutrient deprivation.

4.2. Effects of vetiver rafts and vetiver plants on pH, conductivity, and SO_4^{2-} removal

Acidity and pH are important factors because lower pH inhibits certain AMD treatments like aerobic wetlands and influences metal solubility/availability (RoyChowdhury et al., 2015). For instance, Fe oxidation increases at lower pH from acidophilic microbe activity. Acidophiles operate optimally when $\text{pH} < 4$, whereupon oxidation rates and pH become independent of one another (Akciil and Koldas, 2006). AMD from the SRB possesses more extreme characteristics ($\text{pH} = 2.46$) than the oxidation ditch ($\text{pH} = 2.64$; Kiiskila et al., 2017). Minimal changes in pH by 168 days demonstrate that 6 g L^{-1} was not an optimal density for the SRB. However, higher densities of 16 g L^{-1} and $73 \pm 2 \text{ g L}^{-1}$ show clear and significant improvements, particularly from linear pH increases. Furthermore, the pH reached during the small-scale experiment far surpasses the optimal range for acidophiles and reaches a more ideal range for living organisms.

Our earlier short-term study already reported that vetiver can remove SO_4^{2-} (Kiiskila et al., 2017). Oxidation-reduction pathways can transform SO_4^{2-} into elemental or mixed-valence-state forms of sulfur (Chen et al., 2016b; Balci et al., 2017). Compounds like thiosulfate and tetrathionate have been detected in oxidized sulfide minerals and are thought to have major roles in the sulfur cycle of AMD (Balci et al., 2017). Conversion of SO_4^{2-} to sulfide through dissimilatory sulfate reduction (DSR) is considered a major initial step in detoxification (Chen et al., 2016b; Saad et al., 2016; Balci et al., 2017). Sulfides can then precipitate in the presence of metals, such as Fe from AMD (Chen et al., 2016b).

SO_4^{2-} decreases may result from plant uptake but does not account for external losses. DSR and sulfide oxidation have been reported with constructed wetlands containing *Phragmites australis* and *Juncus effusus*, though DSR was the main removal mechanism (Saad et al., 2016). The SRB and downstream reaches at the Tab-Simco site contain soluble SO_4^{2-} , nanoparticles bearing SO_4^{2-} , and microbially reduced sulfides

(Lefticariu et al., 2017). Moreover, SO_4^{2-} reduction at the site generates H_2S that can form metal sulfides with divalent metals such as Fe^{2+} (Lefticariu et al., 2017). Vetiver has demonstrated affinity for SO_4^{2-} from AMD-impacted waters, where uptake and DSR are possible mechanisms of removal. Sulfide precipitates were not observed; however, sulfurous odors from these experiments could suggest sulfur loss by volatilization, perhaps in the form of H_2S or sulfur dioxide.

4.3. Metal removal by vetiver rafts and vetiver plants

Vetiver has demonstrated high removal of Fe from Tab-Simco AMD; however, removal of the other metals was not entirely clear (Kiiskila et al., 2017). Vetiver plants from milk factory wastewaters have demonstrated comparable removal from 0.49 mg Mn L^{-1} (34%) and 4.1 mg Zn L^{-1} (53%), but lesser from 16 mg Fe L^{-1} (28%) and 0.05 mg Pb L^{-1} (8.9%; Roongtanakiat et al., 2007). There are some clear differences, though rapid Pb removal has been observed in multiple cases (Andra et al., 2009; Chen et al., 2012; Singh et al., 2015). For instance, vetiver roots have accumulated up to 13,240 and 19,800 mg Pb kg^{-1} from 400 and 1200 mg Pb L^{-1} , respectively, within 7 days (Andra et al., 2009). Inconsistencies in Cu removal were also unexpected as previous uptake estimates from Tab-Simco AMD ranged from 58.5 to >100% (Kiiskila et al., 2017). Reports of removal by vetiver have included 36.70% from 5 mg Cu L^{-1} in hydroponic media (Chen et al., 2012) and 87.5% from 118.92 mg Cu L^{-1} in ink manufacturing wastewater (Roongtanakiat et al., 2007). Initial removal during the small-scale experiment could suggest Cu had simply reentered the water following a decay phase. Therefore, a plant turnover would likely have improved Cu and Pb removal during the small-scale experiment.

Accumulations of Cr were unexpected from minimal concentrations; however, vetiver has demonstrated 77 to 79% removal of 5 to 20 mg Cr L^{-1} from synthetic wastewater as dichromate, $\text{Cr}_2\text{O}_7^{2-}$ (Singh et al., 2015). Cr would likely be trivalent (Cr^{3+}) or hexavalent (Cr^{6+}) in AMD, where water solubility is generally low. Compounds such as $\text{Cr}_2\text{O}_7^{2-}$ precipitate in the company of Al, Zn, Fe^{2+} and Fe^{3+} , and cupric Cu (Cu^{2+}),

which were present in Tab-Simco AMD. TCLP suggested that Fe and Cr were immobilized in the roots, likely from removal by adsorption. Differences could be explained by length of treatment and variations in characteristics such as metal abundance/solubility and pH. Furthermore, available space can have significant impacts as vetiver roots from the small-scale experiment were able to spread throughout the entire surface area.

4.4. Metal distribution and translocation throughout vetiver

Vetiver has shown some similar metal distributions, including greater root accumulations of $\geq 85\%$ Fe (Roongtanakiat et al., 2007; Banerjee et al., 2016; Kiiskila et al., 2017) and $\geq 83\%$ Al (Banerjee et al., 2016; Kiiskila et al., 2017). Additionally, vetiver has previously demonstrated higher translocation of 30 to 50% Zn (Truong et al., 2010; Chen et al., 2012; Banerjee et al., 2016; Kiiskila et al., 2017) and 67% Mn (Roongtanakiat et al., 2007), which are necessary nutrients. Contrasting response in vetiver was reported for Cr, with higher translocation from synthetic wastewaters (Singh et al., 2015), and minimal to more even translocation (1 to $>50\%$) from mine rehabilitation (Truong et al., 2010; Banerjee et al., 2016). Metal plaques on the UT plants are likely responsible for higher root accumulations of Fe, Al and Cr, where growth conditions and metal speciation would affect Cr distributions. Insoluble Cr from AMD would likely accumulate in conjunction with the plaques through adsorption, explaining the reduced translocation. This could suggest that plaques selectively block metals, while not impeding necessary plant nutrients.

There is reportedly greater translocation of 16 to 33% Ni (Truong et al., 2010; Banerjee et al., 2016) and low to moderate translocation of Cu (Roongtanakiat et al., 2007; Truong et al., 2010; Chen et al., 2012). Lower translocation of Ni was expected, though Cu was more evenly distributed in the large-scale experiment with greater translocation in the small-scale experiment. Cu uptake could have been inhibited by plaques, requiring more time to accumulate. Numerous studies have reported low TFs (0.10 to 0.20) for Pb accumulated by vetiver (Roongtanakiat et al., 2007; Andra et al.,

2009; Truong et al., 2010; Chen et al., 2012; Kiiskila et al., 2017). Pb translocation was minimal in the small-scale experiment (TF = 0.33), but higher for the large-scale experiment (TF = 0.67). However, Pb from Tab-Simco AMD is low in comparison to other experiments and could have been removed primarily by rhizofiltration as opposed to phytoextraction.

Plaques are important features of wetland and hydroponic plants, commonly described as amorphous or crystalline structures composed of metal hydroxides (Yang et al., 2014; Li et al., 2017). Under waterlogged conditions, adapted plants are able to transport oxygen to the roots for respiration (Pardo et al., 2016). Excess is released into the rhizosphere, leading to oxidation and precipitation of metals as plaques (Chen et al., 2016a; Li et al., 2017; Jia et al., 2018). Plaque formations are evident from the orange-yellow colorations that appeared on the root surface within 14 days of the experiments. Fe was clearly the most abundant metal in the roots, showing little translocation. Metal uptake is dependent on plaque formation, where greater abundance is positively correlated to accumulation (Yang et al., 2014; Li et al., 2017). Surface areas of high specificity and hydroxyl groups provide plaques with an affinity for certain metals (Li et al., 2017). Therefore, roots with plaques increase metal removal through adsorption and coprecipitation (Marchand et al., 2010; Yang et al., 2014; Pardo et al., 2016; Li et al., 2017; Jia et al., 2018). Immobilization prevents toxic metals from entering live cells and acts as a Fe reservoir for plants (Yang et al., 2014; Jia et al., 2018). The majority of were accumulated in vetiver roots, particularly Fe, Al, Ni and Cr. Higher Cu translocation and Pb retention in UT plants as opposed to FT plants suggests greater Cu permeability but Pb sequestration in the plaques.

Histological staining of *Phragmites australis* from AMD-impacted soils demonstrated that Fe is largely accumulated in the exodermis where Al tends to reach endodermis and stele (Guo and Cutright, 2017). SEM-EDX analysis of hydroponic *P. australis* revealed that Fe gathers on the root surface, with lower internal passage (Pardo et al., 2016). It was suggested that Fe passage may be possible through apoplastic transfer through the cell walls with size exclusion limitations (Pardo et al., 2016). Multiple

microscopy techniques have shown that metal plaques of *Imperata cylindrica* mainly form around/within the epidermal cells, throughout the exodermal-parenchyma, and within the stele (Fuente et al., 2017). Apoplastic Fe was prominent, more so in the exodermal and endodermal parenchyma (Fuente et al., 2017). These findings agree with vetiver in that plaques accumulate at the root surface while permitting some internal passage. Fe, Al and Mn were most abundant in the Tab-Simco AMD, and as such were the most accumulated. Mn and Zn had higher mobility and translocation, thus lesser buildup in the plaques, possibly attributing to their use as nutrients. Rhizofiltration likely played a role for Pb removal as SEM-EDX analysis confirmed that it was present in the metal plaques.

Conclusions

These year-long experiments established the effectiveness of vetiver for treatment of AMD-impacted waters. The primary objective was to test the effectiveness of vetiver grass to remediate AMD-impacted water in a long-term study. In the large-scale experiment, vetiver showed removal of SO_4^{2-} from the UT (28%) and FT (45%) waters, with higher net removal of Fe (81%) and Pb (81%) from the UT waters and moderate to low net removal of Ni (38%), Zn (35%), Mn (27%), Cr (21%), Al (11%) and Cu (8.0%). During the small-scale experiment, vetiver demonstrated more rapid results by near complete removal of SO_4^{2-} (91%) and metals (90-100%), with the exception of Pb (15%) and Cu (0.0%). In both cases vetiver was able to increase pH to a less acidic range (≥ 3.4). Diminished plant health and remediation over the first 168 days of the large-scale experiment confirm that 6 g L^{-1} is not a sufficient planting density for waters from the SRB. This was however mitigated by increasing the planting density to 16 g L^{-1} . Differences between the large- and small-scale experiments reveal the benefits of having greater root coverage. For field purposes, these results have shown that handheld meters for DO and conductivity could serve for on-site monitoring of plant health and metal reduction, respectively. Common trends among metals were observed; from the large-scale experiment, vetiver accumulated greater Fe ($17,300 \mu\text{g g}^{-1}$), Al ($3405 \mu\text{g g}^{-1}$), Mn

(1080 $\mu\text{g g}^{-1}$), Zn (113 $\mu\text{g g}^{-1}$) and Cr (62.3 $\mu\text{g g}^{-1}$), with lower Ni (41.2 $\mu\text{g g}^{-1}$), Cu (24.6 $\mu\text{g g}^{-1}$) and Pb (5.37 $\mu\text{g g}^{-1}$). Prominent metal plaque formations were observed along the root surfaces, largely containing Fe with lesser amounts of Al and Pb. Metals were primarily sequestered in the roots ($\text{TF} \leq 0.67$); however, there was higher translocation of Mn ($\text{TF} = 0.88$) and Zn ($\text{TF} = 0.93$), possibly attributed to their use as nutrients. TCLP confirmed that vetiver tissues were not RCRA hazards and therefore are safe for disposal. These results show that vetiver is an ideal candidate for developing plant-based treatment systems in AMD-impacted mine sites.

Acknowledgements

The Office of Surface Mining Reclamation and Enforcement is acknowledged for funding this study (Grant #S12AC20001). Special thanks are due to David Kiiskila for design and construction of the rafts, technical assistance in the greenhouse, and transportation of the Tab-Simco AMD. Thanks to Emili Heikkinen and Matthew Adler for assistance with the initial collection and transportation of the AMD, Laurie Kiiskila and Dan O'Connell for transportation of the AMD for filtration, Dr. Abhishek RoyChowdhury for providing the plug filter, and Dr. Amy Marcarelli for suggestions on statistical analyses. Thanks to undergraduate students Yi Cui, Mona Fabian, Reginald Mullen, Alexis Rule, Nikki Saari, and Amanda Turcotte for assistance in the greenhouse and laboratory.

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Chapter 3. Metabolic Response of Vetiver Grass (*Chrysopogon zizanioides*) to Acid Mine Drainage^a

Chapter 3 was prepared for submission to *Environmental Science & Technology*.

^a The following chapter is a manuscript submitted to *Environmental Science & Technology*.

Metabolic Response of Vetiver Grass (*Chrysopogon zizanioides*) to Acid Mine Drainage

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Abstract

Acid mine drainage (AMD) is a sulfuric discharge containing metals and particulates that can spread to nearby water sources, imposing toxicity and physical stress to living things. Vetiver grass (*Chrysopogon zizanioides*) is capable of tolerating and treating AMD-impacted water from the abandoned Tab-Simco mining site from southern Illinois, though little is known about its tolerance mechanisms. We conducted metabolomic analyses of vetiver shoots and roots after relatively short- and long-term periods of exposure to Tab-Simco AMD. The metabolic shift of vetiver shoots was dramatic with longer-term AMD exposure, including upregulation of amino acid (AA) and glutathione metabolism, cellular respiration and photosynthesis pathways, with downregulation of phosphorylated metabolites. Meanwhile, the roots demonstrated drastic downregulation of phospholipids and phosphorylated metabolites, cellular respiration, glyoxylate metabolism, and AA metabolism. Responses collectively suggest that oxidative stress, nutrient deprivation and dehydration were the main stresses. Vetiver accumulated ornithine and oxaloacetate in the shoots, which could function for nitrogen storage and intracellular metal-chelation, respectively. It's suspected that organic acids and glutathione were secreted from the roots for rhizospheric metal-chelation, whereas phosphorylated metabolites were recycled for phosphorus. These findings reveal AMD-induced metabolic shifts in vetiver grass, which are seemingly unique in comparison to independent abiotic stresses.

Keywords: acid mine drainage, remediation, vetiver grass, combined abiotic stress, metabolomics

1. Introduction

Acid mine drainage (AMD) is formed from oxidation of sulfide minerals under hydrolytic conditions, releasing Fe, SO_4^{2-} and H^+ .¹ Lower pH increases metal mobility and oxidation, releasing more Fe and metals distinctive to mineralogy.^{1,2} Metal toxicity presents health and environmental concerns from contamination of nearby water sources, reduced biodiversity and spread through biomagnification.¹ Moreover, AMD contains metal hydroxides that accumulate as orange-yellow precipitates,^{1,2} overwhelming living organisms through physical stress.² The abandoned Tab-Simco coal mining site near Carbondale, Illinois spans about 12 hectares,^{3,4} discharging an estimated 35,000 gal AMD d⁻¹ with low pH (2-3) and elevated SO_4^{2-} ,^{3,5} Fe, Al, and Mn.⁴

Several plants have been screened for AMD tolerance, including *Typha latifolia*, *Scirpus validus*, and many others.^{6,7} Plants remove metals through root uptake and/or precipitation,^{1,8} which may accumulate as root plaques composed of metal hydroxides.⁹ Vetiver grass, *Chrysopogon zizanioides*, is a noninvasive and fast-growing perennial grass that tolerates and accumulates metals.^{4,10,11} Vetiver can grow sustainably in hydroponics,^{4,10,11} including acidic waters like AMD.^{1,4} Moreover, vetiver has massive, self-regenerating roots that can form plaques and sequester metals.⁴

Vetiver can remediate Tab-Simco AMD,^{4,5} though little is known about tolerance mechanisms to this complex medium. Single factors like individual metals have been the focus of abiotic stress research; however, plants are exposed to multiple stresses under normal conditions.¹²⁻¹⁵ Combined stresses have come under recent investigations for agricultural purposes, often revealing that they have more severe impacts.¹⁴ Furthermore, plants demonstrate unique metabolic shifts and expression patterns that are incomparable to individual stresses.¹²⁻¹⁵ Therefore, combine stresses could provide critical insights into applied usage of plants and biotechnological advancements.¹⁵

Metabolomics is invaluable for understanding plant stress as it provides large datasets that are not dependent on genetic information. Our objectives were to evaluate

the physiological and metabolic responses of vetiver to Tab-Simco AMD over short- and long-term periods to reveal tolerance mechanisms. Physiology was evaluated through changes in growth, photosynthetic activity, and metal accumulation. Metabolism was evaluated through large-scale metabolomics using liquid chromatography coupled tandem mass spectrometry (LC-MS/MS).

2. Materials and methods

2.1. Hydroponic Experiments and Sample Acquisition

Propagated vetiver was maintained hydroponically, acclimated in media (0.5X Hoagland's solution) for two months, and transferred into light-shielded LDPE bottles containing 450 mL of Tab-Simco AMD (media for comparison) for 7 or 56 days ($n = 6$ replicates). Bottles without plants were also utilized. Metabolomic plants were flash frozen using liquid nitrogen and stored at -80°C ($n = 3$). Remaining plants were measured for fresh biomass and stored at -80°C . Dissolved oxygen (DO) was measured,⁴ then water loss was replenished with deionized water and sampled. Additional plants were transferred into light-shielded 250 mL beakers containing 100 mL of AMD for 7 days ($n = 3$), along with no plants beakers. Water was sampled, flash frozen and stored at -80°C for metabolite analysis.

2.2. Water Characterization and Plant Physiological Response

Water was screened for pH, soluble ions (SO_4^{2-} , NO_3^{1-}) and metals (Fe, Al, Mn, Zn, Cu, Ni, Pb, Ag, Cr, As) following standard methods.⁴ Plants were thawed at room temperature and outer layer grass blades were analyzed for total chlorophyll following standard methods.¹⁶ Remaining tissues were digested and screened for metals following standard methods.⁴ Percent change in fresh biomass and root-to-shoot (R:S) ratios for biomass were calculated.

2.3. Metabolite Extractions and LC-MS/MS

Plants were divided into roots and shoots, broken into pieces and homogenized into fine powders by pre-chilled mortars and pestles. Metabolite extractions followed De Vos et al., 2007 with slight modifications.^{11,17} Extracts (200 μ L) were dried by evaporative centrifugation for metabolite analysis. Water samples were thawed to 4°C and syringe filtered (0.22 micron, Nylon). Subsamples (100 μ L) were treated with 400 μ L of methanol:acetonitrile (1:1) for 10 min at 4°C, centrifuged (16,000 rcf) for 10 minutes, and the supernatant was dried for metabolite analysis. Metabolites were analyzed by LC-MS/MS as described in Pidatala et al., 2016 with a Shimadzu UFLC XR HPLC System (LC-20AD Model) and a SCIEX 5500 QTRAP MS with a Turbo V Electrospray Ionization (ESI) Source.¹¹ 248 metabolites were targeted covering primary metabolic pathways. Chromatographic peaks were manually inspected and integrated using MultiQuant 3.0 software (SCIEX, USA). Peak areas were exported to excel and max/min analysis was performed to ensure data quality.

2.4. Data Processing and Statistical Analysis

Changes in water and physiological characteristics were compared using two-sample t-tests with JMP[®] Pro 13 software ($p < 0.05$). Metabolites were processed using MetaboAnalyst 3.0 (www.metaboanalyst.ca), replacing missing values by the k-NN method and normalizing data through log2 transformation with autoscaling. Metabolites were compared by fold change (≥ 1.5 threshold) with FDR adjusted t-testing ($p < 0.05$) and multivariate comparisons by partial least squares-discriminant analysis (PLS-DA).

3. Results and discussion

3.1. Physiological Response of Vetiver to AMD

Tab-Simco AMD was statistically variable to media (**Error! Reference source not found.a**), containing five soluble metals ($p \leq 0.0002$) and one insoluble metal (Cr). At 7 days the untreated AMD (UA) had a pH of 2.40 with $7.8 \text{ mg O}_2 \text{ L}^{-1}$ and $444 \text{ mg SO}_4^{2-} \text{ L}^{-1}$, whereas treated AMD (TA) had a pH of 2.60 ($p = 0.0138$) with $3.5 \text{ mg O}_2 \text{ L}^{-1}$ ($p = 0.0014$) and $349 \text{ mg SO}_4^{2-} \text{ L}^{-1}$ ($p = 0.0037$) but no changes in metals (Figure 15). At 56 days UA had a pH of 2.43 with $7.3 \text{ mg O}_2 \text{ L}^{-1}$, $400 \text{ mg SO}_4^{2-} \text{ L}^{-1}$, and $64,500 \mu\text{g Fe L}^{-1}$, whereas TA had a pH of 3.23 ($p = 0.0098$) with $4.3 \text{ mg O}_2 \text{ L}^{-1}$ ($p = 0.0227$), $166 \text{ mg SO}_4^{2-} \text{ L}^{-1}$ ($p = 0.0002$), and $6550 \mu\text{g Fe L}^{-1}$ ($p = 0.0022$). Excluding Cr, all metals decreased in TA ($p \leq 0.0064$) though not as significantly as Fe. AMD had $<0.200 \text{ mg NO}_3^{1-} \text{ L}^{-1}$ at 7 and 56 days with no plant-induced changes. Changes to media were less significant, though untreated media had $7.3 \text{ mg O}_2 \text{ L}^{-1}$, $9.94 \text{ mg SO}_4^{2-} \text{ L}^{-1}$, and $162 \text{ mg NO}_3^{1-} \text{ L}^{-1}$ at 56 days, whereas treated media had $1.8 \text{ mg O}_2 \text{ L}^{-1}$ ($p = 0.0003$), $0.181 \text{ mg SO}_4^{2-} \text{ L}^{-1}$ ($p = 0.0008$), and $0.663 \text{ mg NO}_3^{1-} \text{ L}^{-1}$ ($p = 0.0004$) with no trace of Ni or Cr or changes to metals.

Physiological changes included a -27.3% decrease in AMD-grown biomass and -2.92% in media-grown at 7 days ($p = 0.0006$, **Error! Reference source not found.b**). AMD-grown shoots had drying (turgor loss), browning and curling blade tips (Figure 15e) with decreased chlorophyll (1.28 mg g^{-1}) from media-grown (2.62 mg g^{-1} , $p = 0.0069$). R:S ratios were greater (0.47) in AMD-grown vetiver as opposed to media-grown (0.30, $p = 0.0397$) with brown-orange plaques beginning to accumulate along the surface (Figure 15e). AMD-grown biomass decreased -30.2% at 56 days, but increased 31.7% in media ($p = 0.0003$). Shoots demonstrated continual, gradual drying and fresh growth with comparable chlorophyll to media-grown. Plaque development began early at the root-stem transition, gradually extending to the tips while fresh white growth emanated from the transition with no significant difference in R:S ratios. AMD-grown vetiver accumulated >2-fold metals at 7 days and >3-fold at 56 days with variable

translocation (**Error! Reference source not found.c**). Fe and Cr were held in the roots while Mn and Ni were translocated. Al and Zn were originally sequestered but more translocated at 56 days.

Metals are known for macromolecular interference and metal displacement,¹⁸⁻²⁰ where redox active metals such as Fe, Mn and Cr produce reactive oxygen species (ROS).¹⁸ This leads to inhibited growth and reduced biomass,^{18,20,21} decreased photosynthesis and chlorosis, and turgor loss.^{18,21} Reduced biomass and turgor loss were the most noticeable symptoms in AMD-grown vetiver. Furthermore, AMD imparts osmotic imbalances and physical stress from particulates. The mere presence of metal hydroxides are suggested to have the greatest ecological impact from covering available surfaces and overwhelming living things.² It's assumed that physical stress was a major factor as vetiver is tolerant of metals in hydroponics.^{10,11}

Additionally, vetiver must contend with root plaques. Waterlogged plants transport oxygen for root respiration,⁸ releasing excess into the rhizosphere as radial oxygen loss (ROL).^{8,9} This leads to metal oxidation and precipitation, which collect as amorphous plaques along the root surface.^{8,9} Although they sequester metals, plaques also reduce uptake and translocation of resources like nutrients and water.⁸ This could explain why NO_3^{1-} was unchanged in the presence of vetiver. Meanwhile, membrane and cell wall disruption from particulates may affect development and water uptake,⁸ explaining the decrease in vetiver biomass and turgor loss.

Lower pH would further deprive vetiver of essential alkaline nutrients like phosphorus by reducing solubility,^{22,23} while releasing Al as the more toxic Al^{3+} .^{24,25} Plants increase R:S ratios in response to deprivation, seeking out to acquire more resources.^{22,23} Abundant fresh growth and increased R:S ratios establish that vetiver was acclimating to AMD. This might suggest that vetiver was mainly adjusting to nutrient deprivation and dehydration.

Table 8: Characteristics of A) Tab-Simco AMD and media , B) characteristics of vetiver grass after 7 and 56 days in AMD (or media), including root-to-shoot (R:S) ratios, and C) metal contents of vetiver shoots (S) and roots (R) per dry plant mass. Represented are mean ± standard deviation for n = 3 replicates.													
A) Characteristics of AMD and media													
Contents	pH	SO ₄ ²⁻ (mg L ⁻¹)		Metals (µg L ⁻¹)									
				Fe	Al	Mn	Zn	Ni	Cr				
AMD	2.39 ±0.0216	398 ±3.79		74200 ±1920	68000 ±1850	21400 ±544	1250 ±36.8	647 ±17.0	10.0 ±0				
Media	5.36 ±0.00471	11.2 ±1.62		117 ±4.71	40.0 ±14.1	420 ±14.1	36.7 ±4.71	0 ±0	0 ±0				
B) Physiological response of AMD and media grown vetiver													
Treatment	Time (days)	Change in biomass (%)				R:S		Total chlorophyll (mg g ⁻¹)					
AMD	7	-27.3±2.19				0.47±0.077		1.28±0.200					
	56	-30.2±6.47				0.40±0.10		2.48±0.667					
Media	7	-2.92±3.06				0.30±0.025		2.62±0.336					
	56	31.7±1.32				0.40±0.046		2.66±0.375					
C) Metal content (µg g ⁻¹) of AMD and media grown vetiver tissues													
Treatment	Time (days)	Fe		Al		Mn		Zn		Ni		Cr	
		S	R	S	R	S	R	S	R	S	R	S	R
AMD	7	671 ±40.6	3320 ±647	714 ±43.6	2337 ±1394	397 ±94.9	244 ±69.2	47.6 ±6.80	55.3 ±8.66	6.99 ±0.818	5.63 ±1.25	2.66 ±0.468	23.5 ±13.7
	56	1310 ±299	13100 ±1960	2720 ±221	4180 ±2180	527 ±55.8	254 ±94.0	93.2 ±6.99	61.5 ±8.46	10.3 ±0.447	6.62 ±1.24	14.6 ±3.84	53.3 ±23.3
Media	7	57.9 ±5.95	946 ±84.4	71.1 ±10.3	1870 ±193	125 ±54.3	160 ±39.6	20.2 ±2.47	27.9 ±3.78	0.331 ±0.468	1.66 ±0.466	0.661 ±0.468	17.6 ±1.68
	56	118 ±50.1	1860 ±1421	148 ±83.6	4110 ±3228	71.2 ±17.1	317 ±124	28.8 ±9.15	53.9 ±21.3	0 ±0	1.99 ±0.812	1.98 ±1.40	37.6 ±28.5

Figure 15: Effect of vetiver on AMD and media for A) dissolved oxygen (DO), B) pH, C) SO_4^{2-} , and D) Fe among untreated AMD (UA), treated AMD (TA), untreated media (UM) and treated media (TM), and E) the appearance of AMD-grown vetiver shoots and roots at 0, 7, and 56 days. Numerical representations include mean $\pm$ standard deviation for $n = 3$ replicates.

3.2. Metabolic Profiling of Vetiver Shoots in Response to AMD

Following 7 days, AMD-grown vetiver shoots exhibited differential regulation of eight metabolites, with seven upregulated and one downregulated. Metabolic changes were visualized using PLS-DA, where AMD-grown shoots showed 52.5% variance in component 1 and 30.7% in component 2 (Figure 16a). Meanwhile, there was drastic regulation of 61 metabolites at 56 days, with 47 upregulated and 14 downregulated. The AMD-grown shoots showed greater variance with 59.2% in component 1 and 40.8% in component 2 (Figure 16b), demonstrating clear differences between AMD- and media-grown shoots.

Most upregulated metabolites were amino acids (AAs), including downregulation of proline metabolism, upregulation of ornithine and its relatives, sulfur-containing AAs, and glutathione metabolism (Figure 17a). In addition, there was upregulation of metabolites relating to cellular respiration and C₄ photosynthesis (Figure 17). Meanwhile, there was differential regulation of purine and phospholipid metabolism.

3.1.1 Upregulation of Amino Acid Metabolism and Downregulation of Proline

Proline metabolism was downregulated, where 1-pyrroline-5-carboxylate decreased 12-fold at 7 days ($p = 0.0080$) and 6.9-fold at 56 days ($p = 0.0074$), and proline decreased 2.8-fold at 56 days ($p = 0.0298$). From the shikimate pathway, shikimate decreased 4.7-fold ($p = 0.0109$) and phenylalanine increased 3.6-fold ($p = 0.0180$). From the TCA cycle, aspartate increased 6.2-fold ($p = 0.0079$) and glutamate increased 2.8-fold ($p = 0.0301$). Additionally, asparagine increased 14-fold ($p = 0.0038$), histidine increased 12-fold ($p = 0.0040$), and two branched chain AAs (BCAAs), leucine and isoleucine were upregulated.

Figure 16: PLS-DA comparing AMD- and media-grown vetiver (n = 3 plants), including two-dimensional score plots for A) 7 day shoots, B) 56 day shoots, C) 7 day roots, and D) 56 day roots.

AAs can relate to protein synthesis/degradation or more targeted responses. For instance, phenylalanine could relate to lignin biosynthesis and strengthening cell walls,¹³ or flavonoid biosynthesis through the phenylpropanoid pathway.^{13,26} BCAAs may accumulate as osmolytes for drought stress or serve as alternative electron donors for respiration.¹³ Downregulation of proline and 1-pyrroline-5-carboxylate was not predicted, though not entirely surprising. Proline is commonly upregulated under abiotic stress,^{21,25,27,28} aiding with cell structure, protein stabilization,²⁷ and ROS scavenging.^{25,27} However, proline is reportedly inhibited when drought conditions are combined with heat,¹² emphasizing the importance of investigating combined stresses or natural environments.

3.1.1 Upregulation of Ornithine and Related Metabolites

Urea cycle metabolites were upregulated, where argininosuccinate increased 12-fold ($p = 0.0040$), arginine increased 30-fold ($p = 0.0024$), citrulline increased 71-fold ($p = 0.0018$) and ornithine increased 207-fold ($p = 0.0014$). From the urea cycle, spermine increased 5.7-fold ($p = 0.0079$), dimethylarginine increased 11-fold ($p = 0.0041$), and ureidopropionate increased 18-fold ($p = 0.0031$). Time-dependent comparisons of AMD-grown vetiver were minor; however, with media-grown argininosuccinate decreased 12-fold ($p = 0.0081$), arginine decreased 13-fold ($p = 0.0081$), citrulline decreased 14-fold ($p = 0.0081$), and ornithine decreased 40-fold ($p = 0.0042$). Therefore, AMD-grown vetiver was accumulating and maintaining higher levels of these metabolites while media-grown were not.

Arginine is used for storage and transport of organic nitrogen, containing the highest nitrogen-to-carbon ratio of proteogenic AAs,²⁸ and also a nitric oxide precursor for signaling.^{28,29} Ornithine is a precursor to arginine,^{28,29} which is synthesized in chloroplasts.²⁸ Reactions from arginine or decarboxylation of ornithine generates polyamines, important for development and stress response.^{28,29} Furthermore, overexpression of SINAGS1 in *Arabidopsis thaliana* results with leaf accumulations of ornithine that provides drought tolerance.²⁸ Significant upregulation suggests a critical role for ornithine in AMD-grown vetiver. Time-dependent downregulation in media-grown vetiver likely indicates lesser requirements for ornithine. Given the lack of NO_3^{1-} in AMD and absence of removal, ornithine would be a good nitrogen store for shoot chloroplasts in vetiver.

3.1.1 Upregulation of Sulfur-Containing Amino Acids and Glutathione Metabolism

Sulfur is taken in by plants roots as SO_4^{2-} and transported for assimilation into organic compounds.^{30,31} Methionine increased 7.7-fold at 56 days ($p = 0.0064$), where methionine sulfoxide (metSO) increased 16-fold at 7 days ($p = 0.0144$) and 5.7-fold at 56 days ($p = 0.0079$). At 56 days, reduced glutathione (GSH) increased 3.0-fold ($p = 0.0253$) while oxidized glutathione (GSSG) increased 14-fold ($p = 0.0038$). Additional GSH precursors included 3.0-to-4.0-fold increases in serine, sarcosine, and glycine. Ethanolamine increased 49-fold ($p = 0.0018$), though time-dependent comparisons revealed equal downregulation in the media-grown vetiver. Surprisingly, taurine increased 5.7-fold ($p = 0.0079$).

Methionine is one of two sulfur-containing, proteogenic AAs.³¹ ROS readily oxidizes methionine into metSO,^{32,33} converting its hydrophobic S-methyl thioether into a polar group, resulting with misfolded proteins.³² Differential changes in methionine and metSO at 56 days could suggest that metSO was being reduced to its native state. Glutathione is a tripeptide with high reductive potential from cysteine and its free thiol.^{19,31} GSH is ideal for sulfur storage with long range transport capabilities,³¹ with primary use for ROS scavenging and phytochelatin biosynthesis.^{10,19,20,31} GSH is oxidized to GSSG, a dimer of glutathione that accumulates under oxidative stress, where the GSH:GSSG ratio functions as an indicator of redox status.^{19,31} Plants use glutathione, phytochelatins and organic acids (OAs) to bind metals for transport into vacuoles, reducing intracellular damage.^{10,18,20} Taurine is not knowingly synthesized by plants but is derived from SO_4^{2-} and serine in algae for osmotic imbalances.³⁴ Taurine could have come from microbial activity, with the alternative being that vetiver has biosynthetic capabilities.

3.1.1 Upregulation of Cellular Respiration and Photosynthesis Metabolites

TCA cycle intermediates were upregulated at 56 days (Figure 17a), where citrate increased 4.5-fold ($p = 0.0118$), succinate increased 6.6-fold ($p = 0.0077$), and oxaloacetate (OAA) increased 233-fold ($p = 0.0014$). Succinate additionally increased 13-fold at 7 days ($p = 0.0080$). Time-dependence revealed that OAA decreased 57-fold by 56 days in media-grown vetiver ($p = 0.0042$). Early glycolytic intermediates were downregulated, where hexose sugars decreased 3.4-fold ($p = 0.0197$) and hexose diphosphate sugars decreased 12-fold ($p = 0.0040$). Meanwhile, glyceraldehyde-3-phosphate increased 4.0-fold ($p = 0.0141$) and phosphoenolpyruvate (PEP) increased 2.5-fold ($p = 0.0394$), and a terpenoid precursor, mevalonate decreased 5.7-fold ($p = 0.0079$).³⁵ Alternatively, CO₂ could be captured as OAA during C₄ photosynthesis (Figure 17b),³⁶ which is converted into a C₄-carrier (aspartate) and moved to the bundle sheath for reconversion to OAA.³⁶ OAA is finally broken into pyruvate and CO₂ for carbon fixation.³⁶ Furthermore, octulose-phosphate, a cycling intermediate of the pentose phosphate pathway relating to photosynthesis,³⁷ increased 49-fold ($p = 0.0018$).

OAs are upregulated in response to metals,^{21,24} where carboxy binding partakes in detoxification.^{18,21} Cytosolic chelation of Al³⁺ under acidic conditions reportedly reduces toxicity while freeing up phosphours.²⁴ Succinate and OAA demonstrated greater upregulation in AMD-grown vetiver, which could simply be for metabolic precursors. Furthermore, OAA is thought to negate the effects of salinity by precipitating Na⁺.³⁸ Photosynthetic decreases were evident, potentially relating to inhibited carbon fixation^{18,21} or pigment breakdown from metals,^{15,21} or inhibited ribulose-1,5-bisphosphate regeneration from phosphorus deficiency.²² However, chlorophyll returned to normal at 56 days, along with increased octulose-phosphate.³⁷

3.1.1 *Purine and Phospholipid Metabolism in Shoots*

Purine and purine nucleosides were upregulated at 56 days, including 3.0-to-8.4-fold increases in adenosine, deoxyadenosine, guanosine, deoxyguanosine, and xanthine. Damaged purines, 1-methyladenosine and 1-methyladenine, increased 2.6-fold ($p = 0.0349$) and 8.9-fold ($p = 0.0055$), respectively. Conversely, nucleotides inosine monophosphate and cyclic adenosine monophosphate (cAMP) decreased 4.5-fold ($p = 0.0118$) and 19-fold ($p = 0.0031$), respectively, while ribose-5-phosphate decreased 3.2-fold ($p = 0.0212$). Pyrimidines were largely unaffected, though thymidine increased 21-fold ($p = 0.0031$). Meanwhile, lipolysis intermediates of phosphatidylcholine (PC) increased at 7 days, where lysoPC(18:0) increased 4.2-fold ($p = 0.0354$), lysoPC(16:0) increased 7.0-fold ($p = 0.0440$), and linoleate increased 24-fold ($p = 0.0080$). Phospholipids were downregulated at 56 days, including 3.3-to-5.4-fold decreases in PC, phosphatidylglycerol (PG), and phosphatidylinositol (PI). Meanwhile, carnitine increased 44-fold ($p = 0.0018$).

Membrane damage from lipid peroxidation was possible,^{15,19,20} though no peroxidation products were detected. Phosphorus deprived plants rely on root uptake of PO_4^{3-} and remobilization from older tissues.^{22,23} Therefore, we hypothesize that vetiver utilized purine nucleotides, phosphorylated sugars, and phospholipids to remobilize PO_4^{3-} . Mature plant regions provide a potential source for actively growing tissues to recycle phosphorus.^{23,30} Although the shoots dried from AMD exposure, vetiver was able to acclimate and substitute fresh growth. Remobilization of internal nitrogen and phosphorus offers an efficient use of resources through recycling as opposed to uptake from impoverished environments. Phospholipid downregulation was minor, though upregulation of lysoPCs and fatty acids (FAs) suggest lipolysis with carnitine serving as a catabolic transporter.

Resource remobilization and peroxidation requires replacement and/or remodeling of phospholipid membranes. Non- PO_4^{3-} lipids would decrease need for PO_4^{3-} , increasing availability.³⁹ Plants have demonstrated replacement using glycolipids,³⁹ along

with sulfolipids that serve for sulfur storage.^{23,31} SO_4^{2-} from AMD would provide a source for sulfolipid replacement, though this would need to be further investigated. Downregulated metabolites mainly contained PO_4^{3-} , suggesting a limited resource as expected from the low pH of AMD.

3.3. Metabolic Profiling of Vetiver Roots in Response to AMD

After 56 days, AMD-grown vetiver roots demonstrated differential regulation of 44 metabolites, with 40 downregulated and four upregulated. PLS-DA revealed distinct changes with 54.1% variance in component 1 and 16.8% in component 2 (Figure 16c). There was differential regulation of 83 metabolites at 56 days, with 71 downregulated and 12 upregulated. PLS-DA revealed 64.7% variance in component 1 and 14.6% in component 2 (Figure 16d).

More than half of the downregulated metabolites contained PO_4^{3-} , including 21 of 40 metabolites at 7 days and 34 of 71 at 56 days. Major pathways included phospholipid and purine metabolism, cellular respiration and glyoxylate metabolism, AA metabolism and GSH (Figure 18).

3.1.1 Phospholipid and Purine Metabolism in Roots

Root phospholipids were significantly impacted, with 2.4-to-9.1-fold decreases in PG and four phosphatidylethanolamine (PE) at 7 days, and greater 4.1-to-72-fold decreases in three PI, three PG, four PC and six PE at 56 days. LysoPC(18:0) increased 3.1-fold ($p = 0.0255$) at 7 days and 7.7-fold ($p = 0.0091$) at 56 days. Carnitine increased 5.1-fold ($p = 0.0096$) and linoleate increased 2.9-fold ($p = 0.0463$) at 56 days. There were minor changes in glycerate, dihydroxyacetone phosphate, myoinositol, and uridine diphosphate-glucose. However, glycerol-3-phosphate and phosphorylcholine decreased 26-fold ($p = 0.0138$) and 70-fold ($p = 0.0138$) at 7 days, and 16- and 82-fold at 56 days ($p = 0.0003$), respectively.

Figure 18: Correlations of vetiver root metabolites in response to AMD, including A) pathways with regards to primary respiration and B) phospholipid metabolism. Compounds of interest contain 7 and/or 56 to represent respective days of treatment, where green shading represents upregulation while magenta shading represents downregulation, including fold change increments for 1.5-to-10-fold, 11-to-50-fold, and >50-fold.

Nucleosides and nucleotides of adenine, guanine, uracil and xanthine were primarily downregulated but less than phospholipids. Adenine was most impacted, where adenosine monophosphate (AMP) decreased 6.7-fold ($p = 0.0370$) and adenosine diphosphate decreased 8.1-fold ($p = 0.0209$) at 7 days. There was 2.4-to-13-fold downregulation of adenosine, AMP, deoxyadenosine, deoxyadenosine monophosphate, and cAMP at 56 days, where cAMP decreased the most ($p = <0.0001$). 5-methylthioadenosine decreased 14- and 51-fold at 7 ($p = 0.0389$) and 56 days ($p = 0.0032$), respectively, while 1-methyladenine increased 4.5-fold at 56 days ($p = 0.0337$).

Pyrimidines were largely unaffected, though deoxycytidine diphosphate decreased 35-fold at 7 days ($p = 0.0138$).

Root surfaces contain negatively charged phospholipid membranes and pectin cell walls with carboxy groups, where metals bind and interfere with neutralizing cations.³⁹ AMD-derived plaques on vetiver roots have been characterized primarily as Fe and Al,⁴ the most abundant metals. Plaques provide a mechanism for reducing toxicity, though they interfere with resource acquisition.¹⁸ Al reduces cell division and membrane integrity at the zone of elongation and root tips,²⁵ explaining why fresh growth originated from the root-stem transition. Oxidative stress and lipid peroxidation are common with metals,^{15,19,20,25} explaining why phospholipid metabolism was more affected. Meanwhile, most downregulated metabolites contained PO_4^{3-} , providing rationale for remobilization and recycling from older tissues.^{23,30}

3.1.1 Downregulation of Cellular Respiration and Glyoxylate Metabolism

Numerous respiration intermediates were downregulated (Figure 18), where fumarate decreased 10-fold ($p = 0.0179$), glyoxylate decreased 13-fold ($p = 0.0138$), isocitrate decreased 22-fold ($p = 0.0138$), and citrate decreased 50-fold ($p = 0.0138$) at 7 days. Meanwhile, malate decreased 10-fold ($p = 0.0011$), fumarate decreased 15-fold ($p = 0.0001$), and glyoxylate decreased 59-fold ($p = 0.0004$) at 56 days. Sugar metabolism was downregulated, where 3-PG decreased 7.2-fold ($p = 0.0278$) and PEP decreased 21-fold ($p = 0.0138$) at 7 days, whereas hexose sugars decreased 28-fold at 56 days ($p = 0.0043$). Moreover, ribose-phosphate decreased 15-fold ($p = 0.0396$) and fructose-phosphate decreased 37-fold ($p = 0.0138$) at 7 days, sucrose-phosphate decreased 54-fold ($p < 0.0001$) and 13-fold ($p = 0.0010$) at 7 and 56 days, respectively, and N-acetylglucosamine-1-phosphate decreased 21-fold ($p = 0.0138$) and 15-fold ($p = 0.0031$) at 56 days, respectively.

Interestingly, hexose diphosphate sugars increased 13-fold ($p = 0.0385$) and pyrophosphate increased 9.3-fold ($p = 0.0125$) at 56 days; however, time-dependence

revealed hexose diphosphate sugars decreased 42-fold ($p = 0.0115$) while pyrophosphate decreased 26-fold ($p = 0.0115$) in media-grown vetiver. There was downregulation of coenzyme metabolites, including pantothenate, riboflavin, flavin mononucleotide, flavin adenine dinucleotide and nicotinamide adenine dinucleotide (NAD). NAD decreased 7.0-fold ($p = 0.0255$) at 7 days and 16-fold at 56 days ($p = 0.0017$).

Downregulation of OAs could indicate root exudation; exudates are common in response to metals,^{18,23,24} PO_4^{3-} deprivation,^{23,30} and low pH.¹⁸ OAs like citrate, fumarate and malate could allow for rhizospheric metal chelation as opposed to cytosolic in the shoots,^{18,23,24,30,39} and may even function for pH adjustment.⁴¹ Glyoxylate is a monocarboxylate and less functional as an exudate, though could serve in conversion of FAs to carbohydrates.⁴² Al-OA complexes from chelation reportedly reduce Al^{3+} toxicity by 5-to-20-times.²⁴ Therefore, ROL and root exudates could account for metal precipitation and subsequent or concurrent formation of root plaques. Furthermore, OAs have lower affinity for pyrophosphate, increasing availability.²⁴ Upregulation of pyrophosphate and hexose diphosphate sugars at 56 days could suggest that PO_4^{3-} was more readily available.

3.1.1 Downregulation of Amino Acid Metabolism and Glutathione

Minor downregulation of AA metabolism included 2.0-to-7.9-fold decreases in common AAs and a variety of intermediates (Figure 18). Phosphorylated intermediates were significantly impacted, with carbamoylphosphate decreasing 301-fold at 7 days ($p = 0.0095$) and O-phosphotyrosine decreasing 81-fold at 7 days ($p = 0.0138$) and 38-fold at 56 days ($p = 0.0042$). Additionally, GSH decreased 45-fold ($p = 0.0371$) at 7 days. Meanwhile, time-dependence revealed that GSH decreased 29-fold ($p = 0.0080$) in media-grown vetiver, while GSSG decrease 24-fold ($p = 0.0491$).

Decreased carbamoylphosphate and O-phosphotyrosine likely resulted from phosphorus deprivation, where reduced downregulation at 56 days could suggest that PO_4^{3-} demands were better met. GSH was highly downregulated at 7 days while GSSG

went unchanged, which might suggest that GSH was being secreted as an exudate. In contrast, GSH could be used for phytochelatin biosynthesis or intracellular chelation.^{19,20,31} Time-dependent downregulation of glutathione in media-grown vetiver was unexpected but potentially explained by lower SO_4^{2-} and less demand for GSH. AMD-grown vetiver had a surplus of SO_4^{2-} at 56 days while GSH was in higher demand for stress management.

3.4. Root Exudates and Rhizospheric Metabolites from AMD

Few metabolites from AMD were within detection limits, though 30 increased or were only present in the company of vetiver. Minor increases included threonine, alanine, glycerate, cytosine, G-3-P, 2-ketogluconate, isocitrate, glucose, citrate, azelaic acid, pyridoxine, glycerol, ethanolamine, S-adenosylhomocysteine, shikimate-3-phosphate, and erythrose-4-phosphate, along with greater increases in adenosine, pyrophosphate, proline, and linoleate. Additionally, 10 metabolites were only detected with vetiver, including 2-methylcitrate, quinolinate, hexose-phosphate, methylsuccinate, 2-isopropylmalate, 2-hydroxyglutarate, citramalate, succinate, O-phosphorylethanolamine, and aconitate.

AMD metabolites are presumed root exudates from vetiver and rhizospheric metabolites from microbial activity, mostly consisting of OAs, sugars, and AAs. OAs and other carboxy-containing metabolites like AAs or FAs could function for metal chelation and precipitation,^{18,23,24,30,39} or influencing pH.⁴¹ Citrate, aconitate and isocitrate were suspected exudates from their downregulation in the roots. Shifts in pH have also been observed following exudation of inorganic anions like PO_4^{3-} .¹⁸ Carboxy- and amine-containing metabolites could have influenced pH of the AMD as there were mild but evident increases by 7 days. Moreover, increased pyrophosphate could be an indicator that phosphorus solubility had improved as previously hypothesized. Increases in proline were unexpected from shoot downregulation, though they likely served for ROS scavenging.^{25,27} Perhaps proline was transported from the shoots to be used as an exudate, explaining its downregulation.

Acknowledgements

The Office of Surface Mining Reclamation and Enforcement is acknowledged for funding this study (Grant #S12AC20001). Thanks to Saumik Panja for performing ion and metal analyses. Additional thanks to Yi Cui, Mona Fabian, Reginald Mullen, Alexis Rule, Nikki Saari, and Amanda Turcotte for assistance in the greenhouse and laboratory.

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Supplemental Information

Table S1: Differentially regulated metabolites demonstrating ≥ 1.5 -fold up- or downregulation ($p < 0.05$) between AMD- and media-grown vetiver shoots and roots at 7 and 56 days of growth.

Metabolite	Tissue	Time (d)	FC	p-value	Metabolite	Tissue	Time (d)	FC	p-value
linoleate	shoot	7	24	0.0080	threonine	shoot	56	6.7	0.0076
metSO	shoot	7	16	0.0144	succinate	shoot	56	6.6	0.0077
succinate	shoot	7	13	0.0080	aspartate	shoot	56	6.2	0.0079
ethanolamine	shoot	7	7.0	0.0331	xanthine	shoot	56	5.7	0.0079
lysoPC(16:0)	shoot	7	7.0	0.0440	metSO	shoot	56	5.7	0.0079
urea	shoot	7	4.4	0.0371	taurine	shoot	56	5.7	0.0079
lysoPC(18:0)	shoot	7	4.2	0.0354	mevalonate	shoot	56	5.7	0.0079
1-pyrroline-5-carboxylate	shoot	7	-12	0.0080	spermine	shoot	56	5.7	0.0079
OAA	shoot	56	233	0.0014	deoxyguanosine	shoot	56	5.7	0.0079
ornithine	shoot	56	207	0.0014	citrate	shoot	56	4.5	0.0118
citrulline	shoot	56	71	0.0018	riboflavin	shoot	56	4.1	0.0141
ethanolamine	shoot	56	49	0.0018	G-3-P	shoot	56	4.0	0.0141
octulose-P	shoot	56	49	0.0018	serine	shoot	56	4.0	0.0141
carnitine	shoot	56	44	0.0018	leucine	shoot	56	3.9	0.0150
arginine	shoot	56	30	0.0024	guanosine	shoot	56	3.7	0.0174
thymidine	shoot	56	21	0.0031	isoleucine	shoot	56	3.6	0.0174
ureidopropionate	shoot	56	18	0.0031	phenylalanine	shoot	56	3.6	0.0180
GSSG	shoot	56	14	0.0038	purine	shoot	56	3.3	0.0212
asparagine	shoot	56	14	0.0038	glycine	shoot	56	3.1	0.0243
argininosuccinate	shoot	56	12	0.0040	sarcosine	shoot	56	3.0	0.0253
histidine	shoot	56	12	0.0040	cytidine	shoot	56	3.0	0.0253
dimethylarginine	shoot	56	11	0.0041	GSH	shoot	56	3.0	0.0253
1-methyladenine	shoot	56	8.9	0.0055	adenosine	shoot	56	3.0	0.0253
deoxyadenosine	shoot	56	8.4	0.0059	ADP	shoot	56	2.9	0.0273
methionine	shoot	56	7.7	0.0064	glutamate	shoot	56	2.8	0.0301

Metabolite	Tissue	Time (d)	FC	p-value	Metabolite	Tissue	Time (d)	FC	p-value
alanine	shoot	7	2.7	0.0312	dCDP	root	7	35	0.0138
cytosine	shoot	7	2.7	0.0312	glycerol-3-P	root	7	26	0.0138
1-methyladenosine	shoot	7	2.6	0.0349	isocitrate	root	7	22	0.0138
PEP	shoot	7	2.5	0.0394	PEP	root	7	21	0.0138
uridine	shoot	7	2.5	0.0394	N-acetylglucosamine-1-P	root	7	21	0.0138
cAMP	shoot	7	19	0.0031	ribose-5-P	root	7	15	0.0396
hexose diphosphate	shoot	7	12	0.0040	5-methylthioadenosine	root	7	14	0.0389
1-pyrroline-5-carboxylate	shoot	7	6.9	0.0074	glyoxylate	root	7	13	0.0138
PG(34:2)	shoot	56	5.4	0.0083	UDP-glucose	root	7	11	0.0209
shikimate	shoot	56	4.7	0.0109	fumarate	root	7	10	0.0179
IMP	shoot	56	4.5	0.0118	PE(34:2)	root	7	9.1	0.0315
lysoPC(16:0)	shoot	56	4.4	0.0118	ADP	root	7	8.1	0.0209
hexose	shoot	56	3.4	0.0197	PG(34:1)	root	7	7.2	0.0322
PC(36:0)	shoot	56	3.3	0.0212	3-PG	root	7	7.2	0.0278
ribose-5-P	shoot	56	3.2	0.0212	NAD	root	7	7.0	0.0255
PI(34:0)	shoot	56	3.0	0.0253	PE(34:1)	root	7	6.8	0.0252
proline	shoot	56	2.8	0.0298	AMP	root	7	6.7	0.0370
inosine	shoot	56	2.5	0.0354	phenyllactate	root	7	6.7	0.0209
4-pyridoxate	shoot	56	2.3	0.0461	aconitate	root	7	6.0	0.0191
lysoPC(18:0)	root	7	3.1	0.0255	7-methylguanosine	root	7	5.9	0.0250
pyridoxine	root	7	2.9	0.0314	malate	root	7	5.7	0.0138
2-ketobutyrate	root	7	1.6	0.0371	gluconate	root	7	5.6	0.0138
DHAP	root	7	1.5	0.0255	glucosamine	root	7	5.0	0.0291
carbamoyl-P	root	7	301	0.0095	aspartate	root	7	4.4	0.0389
O-phosphotyrosine	root	7	81	0.0138	glutamate	root	7	4.2	0.0255
phosphorylcholine	root	7	70	0.0138	azelaic acid	root	7	4.1	0.0371
sucrose-P	root	7	54	<0.0001	myoinositol	root	7	3.9	0.0164
citrate	root	7	50	0.0138	PE(36:3)	root	7	3.8	0.0444
GSH	root	7	45	0.0371	deoxyribose-P	root	7	2.6	0.0388
fructose-P	root	7	37	0.0138	PE(36:2)	root	7	2.4	0.0370

Metabolite	Tissue	Time (d)	FC	p-value	Metabolite	Tissue	Time (d)	FC	p-value
glycerate	root	7	2.0	0.0493	uracil	root	56	2.0	0.0176
hexose disaccharide	root	7	1.9	0.0278	hypoxanthine	root	56	2.0	0.0408
N-acetylserine	root	7	1.8	0.0371	guanine	root	56	1.6	0.0166
hexose diphosphate	root	56	13	0.0385	PE(34:1)	root	56	10	0.0019
pyrophosphate	root	56	9.3	0.0125	myoinositol	root	56	9.5	0.0038
lysoPC(18:0)	root	56	7.7	0.0091	PG(34:1)	root	56	9.1	0.0102
carnitine	root	56	5.1	0.0096	4-hydroxybenzoate	root	56	9.0	0.0051
1-methyladenine	root	56	4.5	0.0337	gluconate	root	56	8.9	0.0254
phosphorylcholine	root	56	82	0.0003	AMP	root	56	8.3	<0.0001
PG(34:2)	root	56	72	0.0004	histidine	root	56	7.9	0.0060
glyoxylate	root	56	59	0.0004	shikimate	root	56	7.8	0.0066
5-methylthioadenosine	root	56	51	0.0032	aspartate	root	56	7.5	0.0102
O-phosphotyrosine	root	56	38	0.0042	PC(34:2)	root	56	7.4	0.0035
hexose	root	56	28	0.0043	glucosamine	root	56	7.0	0.0124
PI(36:2)	root	56	22	0.0116	citrulline	root	56	7.0	0.0181
PI(34:1)	root	56	19	0.0114	PE(36:2)	root	56	6.8	0.0035
glycerol-3-P	root	56	16	0.0003	dIMP	root	56	6.0	0.0104
NAD	root	56	16	0.0017	1-methylhistidine	root	56	6.0	0.0059
fumarate	root	56	15	0.0001	FAD	root	56	5.8	0.0066
N-acetylglucosamine-1-P	root	56	15	0.0031	dAMP	root	56	5.8	0.0059
cAMP	root	56	13	<0.0001	fructose-P	root	56	5.7	0.0008
PE(34:2)	root	56	13	0.0235	pantothenate	root	56	5.7	0.0030
sucrose-P	root	56	13	0.0010	acetylcholine	root	56	5.6	0.0105
phenyllactate	root	56	11	0.0091	arginine	root	56	5.5	0.0151
PC(34:1)	root	56	10	0.0035	glutamate	root	56	5.3	0.0026
malate	root	56	10	0.0011	S-adenosylhomocysteine	root	56	5.3	0.0135
betaine	root	56	4.2	0.0102	PE(36:3)	root	56	5.2	0.0011
linoleate	root	56	2.9	0.0463	PE(36:1)	root	56	5.2	0.0026
2-hydroxyglutarate	root	56	2.8	0.0114	PG(36:2)	root	56	5.1	0.0035
azelaic acid	root	56	2.1	0.0011	PC(36:2)	root	56	5.1	<0.0001

Metabolite	Tissue	Time (d)	FC	p-value
UDP-glucose	root	56	4.7	0.0030
PI(34:0)	root	56	4.6	0.0413
glycine	root	56	4.4	0.0044
PC(36:4)	root	56	4.2	0.0051
PE(36:4)	root	56	4.1	0.0106
xanthosine	root	56	4.1	0.0100
dGMP	root	56	3.7	0.0102
N-acetylaspartate	root	56	3.7	0.0187
ribose-5-P	root	56	3.7	0.0130
deoxyadenosine	root	56	3.6	0.0026
dITP	root	56	3.5	0.0082
glycerate	root	56	3.5	0.0204
riboflavin	root	56	3.3	0.0059
dGDP	root	56	3.0	0.0321
deoxyguanosine	root	56	3.0	0.0084
inosine	root	56	3.0	0.0126
FMN	root	56	3.0	0.0004
thiamine	root	56	2.7	0.0127
serine	root	56	2.4	0.0497
adenosine	root	56	2.4	0.0365
guanosine	root	56	2.3	0.0308
N-acetylserine	root	56	2.1	0.0332
tryptophan	root	56	2.1	0.0114
valine	root	56	2.0	0.0106
aconitate	root	56	2.0	0.0259
sucrose	root	56	2.0	0.0038

Summary

The short-term (30-day) bench-scale experiment from Chapter 1 demonstrated the potential of vetiver for the remediation of AMD-impacted waters from the Tab-Simco site. Vetiver was not only tolerant of the AMD but able to increase pH while significantly decreasing conductivity, SO_4^{2-} and Fe. Vetiver demonstrated higher uptake of Fe, Zn and Cu, particularly at higher planting densities, with lower uptake of Al, Ni and Pb. Translocation was minimal for Fe, Al and Pb, likely due to rhizofiltration and Fe plaque formations, while greater for Cu, Ni and Zn were translocated. The higher planting densities of 6 to 8 g L⁻¹ (three to four plants per liter of AMD) were more efficient, suggesting that a density of ≥ 6 g L⁻¹ would be effective for remediation.

The most significant limitation from the short-term bench-scale experiment came from the air circulator interference, which made it impossible to determine the removal efficiency for SO_4^{2-} and metals. Limitations were also apparent with the short experiment length and relatively minor variations in planting density. These limitations were further explored through the experiments described in Chapter 2, initially by removing the air circulators and significantly extending the time for treatment. Limitations in planting density were explored by increasing the planting density, particularly with the microcosm experiment by ensuring complete root coverage.

The year-long mesocosm and microcosm experiments from Chapter 2 demonstrated the effective of vetiver for more applied remediation of AMD-impacted waters. From the larger-scale mesocosm experiment, there was high net removal of SO_4^{2-} from the unfiltered AMD (28%) as well as the filtered AMD (45%) as a result of the vetiver FTW rafts. There was also high removal of Fe (81%) and Pb (81%) from the unfiltered AMD, with lower removal of Ni (38%), Zn (35%), Mn (27%), Cr (21%), Al (11%) and Cu (8.0%). From the smaller-scale microcosm experiment, there was near complete removal of SO_4^{2-} (91%) and metals (90-100%), excluding Pb (15%) and Cu (0.0%). This demonstrates the benefits of having greater root coverage. In both the

mesocosm and microcosm experiments, vetiver was able to increase pH to a less acidic range (≥ 3.4). The reduction in plant health and remediation that was observed over the first 168 days of the mesocosm experiment confirmed 6 g L^{-1} was not a sufficient planting density for waters from the SRB. However, increasing the planting density to 16 g L^{-1} alleviated the outcome. These results have also demonstrated the benefits that handheld probes, such as those for DO and conductivity, could serve for on-site monitoring. Harvested vetiver biomass was confirmed to not be an RCRA hazard by TCLP, confirming they are safe for disposal.

Vetiver from the mesocosm experimented had greater accumulations of Fe ($17,300 \text{ } \mu\text{g g}^{-1}$), Al ($3405 \text{ } \mu\text{g g}^{-1}$), Mn ($1080 \text{ } \mu\text{g g}^{-1}$), Zn ($113 \text{ } \mu\text{g g}^{-1}$) and Cr ($62.3 \text{ } \mu\text{g g}^{-1}$), with lower Ni ($41.2 \text{ } \mu\text{g g}^{-1}$), Cu ($24.6 \text{ } \mu\text{g g}^{-1}$) and Pb ($5.37 \text{ } \mu\text{g g}^{-1}$). Similar trends were observed with the microcosm experiment. Metals were mainly accumulated along the root surface from rhizofiltration as plaques ($\text{TF} \leq 0.67$), which had an amorphous appearance with an abundance of Fe and lesser Al and Pb. In opposition, there was higher translocation of Zn ($\text{TF} = 0.93$) and Mn ($\text{TF} = 0.88$).

The most significant limitation from the mesocosm experiment was the lack of statistical replication, which was partly addressed by the microcosm experiment. The microcosm suggested that complete root coverage would ensure significant improvements in water quality, demonstrating that deeper and more densely rooted vetiver would better serve for field application. Additionally, the mesocosm verified that the plywood surfaces of the rafts were sufficient for supporting the plants. However, the plywood was a convenient substrate for microbial growth and was not suitable for reuse after being removed from the treated waters. Therefore, plywood surfaces would serve well for single application but not for repeated use.

Limitations in both mesocosms and microcosms came from lack of knowledge on speciation of redox active metals, particularly Fe and Cr, as well as the ultimate fates of SO_4^{2-} , Cu, and Pb. Speculation has sparked some potentially valid hypotheses; however, these particular limitations would require further investigation. One final limitation with

these remediation experiments comes from the lack of tolerance that vetiver has for colder temperatures. Therefore, vetiver would not likely be a suitable candidate for the remediation of AMD-impacted waters under extremely temperate conditions.

Metabolomics from Chapter 3 suggested that oxidative stress, resource deprivation, and dehydration were among the main forms of abiotic stress in AMD. Dramatic shifts were observed in vetiver shoot metabolism after longer-term AMD exposure (56-days), mainly through the upregulation of AA and glutathione metabolism, along with metabolites relating to respiration and photosynthesis, and downregulation of phosphorylated metabolites, including phospholipids and nucleotides. There was particular upregulation of ornithine and OAA, which were thought to function for nitrogen storage and intracellular metal-chelation, respectively. Interestingly, there was prominent downregulation of 1-pyrroline-5-carboxylate, and minor downregulation of proline, which are typically upregulated in response to abiotic stresses.

In opposition, a majority of root metabolites were downregulated, with a particular emphasis on phosphorylated metabolites such as phospholipids and phosphorylated amino acids. This suggested that phosphorus deficiency was of particular importance, which was suspected from the low pH of AMD. Moreover, recycling of phosphorylated compounds would allow vetiver to meet demands for phosphorus. There was also downregulation of OAs relating to respiration and glyoxylate, which were thought to have been secreted from the roots for rhizospheric metal-chelation. These findings have revealed some of the metabolic shifts induced by AMD in vetiver, which could lead to improved understanding and application of FTWs.

An obvious limitation of metabolomics comes from the aspect of data mining in opposition to testing individual hypotheses. Nevertheless, hypotheses derived from metabolomics could be further investigated using additional broad-spectrum systems analyses such as transcriptomics or proteomics, along with follow-up confirmation of differential transcripts and proteins. Additionally, the outcome of combined stresses such as presented in AMD could be better elucidated by comparing with individual stresses.

However, this would be virtually impossible to address given the great number of known variables along with the potential of unknowns.

Appendix A

The figures and tables included in this appendix comprise the entirety of qualitative and quantitative records collected throughout the phytoremediation experiments described and presented in Chapters 1 and 2 of this dissertation.

Figure A1: Images of the short-term bench-scale experiment setup from Chapter 1, including A) direct and B) angled images of the hydroponic containers with vetiver at varying densities.

Table A1: Comprehensive numerical data collected for water sample characterization throughout the short-term bench-scale experiment from Chapter 1.

Container	Water type	Treatment	Time (d)	pH	EC ($\mu\text{S cm}^{-1}$)	SO_4^{2-} (mg L^{-1})	Metals ($\mu\text{g L}^{-1}$)					
							Fe	Al	Zn	Ni	Cu	Pb
01	AMD	4 plants	0	2.99	2997	2641	6616	19570	495.70	449.50	33.50	12.97
02	AMD	4 plants	0	2.69	3173	2730	10380	14250	403.40	397.10	16.96	8.11
03	AMD	4 plants	0	2.62	3350	2700	13250	11880	419.50	388.10	21.30	14.22
04	AMD	3 plants	0	2.65	3317	2650	11220	11170	351.90	368.30	15.70	5.82
05	AMD	3 plants	0	2.79	3240	2650	8490	14400	374.90	378.40	15.65	8.64
06	AMD	3 plants	0	2.64	3360	2710	10940	11630	369.70	378.10	13.66	7.60
07	AMD	2 plants	0	2.54	3447	2710	11270	10280	404.90	397.10	19.06	10.18
08	AMD	2 plants	0	2.57	3423	2680	11950	11760	412.80	391.00	19.48	14.53
09	AMD	2 plants	0	2.56	3370	2650	11190	10070	351.50	379.90	19.73	7.85
10	AMD	No plants	0	2.57	3440	2610	14250	8134	377.60	373.40	17.53	9.20
11	AMD	No plants	0	2.63	3487	2590	12180	8024	350.20	369.70	31.68	9.09
12	AMD	No plants	0	2.57	3527	2620	13980	8237	372.30	375.10	32.26	6.33
13	Media	4 plants	0	5.26	764	82.60	405	50.12	27.02	2.82	22.61	1.39
14	Media	4 plants	0	5.05	836	89.39	381.1	57.15	19.71	2.99	19.21	0.74
15	Media	4 plants	0	5.03	798	85.54	362.4	62.54	19.57	2.81	25.31	0.51
16	Media	3 plants	0	5.08	894	98.28	359.2	43.40	19.96	3.12	15.77	2.18
17	Media	3 plants	0	5.07	924	106.1	353.6	53.26	21.91	3.15	18.66	0.27
18	Media	3 plants	0	5.11	961	118.7	316.9	47.95	21.03	3.23	21.23	0.26
19	Media	2 plants	0	5.13	840	77.34	395.3	67.82	38.72	6.63	24.89	0.44
20	Media	2 plants	0	5.06	821	75.41	353.8	55.69	22.69	4.51	15.91	0.24
21	Media	2 plants	0	5.14	946	106.0	308.9	33.61	22.02	4.81	18.27	0.21
22	Media	No plants	0	5.09	829	94.40	388.4	2.51	23.02	4.21	14.95	0.43
23	Media	No plants	0	5.03	797	67.65	266.2	2.66	14.55	3.42	17.03	0.27
24	Media	No plants	0	5.06	781	73.72	247.5	1.60	13.25	3.34	19.40	0.22

01	AMD	4 plants	2	3.60	2883	NT	NT	NT	NT	NT	NT	NT
02	AMD	4 plants	2	3.10	3007	NT	NT	NT	NT	NT	NT	NT
03	AMD	4 plants	2	3.15	3063	NT	NT	NT	NT	NT	NT	NT
04	AMD	3 plants	2	3.06	2967	NT	NT	NT	NT	NT	NT	NT
05	AMD	3 plants	2	3.26	2923	NT	NT	NT	NT	NT	NT	NT
06	AMD	3 plants	2	2.98	3060	NT	NT	NT	NT	NT	NT	NT
07	AMD	2 plants	2	2.67	3287	NT	NT	NT	NT	NT	NT	NT
08	AMD	2 plants	2	2.78	3200	NT	NT	NT	NT	NT	NT	NT
09	AMD	2 plants	2	2.73	3260	NT	NT	NT	NT	NT	NT	NT
10	AMD	No plants	2	2.58	3553	NT	NT	NT	NT	NT	NT	NT
11	AMD	No plants	2	2.59	3537	NT	NT	NT	NT	NT	NT	NT
12	AMD	No plants	2	2.59	3567	NT	NT	NT	NT	NT	NT	NT
13	Media	4 plants	2	5.42	829	NT	NT	NT	NT	NT	NT	NT
14	Media	4 plants	2	5.12	884	NT	NT	NT	NT	NT	NT	NT
15	Media	4 plants	2	5.16	854	NT	NT	NT	NT	NT	NT	NT
16	Media	3 plants	2	5.17	940	NT	NT	NT	NT	NT	NT	NT
17	Media	3 plants	2	5.12	942	NT	NT	NT	NT	NT	NT	NT
18	Media	3 plants	2	5.09	1020	NT	NT	NT	NT	NT	NT	NT
19	Media	2 plants	2	5.10	873	NT	NT	NT	NT	NT	NT	NT
20	Media	2 plants	2	5.09	859	NT	NT	NT	NT	NT	NT	NT
21	Media	2 plants	2	5.10	983	NT	NT	NT	NT	NT	NT	NT
22	Media	No plants	2	5.17	883	NT	NT	NT	NT	NT	NT	NT
23	Media	No plants	2	5.17	847	NT	NT	NT	NT	NT	NT	NT
24	Media	No plants	2	5.20	824	NT	NT	NT	NT	NT	NT	NT
01	AMD	4 plants	5	3.88	2770	NT	NT	NT	NT	NT	NT	NT
02	AMD	4 plants	5	3.06	3070	NT	NT	NT	NT	NT	NT	NT
03	AMD	4 plants	5	3.10	3030	NT	NT	NT	NT	NT	NT	NT
04	AMD	3 plants	5	3.39	2910	NT	NT	NT	NT	NT	NT	NT
05	AMD	3 plants	5	3.38	2900	NT	NT	NT	NT	NT	NT	NT
06	AMD	3 plants	5	3.01	3020	NT	NT	NT	NT	NT	NT	NT

07	AMD	2 plants	5	2.78	3240	NT	NT	NT	NT	NT	NT	NT
08	AMD	2 plants	5	2.83	3140	NT	NT	NT	NT	NT	NT	NT
09	AMD	2 plants	5	2.77	3230	NT	NT	NT	NT	NT	NT	NT
10	AMD	No plants	5	2.60	3630	NT	NT	NT	NT	NT	NT	NT
11	AMD	No plants	5	2.65	3440	NT	NT	NT	NT	NT	NT	NT
12	AMD	No plants	5	2.60	3560	NT	NT	NT	NT	NT	NT	NT
13	Media	4 plants	5	4.98	832	NT	NT	NT	NT	NT	NT	NT
14	Media	4 plants	5	4.92	911	NT	NT	NT	NT	NT	NT	NT
15	Media	4 plants	5	4.90	867	NT	NT	NT	NT	NT	NT	NT
16	Media	3 plants	5	4.78	950	NT	NT	NT	NT	NT	NT	NT
17	Media	3 plants	5	4.77	1020	NT	NT	NT	NT	NT	NT	NT
18	Media	3 plants	5	5.01	1050	NT	NT	NT	NT	NT	NT	NT
19	Media	2 plants	5	4.84	961	NT	NT	NT	NT	NT	NT	NT
20	Media	2 plants	5	4.89	903	NT	NT	NT	NT	NT	NT	NT
21	Media	2 plants	5	4.79	1070	NT	NT	NT	NT	NT	NT	NT
22	Media	No plants	5	4.98	935	NT	NT	NT	NT	NT	NT	NT
23	Media	No plants	5	4.91	926	NT	NT	NT	NT	NT	NT	NT
24	Media	No plants	5	4.84	852	NT	NT	NT	NT	NT	NT	NT
01	AMD	4 plants	10	3.89	2820	2630	NT	NT	NT	NT	NT	NT
02	AMD	4 plants	10	3.02	3170	2610	NT	NT	NT	NT	NT	NT
03	AMD	4 plants	10	3.04	3130	2580	NT	NT	NT	NT	NT	NT
04	AMD	3 plants	10	3.37	3010	2550	NT	NT	NT	NT	NT	NT
05	AMD	3 plants	10	3.35	2900	2570	NT	NT	NT	NT	NT	NT
06	AMD	3 plants	10	3.52	2920	2540	NT	NT	NT	NT	NT	NT
07	AMD	2 plants	10	2.80	3280	2570	NT	NT	NT	NT	NT	NT
08	AMD	2 plants	10	2.81	3300	2580	NT	NT	NT	NT	NT	NT
09	AMD	2 plants	10	2.73	3380	2640	NT	NT	NT	NT	NT	NT
10	AMD	No plants	10	2.54	3850	2830	NT	NT	NT	NT	NT	NT
11	AMD	No plants	10	2.60	3680	2750	NT	NT	NT	NT	NT	NT
12	AMD	No plants	10	2.56	3780	2710	NT	NT	NT	NT	NT	NT

13	Media	4 plants	10	4.76	882	NT	NT	NT	NT	NT	NT	NT
14	Media	4 plants	10	4.62	964	NT	NT	NT	NT	NT	NT	NT
15	Media	4 plants	10	4.73	893	NT	NT	NT	NT	NT	NT	NT
16	Media	3 plants	10	4.59	981	NT	NT	NT	NT	NT	NT	NT
17	Media	3 plants	10	4.38	1070	NT	NT	NT	NT	NT	NT	NT
18	Media	3 plants	10	4.74	1090	NT	NT	NT	NT	NT	NT	NT
19	Media	2 plants	10	4.56	1020	NT	NT	NT	NT	NT	NT	NT
20	Media	2 plants	10	4.63	943	NT	NT	NT	NT	NT	NT	NT
21	Media	2 plants	10	4.48	1150	NT	NT	NT	NT	NT	NT	NT
22	Media	No plants	10	4.76	977	NT	NT	NT	NT	NT	NT	NT
23	Media	No plants	10	5.24	996	NT	NT	NT	NT	NT	NT	NT
24	Media	No plants	10	4.58	899	NT	NT	NT	NT	NT	NT	NT
01	AMD	4 plants	20	4.24	1860	1680	NT	NT	NT	NT	NT	NT
02	AMD	4 plants	20	4.26	1800	1588	NT	NT	NT	NT	NT	NT
03	AMD	4 plants	20	4.20	1990	1741	NT	NT	NT	NT	NT	NT
04	AMD	3 plants	20	3.92	2290	1960	NT	NT	NT	NT	NT	NT
05	AMD	3 plants	20	4.30	2300	1968	NT	NT	NT	NT	NT	NT
06	AMD	3 plants	20	4.34	2250	1970	NT	NT	NT	NT	NT	NT
07	AMD	2 plants	20	3.28	2350	1850	NT	NT	NT	NT	NT	NT
08	AMD	2 plants	20	3.30	2430	1925	NT	NT	NT	NT	NT	NT
09	AMD	2 plants	20	3.35	2280	1866	NT	NT	NT	NT	NT	NT
10	AMD	No plants	20	2.80	2870	2056	NT	NT	NT	NT	NT	NT
11	AMD	No plants	20	2.87	2710	2030	NT	NT	NT	NT	NT	NT
12	AMD	No plants	20	2.83	2700	1962	NT	NT	NT	NT	NT	NT
13	Media	4 plants	20	4.72	576	NT	NT	NT	NT	NT	NT	NT
14	Media	4 plants	20	4.70	547	NT	NT	NT	NT	NT	NT	NT
15	Media	4 plants	20	4.71	644	NT	NT	NT	NT	NT	NT	NT
16	Media	3 plants	20	4.58	709	NT	NT	NT	NT	NT	NT	NT
17	Media	3 plants	20	4.57	702	NT	NT	NT	NT	NT	NT	NT
18	Media	3 plants	20	4.56	817	NT	NT	NT	NT	NT	NT	NT

19	Media	2 plants	20	4.49	635	NT	NT	NT	NT	NT	NT	NT
20	Media	2 plants	20	4.65	630	NT	NT	NT	NT	NT	NT	NT
21	Media	2 plants	20	4.43	845	NT	NT	NT	NT	NT	NT	NT
22	Media	No plants	20	5.73	645	NT	NT	NT	NT	NT	NT	NT
23	Media	No plants	20	5.90	644	NT	NT	NT	NT	NT	NT	NT
24	Media	No plants	20	4.74	650	NT	NT	NT	NT	NT	NT	NT
01	AMD	4 plants	30	4.25	1670	1575.26	22.95	7583	276.80	300.50	17.03	0.80
02	AMD	4 plants	30	4.20	1590	1425.23	13.79	8118	288.50	306.10	19.73	0.43
03	AMD	4 plants	30	4.11	1830	1656.36	17.80	9733	350.60	380.00	35.78	0.41
04	AMD	3 plants	30	4.10	2050	1782.03	20.63	10530	355.50	383.90	24.36	0.50
05	AMD	3 plants	30	4.04	2150	1868.39	12.98	9613	318.90	368.10	21.93	0.66
06	AMD	3 plants	30	4.03	1910	1751.51	14.12	10540	376.30	409.70	18.35	0.24
07	AMD	2 plants	30	3.92	1940	1669.17	30.61	11880	359.60	388.70	18.51	0.50
08	AMD	2 plants	30	3.56	1950	1736.79	75.36	11250	362.70	387.70	41.57	0.73
09	AMD	2 plants	30	3.82	1830	1607.77	36.61	10270	311.50	328.90	20.59	1.27
10	AMD	No plants	30	2.83	2340	1595.92	685.4	5741	248.40	278.00	15.06	0.41
11	AMD	No plants	30	2.89	2450	1750.20	627.5	8755	276.70	321.30	19.13	2.06
12	AMD	No plants	30	2.84	2320	1572.33	686.3	7137	267.10	303.70	15.57	0.87
13	Media	4 plants	30	4.50	484	NT	262.7	216.8	14.23	2.86	4.28	0.00
14	Media	4 plants	30	4.51	359	NT	184.3	100.4	2.94	1.85	2.95	0.04
15	Media	4 plants	30	4.31	543	NT	266.8	238.7	6.18	2.21	3.86	0.37
16	Media	3 plants	30	4.25	629	NT	274.8	132.6	6.14	2.19	4.49	1.96
17	Media	3 plants	30	4.20	591	NT	265.8	240.8	4.77	2.54	3.57	0.23
18	Media	3 plants	30	4.23	678	NT	273.5	246.8	6.40	2.66	2.69	0.02
19	Media	2 plants	30	4.09	571	NT	358.9	231.3	22.76	10.69	6.31	0.05
20	Media	2 plants	30	4.31	427	NT	257.1	95.86	8.50	3.30	4.10	0.03
21	Media	2 plants	30	4.04	706	NT	355.7	277.4	33.31	4.23	3.93	0.03
22	Media	No plants	30	6.19	394	NT	165.7	2.54	4.99	1.01	5.05	0.26
23	Media	No plants	30	6.80	428	NT	142.8	6.87	15.83	1.42	3.66	0.51
24	Media	No plants	30	4.64	600	NT	176.3	28.49	15.14	1.75	5.37	0.22

Table A2: Fresh biomass, shoot (S) length, and root (R) length measurements for vetiver throughout the short-term bench-scale experiment from Chapter 1.

Container	Water type	Treatment	Plant ID	Time (d)	Fresh biomass (g)	Length (cm)	
						S	R
01	AMD	4 plants	V01	0	5.51	33.6	32.6
01	AMD	4 plants	V02	0	6.25	36.9	30.0
01	AMD	4 plants	V03	0	9.80	39.0	26.1
01	AMD	4 plants	V04	0	4.09	38.2	18.6
02	AMD	4 plants	V05	0	6.10	27.6	26.2
02	AMD	4 plants	V06	0	4.02	14.4	29.1
02	AMD	4 plants	V07	0	9.12	33.1	23.5
02	AMD	4 plants	V08	0	6.68	42.3	20.9
03	AMD	4 plants	V09	0	8.38	43.2	37.0
03	AMD	4 plants	V10	0	3.67	12.2	41.6
03	AMD	4 plants	V11	0	5.32	31.5	29.5
03	AMD	4 plants	V12	0	6.82	35.9	28.0
04	AMD	3 plants	V13	0	6.36	42.4	32.0
04	AMD	3 plants	V14	0	4.97	32.9	28.7
04	AMD	3 plants	V15	0	9.17	30.9	27.5
05	AMD	3 plants	V16	0	9.24	22.4	35.6
05	AMD	3 plants	V17	0	4.16	21.6	31.0
05	AMD	3 plants	V18	0	6.37	43.8	14.2
06	AMD	3 plants	V19	0	4.07	26.6	35.0
06	AMD	3 plants	V20	0	8.99	45.8	25.5
06	AMD	3 plants	V21	0	6.74	44.3	22.8
07	AMD	2 plants	V22	0	6.88	40.7	49.2
07	AMD	2 plants	V23	0	5.81	34.0	21.6
08	AMD	2 plants	V24	0	8.10	36.1	33.6
08	AMD	2 plants	V25	0	6.15	33.4	30.0
09	AMD	2 plants	V26	0	8.96	31.5	39.2
09	AMD	2 plants	V27	0	6.42	38.2	31.9
13	Media	4 plants	V28	0	8.59	36.0	37.3
13	Media	4 plants	V29	0	5.36	38.6	28.1
13	Media	4 plants	V30	0	4.21	32.0	31.5
13	Media	4 plants	V31	0	6.69	32.4	27.0
14	Media	4 plants	V32	0	8.33	38.6	37.2
14	Media	4 plants	V33	0	7.09	37.9	26.5
14	Media	4 plants	V34	0	3.99	37.2	22.1
14	Media	4 plants	V35	0	5.59	38.4	20.3
15	Media	4 plants	V36	0	7.50	31.8	31.4
15	Media	4 plants	V37	0	7.88	33.9	33.6
15	Media	4 plants	V38	0	5.72	25.0	22.9
15	Media	4 plants	V39	0	4.78	33.1	12.5
16	Media	3 plants	V40	0	3.76	35.9	20.3
16	Media	3 plants	V41	0	6.63	34.9	20.2
16	Media	3 plants	V42	0	9.01	32.1	17.5

17	Media	3 plants	V43	0	8.77	37.8	35.6
17	Media	3 plants	V44	0	7.28	31.5	25.9
17	Media	3 plants	V45	0	3.35	36.1	19.0
18	Media	3 plants	V46	0	7.67	35.8	37.4
18	Media	3 plants	V47	0	7.42	34.6	35.8
18	Media	3 plants	V48	0	3.07	35.9	19.7
19	Media	2 plants	V49	0	6.65	39.9	40.5
19	Media	2 plants	V50	0	9.05	39.8	17.9
20	Media	2 plants	V51	0	6.42	9.4	36.5
20	Media	2 plants	V52	0	7.37	39.1	22.3
21	Media	2 plants	V53	0	7.45	36.9	32.3
21	Media	2 plants	V54	0	8.16	37.5	26.4
01	AMD	4 plants	V01	5	5.67	34.4	33.0
01	AMD	4 plants	V02	5	5.73	37.7	29.5
01	AMD	4 plants	V03	5	9.76	39.1	26.0
01	AMD	4 plants	V04	5	4.18	39.8	18.2
02	AMD	4 plants	V05	5	6.06	28.5	25.5
02	AMD	4 plants	V06	5	4.04	14.7	28.9
02	AMD	4 plants	V07	5	9.54	38.8	23.6
02	AMD	4 plants	V08	5	6.75	43.5	22.0
03	AMD	4 plants	V09	5	8.33	43.2	36.8
03	AMD	4 plants	V10	5	3.30	12.1	41.7
03	AMD	4 plants	V11	5	5.37	32.5	28.5
03	AMD	4 plants	V12	5	6.66	36.5	26.1
04	AMD	3 plants	V13	5	6.74	47.2	32.4
04	AMD	3 plants	V14	5	5.05	33.2	28.8
04	AMD	3 plants	V15	5	9.65	33.9	27.5
05	AMD	3 plants	V16	5	8.53	22.1	35.1
05	AMD	3 plants	V17	5	4.27	21.9	31.1
05	AMD	3 plants	V18	5	6.60	44.3	15.6
06	AMD	3 plants	V19	5	4.19	28.1	34.8
06	AMD	3 plants	V20	5	8.84	37.0	24.1
06	AMD	3 plants	V21	5	6.39	44.4	22.5
07	AMD	2 plants	V22	5	6.84	43.0	49.1
07	AMD	2 plants	V23	5	5.73	34.3	25.8
08	AMD	2 plants	V24	5	8.18	37.0	33.6
08	AMD	2 plants	V25	5	6.51	35.4	30.0
09	AMD	2 plants	V26	5	8.96	32.5	38.9
09	AMD	2 plants	V27	5	6.45	40.9	33.3
13	Media	4 plants	V28	5	8.50	36.6	36.9
13	Media	4 plants	V29	5	5.55	40.3	27.9
13	Media	4 plants	V30	5	4.25	32.3	21.3
13	Media	4 plants	V31	5	6.57	33.2	26.2
14	Media	4 plants	V32	5	8.28	41.8	36.1
14	Media	4 plants	V33	5	6.73	37.0	26.2
14	Media	4 plants	V34	5	4.06	38.5	21.6
14	Media	4 plants	V35	5	5.48	38.5	19.4

15	Media	4 plants	V36	5	7.59	31.8	31.0
15	Media	4 plants	V37	5	7.87	34.4	34.3
15	Media	4 plants	V38	5	5.61	26.0	22.8
15	Media	4 plants	V39	5	4.80	36.1	13.5
16	Media	3 plants	V40	5	3.66	37.4	17.3
16	Media	3 plants	V41	5	6.84	39.1	20.9
16	Media	3 plants	V42	5	6.45	32.4	17.1
17	Media	3 plants	V43	5	8.71	38.8	33.2
17	Media	3 plants	V44	5	7.34	33.1	24.9
17	Media	3 plants	V45	5	3.44	38.2	17.7
18	Media	3 plants	V46	5	7.68	38.4	36.2
18	Media	3 plants	V47	5	7.27	35.1	35.4
18	Media	3 plants	V48	5	3.05	37.9	20.2
19	Media	2 plants	V49	5	6.91	43.2	41.0
19	Media	2 plants	V50	5	9.27	42.8	17.8
20	Media	2 plants	V51	5	6.80	9.4	37.2
20	Media	2 plants	V52	5	7.38	40.9	23.1
21	Media	2 plants	V53	5	7.48	39.1	32.5
21	Media	2 plants	V54	5	8.25	40.1	26.8
01	AMD	4 plants	V01	15	6.18	45.9	33.2
01	AMD	4 plants	V02	15	4.48	37.7	30.4
01	AMD	4 plants	V03	15	7.28	39.1	26.0
01	AMD	4 plants	V04	15	4.39	41.2	18.4
02	AMD	4 plants	V05	15	6.08	28.3	25.7
02	AMD	4 plants	V06	15	3.87	14.7	28.5
02	AMD	4 plants	V07	15	9.77	54.5	23.7
02	AMD	4 plants	V08	15	6.88	45.1	22.3
03	AMD	4 plants	V09	15	7.89	43.3	36.1
03	AMD	4 plants	V10	15	3.10	12.5	41.6
03	AMD	4 plants	V11	15	5.42	33.5	28.7
03	AMD	4 plants	V12	15	6.77	38.4	26.4
04	AMD	3 plants	V13	15	6.96	52.6	31.7
04	AMD	3 plants	V14	15	8.93	34.4	28.2
04	AMD	3 plants	V15	15	10.28	45.5	27.0
05	AMD	3 plants	V16	15	6.24	22.0	34.7
05	AMD	3 plants	V17	15	4.30	21.9	30.9
05	AMD	3 plants	V18	15	7.06	44.4	15.8
06	AMD	3 plants	V19	15	4.34	34.9	30.1
06	AMD	3 plants	V20	15	8.85	39.0	24.4
06	AMD	3 plants	V21	15	6.41	47.7	22.0
07	AMD	2 plants	V22	15	6.98	53.2	48.5
07	AMD	2 plants	V23	15	5.44	35.1	26.1
08	AMD	2 plants	V24	15	8.18	37.6	34.0
08	AMD	2 plants	V25	15	6.46	37.4	30.0
09	AMD	2 plants	V26	15	8.46	32.5	39.0
09	AMD	2 plants	V27	15	5.91	42.9	33.4

13	Media	4 plants	V28	15	7.89	38.5	36.6
13	Media	4 plants	V29	15	5.33	41.1	26.0
13	Media	4 plants	V30	15	4.38	33.4	21.2
13	Media	4 plants	V31	15	6.49	33.2	25.9
14	Media	4 plants	V32	15	8.24	45.6	36.6
14	Media	4 plants	V33	15	6.61	37.2	25.8
14	Media	4 plants	V34	15	4.14	40.3	22.0
14	Media	4 plants	V35	15	5.62	40.9	20.1
15	Media	4 plants	V36	15	7.25	32.4	31.6
15	Media	4 plants	V37	15	7.91	34.9	34.2
15	Media	4 plants	V38	15	5.28	26.7	20.5
15	Media	4 plants	V39	15	4.71	40.6	13.8
16	Media	3 plants	V40	15	3.74	41.4	15.1
16	Media	3 plants	V41	15	7.30	48.6	19.4
16	Media	3 plants	V42	15	3.96	32.4	17.2
17	Media	3 plants	V43	15	8.59	42.6	33.5
17	Media	3 plants	V44	15	7.13	36.5	25.8
17	Media	3 plants	V45	15	3.16	40.8	18.3
18	Media	3 plants	V46	15	7.50	42.1	36.6
18	Media	3 plants	V47	15	7.10	37.7	35.5
18	Media	3 plants	V48	15	3.15	39.8	19.9
19	Media	2 plants	V49	15	7.23	50.6	40.4
19	Media	2 plants	V50	15	9.22	47.2	16.9
20	Media	2 plants	V51	15	6.62	9.2	36.0
20	Media	2 plants	V52	15	7.70	41.4	23.4
21	Media	2 plants	V53	15	7.59	46.9	33.0
21	Media	2 plants	V54	15	8.45	46.3	26.8
01	AMD	4 plants	V01	30	7.31	60.6	34.1
01	AMD	4 plants	V02	30	3.83	37.6	29.7
01	AMD	4 plants	V03	30	7.22	19.1	26.4
01	AMD	4 plants	V04	30	4.90	50.9	25.6
02	AMD	4 plants	V05	30	6.26	28.3	25.8
02	AMD	4 plants	V06	30	3.27	14.8	28.5
02	AMD	4 plants	V07	30	11.08	64.8	23.5
02	AMD	4 plants	V08	30	6.60	55.0	22.5
03	AMD	4 plants	V09	30	6.93	43.8	36.4
03	AMD	4 plants	V10	30	2.82	12.0	41.8
03	AMD	4 plants	V11	30	5.43	35.8	28.6
03	AMD	4 plants	V12	30	6.98	48.4	27.2
04	AMD	3 plants	V13	30	7.56	71.0	31.1
04	AMD	3 plants	V14	30	4.84	39.1	28.3
04	AMD	3 plants	V15	30	11.34	64.2	27.2
05	AMD	3 plants	V16	30	5.76	22.0	34.6
05	AMD	3 plants	V17	30	4.26	23.2	31.2
05	AMD	3 plants	V18	30	8.42	44.3	29.2

06	AMD	3 plants	V19	30	4.44	42.1	27.2
06	AMD	3 plants	V20	30	8.96	54.4	22.6
06	AMD	3 plants	V21	30	6.61	56.9	22.4
07	AMD	2 plants	V22	30	7.13	70.4	48.2
07	AMD	2 plants	V23	30	5.68	41.2	26.6
08	AMD	2 plants	V24	30	8.56	53.9	33.8
08	AMD	2 plants	V25	30	6.79	37.7	28.6
09	AMD	2 plants	V26	30	8.11	32.1	39.2
09	AMD	2 plants	V27	30	6.52	59.3	30.3
13	Media	4 plants	V28	30	8.28	49.6	36.7
13	Media	4 plants	V29	30	5.78	45.5	22.2
13	Media	4 plants	V30	30	4.69	37.7	21.5
13	Media	4 plants	V31	30	6.40	33.5	26.0
14	Media	4 plants	V32	30	8.74	55.1	37.3
14	Media	4 plants	V33	30	6.97	39.6	26.2
14	Media	4 plants	V34	30	4.98	40.8	21.7
14	Media	4 plants	V35	30	5.37	47.6	20.2
15	Media	4 plants	V36	30	7.54	31.4	28.6
15	Media	4 plants	V37	30	8.69	35.0	34.0
15	Media	4 plants	V38	30	5.61	26.1	20.2
15	Media	4 plants	V39	30	5.17	47.0	13.2
16	Media	3 plants	V40	30	4.09	44.2	16.2
16	Media	3 plants	V41	30	8.00	64.0	19.5
16	Media	3 plants	V42	30	3.46	32.0	17.2
17	Media	3 plants	V43	30	9.18	54.9	34.0
17	Media	3 plants	V44	30	7.54	45.0	25.1
17	Media	3 plants	V45	30	3.53	42.1	18.3
18	Media	3 plants	V46	30	8.22	49.2	32.8
18	Media	3 plants	V47	30	7.46	44.7	31.1
18	Media	3 plants	V48	30	3.50	45.7	18.7
19	Media	2 plants	V49	30	6.99	60.0	40.7
19	Media	2 plants	V50	30	10.19	53.1	16.9
20	Media	2 plants	V51	30	6.61	9.4	36.0
20	Media	2 plants	V52	30	8.67	41.9	22.7
21	Media	2 plants	V53	30	7.74	55.4	32.8
21	Media	2 plants	V54	30	9.00	60.5	26.1

Table A3: Comprehensive metal analysis of vetiver from the short-term bench-scale experiment from Chapter 1, including total dry tissue mass for shoot (S) and root (R) of each plant.

Container	Water type	Density	Plant ID	Tissue		Digest mass (g)	Metals ($\mu\text{g L}^{-1}$)					
				Type	Mass (g)		Fe	Al	Zn	Ni	Cu	Pb
01	AMD	4 plant	V01	S	0.95	0.25	1069	1183	89.38	19.13	56.46	0.6200
				R	0.23	0.23	199200	23080	1398	185.4	267.8	0.4300
01	AMD	4 plant	V02	S	0.65	0.25	1445	2302	282.2	86.81	75.11	0.6300
				R	0.31	0.25	93460	6798	721.0	157.3	91.30	16.30
01	AMD	4 plant	V03	S	0.63	0.25	20240	9629	432.4	939.8	956.1	0.9500
				R	0.32	0.25	112500	8680	181.4	104.6	105.2	5.900
01	AMD	4 plant	V04	S	0.57	0.25	1180	1066	145.5	27.80	82.24	0.3600
				R	0.40	0.25	44960	5170	232.6	86.00	182.8	8.300
02	AMD	4 plant	V05	S	0.76	0.25	746.8	966.4	227.0	23.61	73.53	0.4800
				R	0.13	0.13	25790	3255	701.5	29.80	91.40	38.20
02	AMD	4 plant	V06	S	0.35	0.11	2861	4868	723.0	41.39	76.01	0.6900
				R	0.18	0.18	130100	15400	287.5	43.20	114.5	6.500
02	AMD	4 plant	V07	S	1.00	0.25	1275	1154	340.1	21.09	66.96	0.4000
				R	0.68	0.25	41080	5020	701.0	56.50	116.1	9.500
02	AMD	4 plant	V08	S	0.91	0.25	488.6	582.5	107.5	20.65	34.06	0.2900
				R	0.28	0.25	46220	4490	521.3	51.00	88.70	6.100
03	AMD	4 plant	V09	S	0.80	0.25	771.7	1159	705.6	27.77	117.2	0.4600
				R	0.23	0.23	54230	3403	312.9	47.90	114.9	6.200
03	AMD	4 plant	V10	S	0.25	0.25	74.94	70.87	14.75	1.300	1.940	0.06000
				R	0.30	0.25	91790	8375	4259	90.40	162.3	56.70
03	AMD	4 plant	V11	S	0.64	0.25	1433	910.0	262.6	22.45	59.36	0.3800
				R	0.12	0.12	53040	6703	190.1	50.30	95.10	5.400
03	AMD	4 plant	V12	S	0.71	0.25	1750	1710	527.3	25.20	85.36	0.5200
				R	0.32	0.25	85680	7718	347.8	74.30	182.8	8.600

04	AMD	4 plant	V13	S	0.97	0.25	4516	4612	1793	39.19	112.6	1.470
				R	0.25	0.25	196500	35050	385.6	131.9	218.7	6.400
04	AMD	3 plant	V14	S	0.50	0.25	628.0	821.1	361.4	33.34	74.46	0.4700
				R	0.36	0.25	104500	5980	179.6	89.60	107.9	4.700
04	AMD	3 plant	V15	S	1.48	0.25	1705	2665	500.0	35.79	105.9	1.150
				R	0.36	0.25	154400	16850	345.1	157.4	217.8	6.500
05	AMD	3 plant	V16	S	1.09	0.25	288.5	517.2	68.40	15.62	44.86	0.3500
				R	0.37	0.25	74440	3694	13.30	28.50	58.00	20.70
05	AMD	3 plant	V17	S	0.52	0.25	12280	6650	1731	47.00	159.0	1.890
				R	0.15	0.15	52160	17820	258.0	48.20	94.40	4.500
05	AMD	3 plant	V18	S	1.30	0.25	1721	1942	127.0	63.47	74.29	0.9200
				R	0.49	0.25	64530	6222	194.0	125.6	160.6	5.800
06	AMD	3 plant	V19	S	0.54	0.25	1243	579.5	123.5	23.41	49.46	0.4400
				R	0.14	0.14	86710	6025	138.1	67.40	114.8	6.400
06	AMD	3 plant	V20	S	1.14	0.25	431.6	361.1	247.3	16.10	44.04	0.4700
				R	0.21	0.21	75630	10180	732.9	73.30	168.6	10.80
06	AMD	3 plant	V21	S	0.90	0.25	2296	2933	197.5	41.87	50.28	0.5900
				R	0.27	0.25	95830	14680	1077	84.90	125.9	17.40
07	AMD	2 plant	V22	S	0.83	0.25	897.0	594.8	502.9	27.83	78.78	0.2600
				R	0.29	0.25	161000	19130	1930	121.1	159.6	33.50
07	AMD	2 plant	V23	S	0.78	0.25	627.3	518.7	268.9	19.75	50.12	0.2600
				R	0.38	0.25	97430	7478	1424	73.80	106.3	25.60
08	AMD	2 plant	V24	S	0.96	0.25	401.5	408.3	234.7	26.48	45.53	0.3700
				R	0.34	0.25	107100	5286	2688	77.40	143.2	84.50
08	AMD	2 plant	V25	S	0.71	0.06	553.0	535.5	31.33	10.66	22.66	0.1800
				R	0.64	0.25	112200	18610	2014	67.20	95.70	57.60
09	AMD	2 plant	V26	S	1.13	0.25	515.1	756.5	356.1	53.84	35.12	0.3900
				R	0.25	0.25	115700	4897	1868	66.20	81.10	49.80
09	AMD	2 plant	V27	S	0.86	0.25	823.4	515.5	105.1	22.14	42.39	0.2000
				R	0.48	0.25	102500	4804	2148	82.80	112.3	44.90

13	Media	4 plant	V28	S	1.07	0.25	266.7	220.6	326.1	6.470	53.92	0.22
				R	0.22	0.22	529500	7279	21860	1115	10320	708.4
13	Media	4 plant	V29	S	0.59	0.25	296.6	165.6	140.9	6.030	50.32	0.5100
				R	0.36	0.25	363300	404000	15220	1646	10240	557.7
13	Media	4 plant	V30	S	0.49	0.25	286.1	239.1	136.3	6.380	56.54	0.2600
				R	0.23	0.23	306800	286900	10570	987.9	7957	310.5
13	Media	4 plant	V31	S	0.76	0.25	359.1	314.2	136.2	7.030	51.17	0.3200
				R	0.18	0.18	260400	356300	8690	717.2	6207	167.2
14	Media	4 plant	V32	S	0.90	0.25	326.4	216.6	110.7	7.150	36.09	0.1900
				R	0.24	0.24	16670	21670	1773	26.90	132.5	45.50
14	Media	4 plant	V33	S	0.83	0.25	211.1	99.29	278.6	4.920	45.88	0.1900
				R	0.37	0.25	5541	2777	1204	18.90	91.90	37.60
14	Media	4 plant	V34	S	0.49	0.25	406.2	251.8	169.4	6.760	73.15	0.5400
				R	0.28	0.25	20020	23440	1687	36.10	214.0	42.90
14	Media	4 plant	V35	S	0.70	0.25	329.2	274.2	130.8	5.790	55.54	0.3300
				R	0.13	0.13	5841	3446	1008	10.70	94.60	39.70
15	Media	4 plant	V36	S	0.91	0.25	233.8	92.68	197.4	14.76	42.32	0.1900
				R	0.16	0.16	2239	1534	903.3	13.60	73.30	32.40
15	Media	4 plant	V37	S	0.87	0.25	294.1	179.8	142.5	10.21	48.13	0.3300
				R	0.38	0.25	10470	6336	1070	27.30	135.6	36.30
15	Media	4 plant	V38	S	0.36	0.14	360.5	268.6	91.71	7.010	30.43	2.260
				R	0.66	0.25	5044	2786	1021	9.000	106.0	48.30
15	Media	4 plant	V39	S	0.55	0.25	357.1	223.4	146.5	5.840	74.43	0.3700
				R	0.42	0.18	7910	4707	1171	25.30	217.7	36.50
16	Media	3 plant	V40	S	0.50	0.25	250.5	128.1	116.2	5.750	45.95	0.4500
				R	0.15	0.15	3006	2275	1237	7.300	88.90	48.00
16	Media	3 plant	V41	S	0.96	0.25	312.3	218.9	112.4	3.550	66.56	0.5500
				R	0.53	0.25	5023	2962	1217	13.90	249.9	53.80
16	Media	3 plant	V42	S	0.94	0.25	208.2	100.1	154.0	4.060	56.46	1.750
				R	0.14	0.14	4347	1828	896.5	4.400	76.40	54.20

17	Media	3 plant	V43	S	0.89	0.25	191.2	81.75	126.5	5.470	37.54	0.2200
				R	0.26	0.25	8440	5826	1228	39.40	134.1	48.10
17	Media	3 plant	V44	S	0.78	0.25	285.7	117.8	309.8	35.89	62.95	0.5800
				R	0.52	0.25	5381	3207	1412	41.90	127.3	57.80
17	Media	3 plant	V45	S	0.46	0.25	358.3	187.0	157.1	18.12	57.30	0.3900
				R	0.22	0.22	9176	6692	1698	22.20	324.8	52.80
18	Media	3 plant	V46	S	0.94	0.25	338.0	214.4	168.9	13.77	68.22	0.4400
				R	0.20	0.20	8818	5867	1266	19.80	152.2	42.70
18	Media	3 plant	V47	S	0.81	0.25	350.0	304.7	158.0	9.810	46.20	0.2400
				R	0.22	0.22	10790	7463	1227	29.50	142.2	41.50
18	Media	3 plant	V48	S	0.43	0.25	347.9	224.6	123.0	8.940	44.85	0.8500
				R	0.10	0.10	5797	2785	1213	7.000	111.8	35.50
19	Media	2 plant	V49	S	0.94	0.25	250.7	192.6	231.5	4.820	62.01	0.2800
				R	0.30	0.25	10110	6568	1434	29.20	177.2	42.60
19	Media	2 plant	V50	S	1.08	0.25	308.7	201.8	430.7	18.56	78.59	0.4700
				R	0.55	0.25	12550	12360	1289	23.90	223.2	35.80
20	Media	2 plant	V51	S	0.52	0.18	188.9	175.2	296.4	4.840	62.03	0.4300
				R	0.59	0.25	6152	3447	1617	8.600	94.10	39.30
20	Media	2 plant	V52	S	1.12	0.25	418.7	328.2	144.4	7.380	48.06	0.3700
				R	0.45	0.25	6750	2806	1067	19.90	142.0	37.40
21	Media	2 plant	V53	S	0.90	0.25	238.0	115.0	225.9	5.190	56.57	0.8900
				R	0.46	0.25	NT	NT	NT	NT	NT	NT
21	Media	2 plant	V54	S	0.95	0.25	NT	NT	NT	NT	NT	NT
				R	0.34	0.25	NT	NT	NT	NT	NT	NT

Figure A2: Images of the AMD filtration from Chapter 2. Initially presented is A) an image of the Tab-Simco AMD or UU water from the SRB in a mixing container, followed by filter preparation, including B) exterior and C) interior images of the 50-gal drum, and D) an image of the filter material. Lastly are images of the filtration process, including E) the UU water inside the plug filter, F) the FU water exiting the plug filter, and G) before and after images showing the UU water on the left and the FU water on the right.

Figure A4: Images of the long-term large-scale experiment setup from Chapter 2. Presented is the materials assembly, including A) hydroponic containers, mixing containers and water containers, along with images of the B) UU water and C) FU water in their respective mixing containers, and acclimated vetiver being prepared for transfer to the rafts. In addition, the vetiver raft assembly can be observed from E) above and F) below, along with G) direct and H) angled images of the vetiver rafts in their respective containers.

Figure A5: Images of vetiver from the UU water following the long-term large-scale experiment from Chapter 2. Presented are images of the vetiver rafts, including A) the vetiver raft in its hydroponic container, along with B) the underside of the vetiver raft that reveals fresh root growth. In addition, vetiver images show C) vetiver plants after having been removed from the raft and D) SEM images of the vetiver root surface that shows metal plaques.

Figure A6: Images of sample processing and characterization following the long-term large-scale experiment from Chapter 2. Presented are images of the water samples being A) measured for pH and B) undergoing gravity filtration, along with images of vetiver tissues during C) acid digestion prior to metal analysis and D) vacuum filtration at the end of TCLP.

LSE FT	112	4.8	7.52	2.29	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
			7.53	2.51	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
			7.54	2.54	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
LSE FU	112	7.5	8.07	2.94	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
			8.10	2.92	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
			8.10	2.88	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
LSE UT	140	8.1	2.53	3.20	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
			2.53	3.25	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
			2.54	3.24	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
LSE UU	140	7.9	2.39	3.70	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
			2.39	3.88	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
			2.40	3.80	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
LSE FT	140	3.3	7.46	2.38	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
			7.46	2.36	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
			7.46	2.40	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
LSE FU	140	8.0	8.09	2.90	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
			8.09	2.87	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
			8.08	2.83	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
LSE UT	168	7.6	2.54	3.20	340	72410	66520	20460	1260	640	105	110	10	10	0
			2.54	3.23	351	72600	66730	20570	1290	640	105	60	10	10	0
			2.54	3.23	374	71920	66460	20380	1280	620	106	40	10	10	0
LSE UU	168	7.4	2.40	3.83	430	77550	74490	22530	1420	700	99	120	10	10	0
			2.41	3.82	418	82310	79770	23870	1470	750	99	80	10	10	0
			2.41	3.72	419	79540	75800	23100	1420	720	98	110	10	10	0
LSE FT	168	2.9	7.41	2.18	263	40	90	10	100	0	86	40	0	20	10
			7.41	2.27	247	70	60	0	100	0	87	50	0	10	10
			7.41	2.26	251	70	60	10	100	0	87	90	0	10	0
LSE FU	168	7.4	8.13	2.86	358	70	70	0	100	70	88	110	10	20	10
			8.12	2.86	370	70	50	0	100	60	87	50	10	20	10
			8.14	2.89	347	70	50	10	110	50	86	20	0	10	10

LSE UT	196	5.0	2.85	2.73	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
			2.84	2.82	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
			2.84	2.79	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
LSE UU	196	7.3	2.38	3.88	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
			2.38	3.75	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
			2.37	3.89	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
LSE FT	196	3.7	7.37	2.25	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
			7.41	2.22	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
			7.39	2.26	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
LSE FU	196	7.5	8.04	2.87	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
			8.06	2.82	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
			8.06	2.85	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
LSE UT	224	5.1	3.01	2.69	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
			3.01	2.68	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
			3.03	2.67	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
LSE UU	224	8.3	2.38	3.97	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
			2.38	3.95	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
			2.37	3.98	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
LSE FT	224	1.9	7.39	1.85	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
			7.39	1.92	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
			7.40	1.96	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
LSE FU	224	7.4	8.01	2.83	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
			8.09	2.84	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
			8.11	2.79	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
LSE UT	252	4.1	3.22	2.52	289	3690	80150	19080	1190	550	145	130	70	10	0
			3.19	1.97	286	3750	81510	19280	1210	560	147	130	70	10	0
			3.20	2.50	308	3770	82060	19590	1220	560	146	90	70	10	0
LSE UU	252	7.5	2.37	3.99	409	69650	69890	21060	1320	650	98	130	10	0	0
			2.33	3.73	452	68910	68350	20830	1260	650	98	110	10	0	0
			2.33	3.95	418	72990	72290	22000	1350	680	99	100	10	0	0

LSE UT	336	2.2	3.57	1.87	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
			3.56	1.86	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
			3.56	1.89	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
LSE UU	336	7.3	2.42	3.88	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
			2.40	3.91	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
			2.40	3.91	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
LSE FT	336	3.3	7.27	1.70	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
			7.26	1.70	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
			7.27	1.69	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
LSE FU	336	7.7	8.14	2.84	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
			8.15	2.83	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
			8.13	2.88	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
LSE UT	364	1.4	3.47	1.77	230	530	59030	14770	820	410	94	90	40	10	0
			3.45	1.76	225	540	62900	15960	900	440	95	40	40	0	0
			3.45	1.77	211	450	56060	14270	800	400	94	110	30	10	0
LSE UU	364	6.8	2.40	3.90	379	68630	70510	21590	1360	670	99	130	10	10	0
			2.39	3.83	352	65920	67410	20790	1320	650	99	90	10	10	0
			2.38	3.86	404	65430	66980	20640	1240	640	98	110	10	10	0
LSE FT	364	3.4	7.21	1.49	162	40	90	10	80	0	86	40	0	20	0
			7.29	1.49	171	70	50	0	100	0	86	20	0	10	0
			7.30	1.52	154	70	50	0	100	0	86	20	0	0	0
LSE FU	364	8.0	8.10	2.63	372	70	60	10	90	20	87	30	10	20	10
			8.12	2.74	360	70	60	10	90	20	87	10	10	10	10
			8.13	2.75	371	70	50	10	100	20	87	0	0	20	10
SSE UT01	0	NT	2.42	4.01	417	68740	66500	21030	1300	680	70	80	50	30	80
SSE UT02	0	NT	2.40	3.99	403	66100	64810	20170	1190	620	140	100	10	20	40
SSE UT03	0	NT	2.39	3.94	380	67130	66780	20440	1200	620	140	130	10	20	40
SSE UU01	0	NT	2.38	4.01	403	68420	67430	20830	1210	630	140	120	10	20	30
SSE UU02	0	NT	2.37	3.93	407	73110	70670	22180	1310	680	130	90	10	20	30
SSE UU03	0	NT	2.37	3.92	442	67360	65790	20540	1190	630	130	80	10	20	20

SSE UT01	28	NT	2.78	2.18	210	41450	45140	14390	870	420	90	100	20	10	20
SSE UT02	28	NT	2.95	1.63	163	27330	43780	13170	830	370	80	90	20	20	10
SSE UT03	28	NT	2.94	1.81	200	32910	47590	14520	980	410	80	40	20	20	10
SSE UU01	28	NT	2.43	3.81	444	73500	72200	22320	1320	680	130	80	10	10	0
SSE UU02	28	NT	2.40	3.98	432	68820	66580	20960	1240	640	130	100	10	10	0
SSE UU03	28	NT	2.38	4.06	438	69430	67000	21180	1270	650	130	70	10	10	0
SSE UT01	56	NT	3.09	1.30	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
SSE UT02	56	NT	3.25	1.02	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
SSE UT03	56	NT	3.13	1.05	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
SSE UU01	56	NT	2.40	3.79	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
SSE UU02	56	NT	2.40	3.56	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
SSE UU03	56	NT	2.38	3.85	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
SSE UT01	84	NT	3.31	0.78	66.0	1390	20820	6330	410	170	80	20	10	10	10
SSE UT02	84	NT	3.42	0.85	65.6	550	26790	6050	380	160	90	20	10	10	10
SSE UT03	84	NT	3.42	0.62	46.9	480	17570	4660	270	120	100	90	10	20	0
SSE UU01	84	NT	2.37	3.91	440	64100	63440	20120	1220	610	130	110	10	10	10
SSE UU02	84	NT	2.38	3.78	406	62580	62040	19640	1150	600	130	70	10	20	10
SSE UU03	84	NT	2.37	3.87	419	67360	67410	21010	1240	640	130	60	10	10	10
SSE UT01	112	NT	3.52	0.41	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
SSE UT02	112	NT	3.53	0.50	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
SSE UT03	112	NT	3.62	0.40	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
SSE UU01	112	NT	2.37	3.88	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
SSE UU02	112	NT	2.35	3.83	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
SSE UU03	112	NT	2.33	3.97	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
SSE UT01	140	NT	3.68	0.26	14.9	60	6360	1170	0	30	140	70	0	10	10
SSE UT02	140	NT	3.61	0.39	27.2	50	13550	1890	100	50	130	40	0	20	10
SSE UT03	140	NT	3.71	0.29	19.1	40	9170	1650	40	40	130	50	0	20	10
SSE UU01	140	NT	2.41	3.92	407	64190	65250	20330	1240	620	130	80	10	20	10
SSE UU02	140	NT	2.37	3.99	426	68910	69690	21730	1290	660	120	120	10	10	10
SSE UU03	140	NT	2.36	4.08	397	72020	72510	22580	1360	690	130	110	10	10	0

Table A5: Fresh biomass measurements for vetiver throughout the large-scale experiment (LSE) and small-scale experiment (SSE) from Chapter 2, including initial (I) and final (F) measurements.

Container	Plant ID	Fresh biomass (g)	
		I	F
LSE UT (0-168d)	V01	21.0	13.2
LSE UT (0-168d)	V02	12.6	7.80
LSE UT (0-168d)	V03	38.9	19.9
LSE UT (0-168d)	V04	29.7	18.1
LSE UT (0-168d)	V05	46.7	25.7
LSE UT (0-168d)	V06	30.6	20.2
LSE UT (0-168d)	V07	36.9	18.9
LSE UT (0-168d)	V08	55.5	30.7
LSE UT (0-168d)	V09	22.3	15.7
LSE UT (0-168d)	V10	44.0	25.8
LSE UT (0-168d)	V11	34.9	17.3
LSE UT (0-168d)	V12	23.6	10.3
LSE UT (0-168d)	V13	23.1	11.5
LSE UT (0-168d)	V14	18.4	8.00
LSE UT (0-168d)	V15	48.7	34.0
LSE UT (0-168d)	V16	19.3	9.60
LSE UT (0-168d)	V17	14.1	8.00
LSE UT (0-168d)	V18	14.9	10.7
LSE UT (0-168d)	V19	105	52.5
LSE UT (0-168d)	V20	60.1	26.4
LSE UT (0-168d)	V21	28.6	17.9
LSE UT (0-168d)	V22	33.1	17.3
LSE UT (0-168d)	V23	56.3	23.3
LSE UT (0-168d)	V24	67.7	27.5
LSE UT (0-168d)	V25	31.7	17.4
LSE UT (0-168d)	V26	19.1	7.80
LSE UT (0-168d)	V27	27.3	14.9
LSE UT (0-168d)	V28	18.5	12.4
LSE FT	V29	14.2	11.2
LSE FT	V30	24.7	21.0
LSE FT	V31	48.1	19.1
LSE FT	V32	36.9	43.0
LSE FT	V33	22.6	13.1
LSE FT	V34	21.7	11.7
LSE FT	V35	27.4	10.3
LSE FT	V36	19.2	18.0
LSE FT	V37	101	233
LSE FT	V38	23.4	21.4
LSE FT	V39	19.0	5.90
LSE FT	V40	19.8	32.4

LSE FT	V41	57.1	168
LSE FT	V42	38.9	66.2
LSE FT	V43	83.1	202
LSE FT	V44	24.7	27.1
LSE FT	V45	33.3	34.3
LSE FT	V46	30.6	53.2
LSE FT	V47	33.7	78.4
LSE FT	V48	40.2	71.2
LSE FT	V49	54.0	151
LSE FT	V50	55.5	71.1
LSE FT	V51	46.1	85.9
LSE FT	V52	12.5	3.00
LSE FT	V53	29.5	27.4
LSE FT	V54	16.3	46.5
LSE FT	V55	16.7	11.8
LSE FT	V56	32.7	57.0
LSE UT (168-364d)	V57	185	139
LSE UT (168-364d)	V58	67.6	113
LSE UT (168-364d)	V59	122	165
LSE UT (168-364d)	V60	80.3	116
LSE UT (168-364d)	V61	138	189
LSE UT (168-364d)	V62	82.1	88.6
LSE UT (168-364d)	V63	91.6	159
LSE UT (168-364d)	V64	85.6	106
LSE UT (168-364d)	V65	120	187
LSE UT (168-364d)	V66	72.1	107
LSE UT (168-364d)	V67	82.6	143
LSE UT (168-364d)	V68	92.8	119
LSE UT (168-364d)	V69	83.1	118
LSE UT (168-364d)	V70	116	209
LSE UT (168-364d)	V71	79.3	144
LSE UT (168-364d)	V72	79.7	114
LSE UT (168-364d)	V73	86.5	97.6
LSE UT (168-364d)	V74	90.9	130
LSE UT (168-364d)	V75	80.7	104
LSE UT (168-364d)	V76	76.6	143
LSE UT (168-364d)	V77	83.3	174
LSE UT (168-364d)	V78	80.3	121
LSE UT (168-364d)	V79	85.1	145
LSE UT (168-364d)	V80	94.2	136
LSE UT (168-364d)	V81	81.8	130
LSE UT (168-364d)	V82	122	185
LSE UT (168-364d)	V83	94.3	132
LSE UT (168-364d)	V84	90.8	126
SSE UT01	V01	62.8	66.6

SSE UT01	V02	39.2	62.1
SSE UT01	V03	83.1	116
SSE UT01	V04	74.0	116
SSE UT02	V05	49.5	77.0
SSE UT02	V06	84.1	110
SSE UT02	V07	52.9	104
SSE UT02	V08	56.8	143
SSE UT03	V09	55.2	124
SSE UT03	V10	80.2	106
SSE UT03	V11	42.8	106
SSE UT03	V12	81.0	108

Table A6: Comprehensive metal analysis of vetiver through ICP-OES from the large-scale experiment (LSE) and small-scale experiment (SSE) from Chapter 2, including total dry tissue mass for shoot (S) and root (R) of each plant.														
Container	Plant ID	Tissue		Digest mass (g)	Metals ($\mu\text{g L}^{-1}$)									
		Type	Mass (g)		Fe	Al	Mn	Zn	Ni	Cu	Pb	Cr	As	Ag
LSE UT	V57	S	48.6	0.5061	1810	3740	3620	420	50	30	40	30	80	20
		R	9.3	0.5019	118980	13510	3980	360	230	210	30	210	10	0
LSE UT	V58	S	35.1	0.5030	4140	3300	5010	750	40	20	0	30	30	10
		R	7.2	0.5018	96450	21100	5130	460	360	120	20	310	10	0
LSE UT	V59	S	43.3	0.5011	9930	4560	5010	750	50	50	10	40	20	10
		R	11.1	0.5007	127040	22820	5700	430	370	150	20	350	10	0
LSE UT	V60	S	34.0	0.5021	2360	3180	5400	410	40	100	10	20	10	0
		R	6.4	0.5043	199400	25570	5160	440	360	110	30	610	10	0
LSE UT	V61	S	56.3	0.5015	1480	3690	4430	410	30	40	20	20	20	0
		R	11.7	0.5036	213580	26480	5450	730	310	190	50	610	10	0
LSE UT	V62	S	44.2	0.5029	3290	4530	5460	560	30	60	100	30	10	10
		R	8.7	0.5040	112920	18980	4350	450	290	140	20	340	10	0
LSE UT	V63	S	27.6	0.5038	4970	7250	4640	370	50	70	10	40	10	0
		R	7.2	0.5010	221300	33010	6030	710	400	190	50	830	10	0
LSE UT	V64	S	59.6	0.5021	2410	5720	4690	380	40	30	50	20	20	10
		R	10.9	0.5026	150040	27750	7050	790	440	170	20	370	10	0
LSE UT	V65	S	28.3	0.5005	2040	3710	5370	500	40	80	10	20	30	0
		R	7.0	0.5016	181990	29620	6190	440	380	150	10	750	10	0
LSE UT	V66	S	39.6	0.5006	6110	3960	4060	360	30	60	10	30	10	0
		R	8.3	0.5015	142010	23210	5910	540	370	160	60	450	10	0
LSE UT	V67	S	20.9	0.5011	2080	8000	4790	410	40	100	10	20	10	0
		R	7.3	0.5005	186900	20760	4230	920	270	360	30	570	10	0
LSE UT	V68	S	32.8	0.5036	3680	2970	4540	530	30	140	20	30	20	0
		R	8.5	0.5040	189360	28720	5470	350	350	90	40	750	10	0

LSE UT	V69	S	32.5	0.5015	3790	9110	5970	740	70	100	10	50	10	0
		R	9.3	0.5020	195310	34690	6050	390	370	80	40	660	20	0
LSE UT	V70	S	38.7	0.5041	1590	3360	6490	400	30	90	10	20	10	0
		R	10.0	0.5023	110430	33350	6340	870	420	190	50	470	10	0
LSE UT	V71	S	63.1	0.5043	1740	3230	4420	600	40	110	10	20	10	0
		R	10.9	0.5001	167010	39710	7340	1380	450	110	10	600	20	0
LSE UT	V72	S	28.5	0.5003	6650	6650	3760	300	50	60	20	40	0	0
		R	5.4	0.5025	177810	22960	5040	480	290	120	100	540	10	0
LSE UT	V73	S	34.0	0.5037	8340	3790	4030	640	40	70	10	40	10	0
		R	5.9	0.5004	189770	36070	6920	570	460	150	90	800	0	0
LSE UT	V74	S	38.7	0.5017	8440	9420	5530	510	60	90	10	50	10	0
		R	5.6	0.5016	136160	24990	5650	440	340	140	10	460	10	0
LSE UT	V75	S	28.2	0.5029	5790	5460	5490	530	40	70	10	30	10	0
		R	5.7	0.5037	290940	50460	6730	460	390	130	30	1300	10	0
LSE UT	V76	S	42.1	0.5045	3260	2660	5210	590	30	80	10	20	10	0
		R	8.0	0.5014	100940	16320	4030	430	300	440	90	310	10	0
LSE UT	V77	S	49.3	0.5044	3720	10270	6730	580	70	110	10	40	0	0
		R	9.7	0.5032	115550	34780	6510	1020	450	130	30	520	10	0
LSE UT	V78	S	31.6	0.5022	1900	4150	4910	500	50	150	10	20	0	0
		R	6.1	0.5039	163800	33770	7800	650	480	120	20	660	10	0
LSE UT	V79	S	41.0	0.5012	6800	7270	5080	510	60	90	10	40	0	0
		R	8.0	0.5024	180640	46310	7080	830	430	160	40	880	20	0
LSE UT	V80	S	34.1	0.5036	1740	5240	6100	430	40	120	10	30	10	0
		R	9.7	0.5008	161520	38730	5340	440	330	170	30	790	10	0
LSE UT	V81	S	38.3	0.5008	1310	1790	4860	360	20	50	10	10	10	0
		R	5.2	0.5021	103480	27020	7840	600	510	130	20	350	20	0
LSE UT	V82	S	51.1	0.5010	3820	4460	5060	770	40	80	10	30	10	0
		R	12.9	0.5022	256290	31100	5540	840	380	220	40	780	20	0
LSE UT	V83	S	39.0	0.5020	3920	3270	4710	600	30	110	10	30	10	0
		R	7.1	0.5026	271310	30310	4970	640	320	140	60	820	10	0

LSE UT	V84	S	37.3	0.5014	1950	2280	5060	380	30	120	10	20	0	0
		R	6.7	0.5045	200970	28730	5610	970	370	160	10	600	10	0
LSE FT	B01	S	444.2	0.5008	330	920	1110	390	20	40	130	0	20	10
		R	81.0	0.5028	5900	15150	12940	410	570	120	80	150	20	0
LSE FT	B02	S	444.2	0.5002	270	510	790	340	10	40	90	0	20	10
		R	81.0	0.5027	3130	8140	15380	440	690	140	120	100	20	0
LSE FT	B03	S	444.2	0.5030	600	1610	1190	350	20	60	100	10	20	10
		R	81.0	0.5042	3240	7430	12720	430	480	80	130	80	20	10
SSE UT01	V01	S	16.7	0.5044	1700	2700	1090	10	10.0	100	30.0	10.0	10	0
		R	7.1	0.5003	131920	60250	3800	720	170	200	90.0	730	10	0
SSE UT01	V02	S	16.2	0.5043	4260	9950	4260	440	50.0	30.0	20.0	40.0	10	0
		R	6.3	0.5018	163500	69800	3290	710	120	180	30.0	1030	10	0
SSE UT01	V03	S	32.1	0.5039	3660	11680	4480	490	40.0	30.0	50.0	70.0	20	0
		R	14.5	0.5017	133250	73050	5940	940	260	300	110	830	10	0
SSE UT01	V04	S	28.0	0.5039	770	3180	3440	320	20.0	70.0	30.0	10.0	10	10
		R	12.3	0.5033	146010	159930	7320	1200	280	380	60.0	1580	10	0
SSE UT02	V05	S	21.7	0.5036	490	2140	3720	300	20.0	80.0	20.0	10.0	10	10
		R	7.2	0.5032	159150	42970	2940	630	170	250	50.0	670	10	0
SSE UT02	V06	S	9.5	0.5028	2100	11110	4820	410	50.0	30.0	10.0	40.0	20	10
		R	25.5	0.5006	152810	100950	6100	950	270	290	70.0	1190	20	0
SSE UT02	V07	S	23.6	0.5026	1520	6220	4200	400	20.0	50.0	30.0	30.0	20	0
		R	10.0	0.5013	129400	89820	5630	890	300	330	120	1000	50	10
SSE UT02	V08	S	35.6	0.502	1390	7570	3640	330	30.0	40.0	10.0	30.0	20	10
		R	14.8	0.5023	178530	88760	5080	790	250	260	150	1250	10	0
SSE UT03	V09	S	29.4	0.5024	2100	8600	3680	430	40.0	30.0	10.0	30.0	20	10
		R	13.9	0.5011	104830	121880	5960	1120	220	400	100	1200	20	0
SSE UT03	V10	S	30.8	0.5021	1020	3530	4000	290	20.0	60.0	30.0	20.0	20	10
		R	9.7	0.5042	137170	110120	6900	960	330	380	80.0	1150	20	0
SSE UT03	V11	S	27.8	0.502	2700	9510	3170	310	40.0	30.0	22.7	20.0	10	10
		R	10.5	0.5015	153650	157560	6570	1110	150	340	130	1560	20	0

SSE UT03	V12	S	31.9	0.5018	880	2170	2960	270	20.0	70.0	10.0	20.0	10	10
		R	9.5	0.502	194090	148080	6160	1010	340	440	50.0	1560	10	0

Table A7: Comprehensive metal analysis of TCLP digests from the large-scale experiment of Chapter 2, including shoot (S) and root (R) batches from vetiver, along with a blank (B) sample.

Batch ID	Tissue	Metals ($\mu\text{g L}^{-1}$)													
		Fe	Al	Mn	Zn	Ni	Cu	Pb	Cr	Ba	Se	Cd	As	Ag	Hg
TCLP S01	S	1970	12410	22970	2570	210	350	160	30	221	31	4	20	10	161
TCLP S02	S	2230	12050	21910	2820	220	470	210	30	148	12	4	20	10	37
TCLP S03	S	1900	12720	22840	2520	220	390	180	30	188	35	4	20	10	21
TCLP R01	R	6510	43090	18380	1670	910	430	190	80	213	34	26	10	10	17
TCLP R02	R	6390	39710	17310	1550	850	430	210	70	102	13	23	20	0	14
TCLP R03	R	7450	53790	21210	1820	1030	430	150	90	207	7	31	20	10	18
TCLP B	NA	40	80	10	50	0	20	70	10	16	0	0	20	10	25

Appendix B

The figures and tables included in this appendix comprise the entirety of qualitative and quantitative records collected throughout the metabolomics experiments described and presented in Chapters 3 of this dissertation.

Figure B1: Images of the metabolomic experiments setup from Chapter 3. Presented are images of vetiver in their hydroponic containers, including A) distal and B) proximal images from the plant metabolomics experiment, along with C) distal and D) proximal images from the water metabolomics experiment.

Table B1: Comprehensive numerical data collected for water sample characterization throughout the plant metabolomic experiment from Chapter 3, where DO is in mg L ⁻¹ and EC is in mS cm ⁻¹ .																	
Container	Water type	Time (d)	DO	pH	EC	Ions (mg L ⁻¹)		Metals (µg L ⁻¹)									
						SO ₄ ²⁻	NO ₃ ¹⁻	Fe	Al	Mn	Zn	Ni	Cu	Pb	Cr	As	Ag
A01	AMD	0	NA	2.37	3.91	402	NA	71840	65490	20720	1200	630	140	110	10	20	10
A02	AMD	0	NA	2.38	3.88	399	NA	76550	69910	22050	1290	670	140	10	10	20	10
A03	AMD	0	NA	2.42	3.80	393	NA	74140	68580	21330	1250	640	140	100	10	10	10
M01	Media	0	NA	5.36	0.902	13.5	NA	120	60	400	40	0	130	60	0	20	10
M02	Media	0	NA	5.36	0.898	10.1	NA	110	30	430	40	0	120	20	0	10	0
M03	Media	0	NA	5.37	0.824	10.1	NA	120	30	430	30	0	120	50	0	20	10
A07V01	AMD	7	3.1	2.63	3.05	341	0.153	65470	69980	20530	1240	600	80	100	50	20	10
A07V02	AMD	7	4.2	2.53	3.21	339	0.184	67790	65900	20260	1210	600	90	60	20	20	10
A07V03	AMD	7	3.1	2.65	3.06	368	0.131	60220	67600	19840	1350	590	40	80	40	10	10
A07N01	AMD	7	7.6	2.41	3.96	462	0.149	68790	63880	20040	1190	610	140	140	10	20	10
A07N02	AMD	7	7.8	2.39	3.91	416	0.171	75720	72200	21950	1300	670	140	60	10	20	10
A07N03	AMD	7	8.1	2.39	3.92	454	0.190	64730	60730	18910	1110	570	140	110	10	20	10
M07V01	Media	7	2.7	6.33	0.445	7.78	211	130	80	40	20	0	80	20	0	20	10
M07V02	Media	7	3.3	6.10	0.577	9.89	285	60	20	140	0	0	60	40	0	20	10
M07V03	Media	7	3.8	4.53	0.568	9.92	301	190	110	210	20	0	100	40	0	20	10
M07N01	Media	7	8.2	5.36	0.768	12.3	197	90	30	470	10	0	120	60	0	20	10
M07N02	Media	7	7.9	5.45	0.705	10.6	176	90	20	490	30	0	120	20	0	20	10
M07N02	Media	7	8.2	5.51	0.718	12.0	185	110	30	510	20	0	120	30	0	20	10
A56V01	AMD	56	3.2	3.25	1.54	187	0.097	4330	42070	11650	680	320	90	60	30	20	10
A56V02	AMD	56	4.1	3.02	1.53	168	0.148	14250	32770	10180	600	290	30	50	20	20	10
A56V03	AMD	56	5.5	3.41	1.21	144	0.123	1080	34820	9850	580	270	80	50	20	20	10
A56N01	AMD	56	7.3	2.43	3.66	414	0.146	63890	58530	18610	1080	560	140	110	10	20	10
A56N02	AMD	56	7.3	2.43	3.63	392	0.166	64810	59860	18840	1110	560	130	100	10	20	10
A56N03	AMD	56	7.3	2.44	3.64	394	0.098	64890	60070	18890	1130	570	130	110	10	20	10

M56V01	Media	56	1.5	5.09	0.00580	0.195	0.668	30	40	30	80	0	140	30	0	20	10
M56V02	Media	56	2.1	5.17	0.00520	0.203	0.541	80	20	0	80	0	140	70	0	10	10
M56V03	Media	56	1.8	5.18	0.00550	0.144	0.780	120	20	0	80	0	140	40	0	10	10
M56N01	Media	56	7.3	5.66	0.670	10.4	171	150	30	10	100	0	160	190	0	20	10
M56N02	Media	56	7.4	5.65	0.607	10.3	160	150	30	10	100	0	160	150	0	20	10
M56N03	Media	56	7.3	5.69	0.611	9.16	155	120	20	510	10	0	110	40	0	20	10

Table B2: Base plant analysis from the plant metabolomic experiment from Chapter 3, including initial (I) and final (F) fresh biomass, chlorophyll (Chl) in $\mu\text{g g}^{-1}$, and total dry tissue mass for shoot (S) and root (R) of each plant.

Plant ID	Water type	Time (d)	Fresh biomass (g)		Chl	Tissue		Digest mass (g)	Metals ($\mu\text{g L}^{-1}$)									
			I	F		Type	Mass (g)		Fe	Al	Mn	Zn	Ni	Cu	Pb	Cr	As	Ag
A07V1	AMD	7	43.6	32.6	1.17	S	6.2	0.5018	6170	7660	4830	520	70	140	100	30	20	10
						R	3.6	0.5016	42180	42410	3300	660	70	200	70	420	20	0
A07V2	AMD	7	47.5	33.1	1.56	S	7.3	0.5006	6910	6580	2650	530	60	160	0	30	20	0
						R	3.0	0.5047	27020	9170	1610	450	40	200	40	90	20	0
A07V3	AMD	7	41.5	30.6	1.10	S	7.1	0.5002	7090	7210	4460	380	80	100	0	20	20	10
						R	3.0	0.5034	30980	18880	2450	560	60	200	10	200	30	0
M07V1	Media	7	61.5	57.6	2.80	S	11.2	0.5036	1800	2640	960	420	0	110	60	40	20	10
						R	3.0	0.5014	36840	83390	4900	780	30	490	180	740	10	0
M07V2	Media	7	37.1	35.8	2.15	S	6.4	0.5042	1220	1180	620	210	0	120	90	10	10	10
						R	2.1	0.5038	1950	4480	1950	260	10	340	70	40	20	0
M07V3	Media	7	36.8	37.2	2.91	S	7.2	0.5040	560	640	570	240	0	100	120	10	10	0
						R	2.2	0.5029	17180	35930	2700	580	20	450	30	350	20	0
A56V1	AMD	56	50.6	39.0	1.97	S	8.8	0.5040	9840	27450	5970	840	110	250	60	130	20	0
						R	4.2	0.5031	107880	59030	3510	680	80	240	80	650	10	0
A56V2	AMD	56	48.1	29.5	3.42	S	9.1	0.5024	17100	30050	4580	990	100	320	30	200	20	0
						R	2.4	0.5043	130920	11060	1270	500	50	270	70	210	10	0
A56V3	AMD	56	39.8	28.2	2.05	S	6.1	0.5012	12530	24550	5330	980	100	300	80	110	20	10
						R	2.9	0.5041	156340	56080	2900	680	70	240	100	750	20	0
M56V1	Media	56	47.5	65.6	2.17	S	14.5	0.5045	500	570	490	170	0	90	100	0	20	0
						R	5.1	0.5022	9190	17530	1110	330	10	300	70	160	20	0
M56V2	Media	56	48.1	61.1	3.09	S	12.8	0.5034	610	800	1720	210	0	80	90	10	10	10
						R	4.9	0.5007	8670	17240	1620	240	20	190	60	170	20	0
M56V3	Media	56	39.9	51.8	2.70	S	10.0	0.5016	640	780	1570	230	10	100	130	10	10	0
						R	4.6	0.5002	10670	21510	2090	270	20	180	100	200	20	0

Table B3: Comprehensive shoot metabolite data as area under the curve (AUC) collected by LC-MS/MS throughout the plant metabolomic analysis from Chapter 3.												
Metabolites	A7 S1	A7 S2	A7 S3	M7 S1	M7 S2	M7 S3	A56 S1	A56 S2	A56 S3	M56 S1	M56 S2	M56 S3
1-methyladenine	8.17 E+05	1.86 E+06	1.15 E+06	9.79 E+05	5.48 E+05	7.83 E+05	3.85 E+06	5.00 E+06	2.81 E+06	5.49 E+05	3.38 E+05	4.22 E+05
1-methyladenosine	5.61 E+06	9.75 E+06	7.74 E+06	5.54 E+06	3.10 E+06	4.43 E+06	5.54 E+06	7.20 E+06	4.04 E+06	2.69 E+06	1.66 E+06	2.07 E+06
1-methylhistidine	1.72 E+06	3.88 E+06	1.93 E+04	2.02 E+06	1.13 E+06	1.62 E+06	2.66 E+06	3.46 E+06	1.94 E+06	1.55 E+06	9.54 E+05	1.19 E+06
1-pyrroline-5-carboxylate	5.27 E+06	4.42 E+06	4.86 E+06	7.67 E+07	4.30 E+07	6.14 E+07	1.07 E+07	1.39 E+07	7.83 E+06	9.33 E+07	5.74 E+07	7.18 E+07
dGMP	7.18 E+05	9.23 E+05	8.88 E+05	6.02 E+05	3.37 E+05	4.82 E+05	5.59 E+05	7.27 E+05	4.08 E+05	3.79 E+05	2.33 E+05	2.91 E+05
dITP	2.75 E+05	2.85 E+05	3.98 E+05	5.66 E+05	3.17 E+05	4.53 E+05	3.57 E+05	4.64 E+05	2.61 E+05	8.91 E+05	5.48 E+05	6.85 E+05
2-hydroxyglutarate	5.97 E+06	5.72 E+06	4.38 E+06	5.99 E+06	3.35 E+06	4.79 E+06	2.98 E+06	3.88 E+06	2.18 E+06	5.17 E+06	3.18 E+06	3.98 E+06
2-isopropylmalate	3.73 E+06	3.18 E+06	3.12 E+06	2.96 E+06	1.66 E+06	2.37 E+06	2.21 E+06	2.87 E+06	1.61 E+06	2.14 E+06	1.32 E+06	1.65 E+06
2-ketobutyrate	1.45 E+07	1.31 E+07	1.48 E+07	1.42 E+07	7.92 E+06	1.13 E+07	1.39 E+07	1.80 E+07	1.01 E+07	1.60 E+07	9.83 E+06	1.23 E+07
2-ketogluconate	6.21 E+06	7.80 E+06	4.68 E+06	4.04 E+06	2.26 E+06	3.23 E+06	2.39 E+06	3.10 E+06	1.74 E+06	3.78 E+06	2.32 E+06	2.90 E+06
2-methylcitrate	1.74 E+06	2.12 E+06	1.55 E+06	2.85 E+06	1.59 E+06	2.28 E+06	1.78 E+06	2.31 E+06	1.30 E+06	2.87 E+06	1.76 E+06	2.21 E+06
3-PG	1.31 E+05	2.09 E+05	1.14 E+05	5.57 E+05	3.12 E+05	4.45 E+05	4.61 E+05	5.99 E+05	3.36 E+05	2.64 E+05	1.62 E+05	2.03 E+05
4-hydroxybenzoate	1.45 E+07	1.37 E+07	1.44 E+07	2.12 E+07	1.19 E+07	1.70 E+07	1.01 E+07	1.31 E+07	7.38 E+06	1.90 E+07	1.17 E+07	1.46 E+07

4-pyridoxate	2.39 E+07	2.39 E+07	2.50 E+07	2.31 E+07	1.29 E+07	1.85 E+07	2.63 E+07	3.41 E+07	1.92 E+07	7.81 E+07	4.81 E+07	6.01 E+07
5-methylthioadenosine	1.05 E+06	1.45 E+06	9.58 E+05	7.73 E+05	4.33 E+05	6.19 E+05	5.04 E+05	6.55 E+05	3.68 E+05	9.90 E+05	6.09 E+05	7.62 E+05
7-methylguanine	2.06 E+05	2.37 E+05	1.95 E+05	2.57 E+05	1.44 E+05	2.05 E+05	4.56 E+05	5.92 E+05	3.33 E+05	4.90 E+05	3.02 E+05	3.77 E+05
7-methylguanosine	1.68 E+06	2.39 E+06	2.28 E+06	1.40 E+06	7.86 E+05	1.12 E+06	1.54 E+06	2.01 E+06	1.13 E+06	1.00 E+06	6.18 E+05	7.72 E+05
acetylcholine	7.66 E+06	7.93 E+06	8.75 E+06	6.80 E+06	3.81 E+06	5.44 E+06	1.22 E+07	1.58 E+07	8.88 E+06	6.92 E+06	4.26 E+06	5.32 E+06
adenine	1.77 E+07	2.76 E+07	1.16 E+07	9.56 E+06	5.35 E+06	7.65 E+06	1.12 E+07	1.45 E+07	8.15 E+06	8.07 E+06	4.97 E+06	6.21 E+06
adenosine	8.48 E+07	1.22 E+08	7.10 E+07	5.03 E+07	2.82 E+07	4.03 E+07	4.24 E+07	5.51 E+07	3.10 E+07	1.82 E+07	1.12 E+07	1.40 E+07
adenylsuccinate	1.38 E+05	2.00 E+05	2.19 E+05	2.63 E+05	1.47 E+05	2.11 E+05	2.14 E+05	2.78 E+05	1.56 E+05	2.51 E+05	1.55 E+05	1.93 E+05
ADP	3.19 E+05	5.52 E+05	2.55 E+05	4.22 E+05	2.37 E+05	3.38 E+05	4.24 E+05	5.52 E+05	3.10 E+05	1.87 E+05	1.15 E+05	1.44 E+05
alanine	2.48 E+07	1.88 E+07	2.72 E+07	1.25 E+07	7.01 E+06	1.00 E+07	4.89 E+06	6.35 E+06	3.57 E+06	2.28 E+06	1.40 E+06	1.75 E+06
AMP	7.26 E+05	5.24 E+05	9.65 E+05	1.15 E+06	6.45 E+05	9.22 E+05	5.57 E+05	7.24 E+05	4.06 E+05	7.11 E+05	4.38 E+05	5.47 E+05
arginine	2.02 E+07	3.70 E+07	2.59 E+07	1.34 E+08	7.52 E+07	1.07 E+08	2.45 E+08	3.19 E+08	1.79 E+08	1.05 E+07	6.45 E+06	8.06 E+06
argininosuccinate	3.34 E+04	2.37 E+05	7.08 E+04	1.98 E+05	1.11 E+05	1.58 E+05	1.47 E+05	1.90 E+05	1.07 E+05	1.57 E+04	9.68 E+03	1.21 E+04
azelaic acid	4.78 E+06	3.39 E+06	4.41 E+06	3.08 E+06	1.72 E+06	2.46 E+06	3.57 E+06	4.64 E+06	2.61 E+06	5.62 E+06	3.46 E+06	4.32 E+06
betaine	1.84 E+07	2.81 E+07	2.02 E+07	2.14 E+07	1.20 E+07	1.71 E+07	2.79 E+07	3.63 E+07	2.04 E+07	2.15 E+07	1.32 E+07	1.65 E+07

carbamoylphosphate	1.86 E+06	4.60 E+06	1.22 E+06	4.10 E+05	2.29 E+05	3.28 E+05	5.90 E+05	7.66 E+05	4.30 E+05	1.09 E+06	6.72 E+05	8.40 E+05
CDP-choline	7.91 E+04	8.47 E+04	4.66 E+04	1.10 E+05	6.18 E+04	8.83 E+04	3.81 E+04	4.95 E+04	2.78 E+04	6.47 E+04	3.98 E+04	4.97 E+04
aconitate	4.83 E+08	4.43 E+08	4.37 E+08	6.07 E+08	3.40 E+08	4.86 E+08	3.96 E+08	5.14 E+08	2.89 E+08	5.65 E+08	3.48 E+08	4.35 E+08
citramalate	7.19 E+06	5.70 E+06	6.51 E+06	1.22 E+07	6.82 E+06	9.74 E+06	4.24 E+06	5.51 E+06	3.10 E+06	5.75 E+06	3.54 E+06	4.42 E+06
citrate	5.20 E+06	7.78 E+06	2.72 E+06	1.44 E+07	8.08 E+06	1.15 E+07	2.30 E+07	2.99 E+07	1.68 E+07	6.51 E+06	4.01 E+06	5.01 E+06
citrulline	2.49 E+05	5.23 E+05	3.49 E+05	2.48 E+06	1.39 E+06	1.98 E+06	9.86 E+06	1.28 E+07	7.19 E+06	1.77 E+05	1.09 E+05	1.36 E+05
cAMP	1.01 E+05	6.14 E+04	4.48 E+03	1.21 E+05	6.79 E+04	9.70 E+04	1.75 E+04	2.27 E+04	1.28 E+04	4.33 E+05	2.66 E+05	3.33 E+05
cytidine	3.14 E+06	3.55 E+06	2.31 E+06	1.85 E+06	1.03 E+06	1.48 E+06	2.79 E+06	3.63 E+06	2.04 E+06	1.18 E+06	7.28 E+05	9.11 E+05
cytosine	1.46 E+06	1.29 E+06	1.39 E+06	2.18 E+06	1.22 E+06	1.74 E+06	2.33 E+06	3.03 E+06	1.70 E+06	1.09 E+06	6.71 E+05	8.38 E+05
deoxyadenosine	5.19 E+05	1.04 E+06	4.67 E+05	2.38 E+05	1.33 E+05	1.91 E+05	1.08 E+06	1.40 E+06	7.86 E+05	1.64 E+05	1.01 E+05	1.26 E+05
dAMP	2.80 E+05	2.74 E+05	2.46 E+05	8.17 E+05	4.58 E+05	6.54 E+05	6.11 E+05	7.94 E+05	4.46 E+05	3.47 E+05	2.13 E+05	2.67 E+05
dCDP	2.20 E+05	4.16 E+05	1.96 E+05	1.99 E+05	1.12 E+05	1.60 E+05	2.01 E+05	2.62 E+05	1.47 E+05	2.93 E+05	1.80 E+05	2.25 E+05
deoxyguanosine	8.38 E+04	1.53 E+05	5.92 E+04	1.85 E+04	1.04 E+04	1.48 E+04	1.97 E+05	2.56 E+05	1.44 E+05	4.43 E+04	2.72 E+04	3.41 E+04
dGDP	4.20 E+05	8.00 E+05	3.68 E+05	4.21 E+05	2.36 E+05	3.37 E+05	5.05 E+05	6.56 E+05	3.68 E+05	2.97 E+05	1.82 E+05	2.28 E+05
dIMP	2.20 E+07	2.28 E+07	2.00 E+07	2.67 E+07	1.50 E+07	2.14 E+07	3.27 E+07	4.25 E+07	2.39 E+07	4.49 E+07	2.76 E+07	3.45 E+07

deoxyribose-P	1.25 E+05	5.74 E+04	4.97 E+04	2.92 E+05	1.63 E+05	2.34 E+05	6.18 E+04	8.04 E+04	4.51 E+04	6.93 E+04	4.27 E+04	5.33 E+04
G-3-P	1.94 E+05	3.17 E+05	2.88 E+05	2.45 E+05	1.37 E+05	1.96 E+05	2.62 E+05	3.41 E+05	1.92 E+05	8.27 E+04	5.09 E+04	6.36 E+04
DHAP	3.66 E+05	3.76 E+05	1.60 E+05	3.13 E+05	1.75 E+05	2.51 E+05	4.15 E+05	5.40 E+05	3.03 E+05	3.58 E+05	2.20 E+05	2.76 E+05
dimethylglycine	5.36 E+08	7.54 E+08	5.39 E+08	5.04 E+08	2.82 E+08	4.03 E+08	7.05 E+08	9.16 E+08	5.14 E+08	5.20 E+08	3.20 E+08	4.00 E+08
dimethylarginine	1.74 E+07	2.70 E+07	1.71 E+07	9.58 E+06	5.37 E+06	7.67 E+06	4.82 E+07	6.26 E+07	3.52 E+07	5.63 E+06	3.47 E+06	4.33 E+06
erythrose 4-P	3.95 E+05	4.20 E+05	5.18 E+05	7.58 E+05	4.24 E+05	6.06 E+05	5.24 E+05	6.81 E+05	3.82 E+05	7.35 E+05	4.53 E+05	5.66 E+05
ethanolamine	1.89 E+07	2.93 E+07	1.85 E+07	4.05 E+06	2.27 E+06	3.24 E+06	3.16 E+06	4.11 E+06	2.31 E+06	8.21 E+04	5.05 E+04	6.32 E+04
FAD	1.78 E+05	2.38 E+05	1.33 E+05	2.75 E+05	1.54 E+05	2.20 E+05	1.71 E+05	2.23 E+05	1.25 E+05	2.12 E+05	1.30 E+05	1.63 E+05
FMN	6.05 E+03	5.83 E+03	6.72 E+03	8.40 E+03	4.70 E+03	6.72 E+03	1.48 E+04	1.92 E+04	1.08 E+04	1.11 E+04	6.81 E+03	8.51 E+03
fumarate	8.15 E+07	9.69 E+07	8.31 E+07	9.20 E+07	5.15 E+07	7.36 E+07	8.98 E+07	1.17 E+08	6.55 E+07	9.73 E+07	5.99 E+07	7.48 E+07
γ -glutamylalanine	3.00 E+05	5.36 E+05	1.39 E+05	5.27 E+04	2.95 E+04	4.21 E+04	2.33 E+04	3.03 E+04	1.70 E+04	1.86 E+04	1.15 E+04	1.43 E+04
gluconate	1.97 E+07	2.43 E+07	1.33 E+07	3.21 E+07	1.80 E+07	2.57 E+07	3.02 E+07	3.93 E+07	2.21 E+07	3.43 E+07	2.11 E+07	2.64 E+07
glucosamine	2.66 E+06	2.55 E+06	4.37 E+06	3.09 E+06	1.73 E+06	2.47 E+06	2.22 E+06	2.89 E+06	1.62 E+06	2.84 E+06	1.75 E+06	2.18 E+06
GSH	2.11 E+06	1.29 E+06	1.17 E+06	3.56 E+06	2.00 E+06	2.85 E+06	2.61 E+06	3.39 E+06	1.90 E+06	1.11 E+06	6.85 E+05	8.57 E+05
glycerate	1.07 E+07	1.13 E+07	8.11 E+06	7.71 E+06	4.31 E+06	6.16 E+06	5.29 E+06	6.88 E+06	3.86 E+06	1.06 E+07	6.50 E+06	8.13 E+06

glycerol	1.99 E+07	2.07 E+07	1.32 E+07	8.96 E+06	5.02 E+06	7.17 E+06	1.36 E+07	1.77 E+07	9.94 E+06	1.51 E+07	9.32 E+06	1.17 E+07
glycerol-3-P	2.36 E+06	3.15 E+06	2.28 E+06	3.05 E+06	1.71 E+06	2.44 E+06	1.69 E+06	2.20 E+06	1.24 E+06	2.79 E+06	1.72 E+06	2.15 E+06
glycerophosphocholine	1.25 E+08	1.04 E+08	9.50 E+07	3.18 E+08	1.78 E+08	2.54 E+08	1.43 E+08	1.86 E+08	1.04 E+08	1.23 E+08	7.59 E+07	9.49 E+07
glycine	1.21 E+06	1.83 E+06	1.25 E+06	9.12 E+05	5.11 E+05	7.30 E+05	6.01 E+05	7.81 E+05	4.39 E+05	2.46 E+05	1.51 E+05	1.89 E+05
glyoxylate	1.68 E+06	1.84 E+06	1.61 E+06	1.76 E+06	9.84 E+05	1.41 E+06	1.91 E+06	2.48 E+06	1.39 E+06	1.69 E+06	1.04 E+06	1.30 E+06
guanine	1.05 E+06	6.03 E+05	1.04 E+06	9.78 E+05	5.48 E+05	7.82 E+05	2.72 E+06	3.53 E+06	1.98 E+06	4.12 E+06	2.54 E+06	3.17 E+06
guanosine	2.08 E+06	2.46 E+06	2.41 E+06	1.13 E+06	6.31 E+05	9.02 E+05	2.52 E+06	3.27 E+06	1.84 E+06	8.76 E+05	5.39 E+05	6.74 E+05
hexose diphosphate pool	8.49 E+04	5.83 E+03	1.21 E+04	5.83 E+04	3.26 E+04	4.66 E+04	9.41 E+03	1.22 E+04	6.87 E+03	1.47 E+05	9.07 E+04	1.13 E+05
hexose disaccharide monophosphate pool	2.33 E+05	3.26 E+05	1.79 E+05	3.40 E+05	1.90 E+05	2.72 E+05	2.81 E+05	3.65 E+05	2.05 E+05	3.02 E+05	1.86 E+05	2.33 E+05
hexose disaccharide pool	3.12 E+08	3.23 E+08	3.14 E+08	4.94 E+08	2.77 E+08	3.95 E+08	3.42 E+08	4.45 E+08	2.50 E+08	4.44 E+08	2.73 E+08	3.42 E+08
hexose phosphate pool	2.35 E+07	3.25 E+07	1.84 E+07	3.17 E+07	1.78 E+07	2.54 E+07	1.85 E+07	2.40 E+07	1.35 E+07	2.79 E+07	1.72 E+07	2.15 E+07
hexose pool	2.45 E+07	4.22 E+07	4.43 E+07	4.03 E+07	2.26 E+07	3.23 E+07	1.43 E+07	1.86 E+07	1.04 E+07	6.16 E+07	3.79 E+07	4.74 E+07
hydroxyproline	1.24 E+06	1.71 E+06	1.03 E+06	7.23 E+05	4.05 E+05	5.78 E+05	1.60 E+06	2.08 E+06	1.17 E+06	1.84 E+06	1.13 E+06	1.42 E+06
hypoxanthine	1.47 E+05	7.66 E+04	1.26 E+05	8.07 E+04	4.52 E+04	6.45 E+04	1.80 E+05	2.34 E+05	1.31 E+05	1.99 E+05	1.22 E+05	1.53 E+05
IMP	4.24 E+05	2.35 E+05	3.03 E+05	1.09 E+06	6.09 E+05	8.71 E+05	2.06 E+05	2.68 E+05	1.50 E+05	1.18 E+06	7.23 E+05	9.04 E+05

inosine	1.35 E+06	8.93 E+05	7.28 E+05	1.45 E+06	8.13 E+05	1.16 E+06	5.15 E+05	6.70 E+05	3.76 E+05	1.67 E+06	1.03 E+06	1.28 E+06
isocitrate	1.31 E+08	1.98 E+08	8.23 E+07	3.56 E+08	1.99 E+08	2.84 E+08	3.57 E+08	4.63 E+08	2.60 E+08	2.11 E+08	1.30 E+08	1.62 E+08
ITP	2.96 E+06	3.40 E+06	2.56 E+06	2.30 E+06	1.29 E+06	1.84 E+06	9.19 E+05	1.19 E+06	6.71 E+05	1.39 E+06	8.58 E+05	1.07 E+06
ascorbate	4.53 E+06	4.05 E+06	3.99 E+06	3.64 E+06	2.04 E+06	2.91 E+06	2.85 E+06	3.70 E+06	2.08 E+06	2.61 E+06	1.60 E+06	2.01 E+06
asparagine	1.95 E+08	1.86 E+08	2.78 E+08	2.75 E+08	1.54 E+08	2.20 E+08	3.46 E+08	4.50 E+08	2.53 E+08	3.19 E+07	1.96 E+07	2.45 E+07
aspartate	4.72 E+07	7.95 E+07	5.12 E+07	5.22 E+07	2.92 E+07	4.17 E+07	7.62 E+07	9.90 E+07	5.56 E+07	1.56 E+07	9.61 E+06	1.20 E+07
carnitine	3.10 E+05	9.16 E+05	4.62 E+05	2.09 E+05	1.17 E+05	1.67 E+05	6.20 E+05	8.06 E+05	4.53 E+05	1.81 E+04	1.11 E+04	1.39 E+04
glutamate	2.74 E+07	4.21 E+07	3.04 E+07	3.70 E+07	2.07 E+07	2.96 E+07	4.53 E+07	5.89 E+07	3.31 E+07	2.08 E+07	1.28 E+07	1.60 E+07
glutamine	3.03 E+08	3.55 E+08	3.26 E+08	4.04 E+08	2.26 E+08	3.23 E+08	3.41 E+08	4.43 E+08	2.49 E+08	2.53 E+08	1.56 E+08	1.95 E+08
histidine	5.99 E+07	1.35 E+08	7.09 E+07	2.44 E+07	1.37 E+07	1.95 E+07	1.24 E+08	1.61 E+08	9.06 E+07	1.37 E+07	8.42 E+06	1.05 E+07
linoleate	1.28 E+06	1.79 E+06	1.49 E+06	7.90 E+04	4.42 E+04	6.32 E+04	3.20 E+05	4.16 E+05	2.34 E+05	8.69 E+05	5.35 E+05	6.69 E+05
isoleucine	1.71 E+08	2.76 E+08	2.32 E+08	1.82 E+08	1.02 E+08	1.46 E+08	1.84 E+08	2.39 E+08	1.34 E+08	6.41 E+07	3.94 E+07	4.93 E+07
leucine	1.71 E+08	2.78 E+08	2.30 E+08	1.73 E+08	9.71 E+07	1.39 E+08	1.86 E+08	2.41 E+08	1.35 E+08	6.02 E+07	3.71 E+07	4.63 E+07
lysine	5.38 E+07	1.10 E+08	6.08 E+07	3.62 E+07	2.03 E+07	2.90 E+07	1.05 E+08	1.37 E+08	7.69 E+07	1.42 E+08	8.75 E+07	1.09 E+08
methionine	3.94 E+07	8.64 E+07	4.69 E+07	2.21 E+07	1.24 E+07	1.77 E+07	2.96 E+07	3.85 E+07	2.16 E+07	4.86 E+06	2.99 E+06	3.74 E+06

phenylalanine	5.47 E+07	1.03 E+08	6.15 E+07	1.68 E+07	9.41 E+06	1.34 E+07	2.92 E+07	3.80 E+07	2.13 E+07	1.04 E+07	6.42 E+06	8.03 E+06
proline	4.36 E+07	8.36 E+07	5.91 E+07	5.07 E+07	2.84 E+07	4.06 E+07	3.45 E+07	4.48 E+07	2.52 E+07	1.21 E+08	7.42 E+07	9.28 E+07
serine	5.50 E+07	9.31 E+07	6.59 E+07	4.54 E+07	2.54 E+07	3.64 E+07	3.46 E+07	4.49 E+07	2.52 E+07	1.09 E+07	6.73 E+06	8.41 E+06
threonine	1.79 E+07	3.77 E+07	2.06 E+07	1.51 E+07	8.48 E+06	1.21 E+07	1.04 E+07	1.35 E+07	7.61 E+06	1.97 E+06	1.21 E+06	1.52 E+06
tryptophan	2.79 E+07	6.72 E+07	3.66 E+07	2.15 E+07	1.20 E+07	1.72 E+07	3.19 E+07	4.14 E+07	2.33 E+07	3.72 E+07	2.29 E+07	2.86 E+07
tyrosine	3.65 E+06	6.16 E+06	3.79 E+06	1.52 E+06	8.50 E+05	1.21 E+06	1.24 E+06	1.62 E+06	9.08 E+05	8.30 E+05	5.11 E+05	6.38 E+05
valine	1.47 E+06	2.39 E+06	1.84 E+06	2.13 E+06	1.19 E+06	1.70 E+06	2.30 E+06	2.99 E+06	1.68 E+06	1.69 E+06	1.04 E+06	1.30 E+06
lysoPC(16:0)	1.00 E+05	1.98 E+05	1.80 E+05	2.91 E+04	1.63 E+04	2.33 E+04	1.12 E+04	1.46 E+04	8.18 E+03	6.29 E+04	3.87 E+04	4.84 E+04
lysoPC(18:0)	3.29 E+05	3.95 E+05	3.29 E+05	1.07 E+05	5.99 E+04	8.56 E+04	2.00 E+05	2.60 E+05	1.46 E+05	1.47 E+05	9.07 E+04	1.13 E+05
malate	4.19 E+08	4.79 E+08	4.36 E+08	5.40 E+08	3.02 E+08	4.32 E+08	4.47 E+08	5.81 E+08	3.26 E+08	5.66 E+08	3.49 E+08	4.36 E+08
malonate	3.32 E+07	3.14 E+07	3.03 E+07	3.42 E+07	1.92 E+07	2.74 E+07	2.48 E+07	3.22 E+07	1.81 E+07	2.28 E+07	1.40 E+07	1.75 E+07
metSO	1.57 E+07	2.59 E+07	2.74 E+07	1.81 E+06	1.01 E+06	1.45 E+06	2.68 E+06	3.48 E+06	1.95 E+06	5.94 E+05	3.66 E+05	4.57 E+05
methylsuccinate	1.71 E+06	1.82 E+06	2.05 E+06	2.09 E+06	1.17 E+06	1.67 E+06	1.65 E+06	2.15 E+06	1.21 E+06	1.79 E+06	1.10 E+06	1.38 E+06
mevalonate	5.44 E+05	7.86 E+05	8.58 E+05	2.20 E+06	1.23 E+06	1.76 E+06	1.14 E+06	1.49 E+06	8.35 E+05	2.55 E+05	1.57 E+05	1.96 E+05
myoinositol	2.37 E+07	2.27 E+07	2.19 E+07	2.68 E+07	1.50 E+07	2.14 E+07	2.52 E+07	3.28 E+07	1.84 E+07	3.02 E+07	1.86 E+07	2.32 E+07

N-acetylglucosamine-1-P	1.36 E+06	2.02 E+06	1.32 E+06	1.81 E+06	1.01 E+06	1.44 E+06	1.27 E+06	1.65 E+06	9.24 E+05	2.17 E+06	1.34 E+06	1.67 E+06
N-acetylglutamate	1.96 E+06	2.30 E+06	1.43 E+06	1.65 E+06	9.25 E+05	1.32 E+06	1.22 E+06	1.59 E+06	8.91 E+05	1.22 E+06	7.54 E+05	9.42 E+05
N-acetylaspartate	1.29 E+06	1.96 E+06	1.21 E+06	9.44 E+05	5.29 E+05	7.55 E+05	9.58 E+05	1.25 E+06	7.00 E+05	5.78 E+05	3.56 E+05	4.45 E+05
N-acetylserine	2.51 E+06	2.55 E+06	1.89 E+06	2.24 E+06	1.25 E+06	1.79 E+06	9.83 E+05	1.28 E+06	7.18 E+05	1.08 E+06	6.66 E+05	8.32 E+05
NAD	6.88 E+05	8.94 E+05	6.74 E+05	8.52 E+05	4.77 E+05	6.82 E+05	8.79 E+05	1.14 E+06	6.42 E+05	6.12 E+05	3.77 E+05	4.71 E+05
N- α -acetylarginine	4.12 E+06	4.08 E+06	4.47 E+06	2.22 E+06	1.24 E+06	1.78 E+06	1.55 E+06	2.01 E+06	1.13 E+06	1.65 E+06	1.01 E+06	1.27 E+06
nicotinate	1.19 E+05	1.09 E+05	1.69 E+05	1.77 E+05	9.91 E+04	1.42 E+05	2.12 E+05	2.75 E+05	1.55 E+05	1.98 E+05	1.22 E+05	1.52 E+05
octulose-P	8.85 E+04	7.04 E+04	9.01 E+04	6.50 E+04	3.64 E+04	5.20 E+04	2.90 E+05	3.78 E+05	2.12 E+05	7.58 E+03	4.66 E+03	5.83 E+03
oleate	1.65 E+06	2.60 E+06	1.66 E+06	1.19 E+06	6.64 E+05	9.49 E+05	2.57 E+06	3.34 E+06	1.87 E+06	1.75 E+06	1.08 E+06	1.35 E+06
O-phosphoethanolamine	1.56 E+07	1.33 E+07	1.66 E+07	1.21 E+07	6.77 E+06	9.67 E+06	1.49 E+07	1.94 E+07	1.09 E+07	1.64 E+07	1.01 E+07	1.27 E+07
O-phosphotyrosine	6.35 E+05	9.90 E+05	4.43 E+05	9.14 E+05	5.12 E+05	7.31 E+05	5.30 E+05	6.89 E+05	3.87 E+05	7.45 E+05	4.58 E+05	5.73 E+05
ornithine	7.95 E+06	1.97 E+07	1.06 E+07	6.43 E+06	3.60 E+06	5.15 E+06	2.60 E+07	3.38 E+07	1.90 E+07	1.60 E+05	9.82 E+04	1.23 E+05
oxalate	1.13 E+05	9.90 E+04	1.37 E+05	1.54 E+05	8.60 E+04	1.23 E+05	1.82 E+05	2.36 E+05	1.32 E+05	1.05 E+05	6.49 E+04	8.11 E+04
OAA	1.54 E+06	4.13 E+06	1.74 E+06	1.51 E+06	8.45 E+05	1.21 E+06	4.80 E+06	6.24 E+06	3.51 E+06	2.62 E+04	1.61 E+04	2.02 E+04
GSSG	1.92 E+05	3.94 E+05	1.43 E+05	8.79 E+04	4.92 E+04	7.04 E+04	2.23 E+05	2.90 E+05	1.63 E+05	2.04 E+04	1.25 E+04	1.57 E+04

pantothenate	7.10 E+06	8.84 E+06	6.10 E+06	7.03 E+06	3.93 E+06	5.62 E+06	5.52 E+06	7.17 E+06	4.03 E+06	1.19 E+07	7.33 E+06	9.16 E+06
PC(34:1)	1.14 E+06	1.20 E+06	1.41 E+06	2.48 E+06	1.39 E+06	1.98 E+06	1.59 E+06	2.06 E+06	1.16 E+06	2.76 E+06	1.70 E+06	2.12 E+06
PC(34:2)	5.00 E+06	6.17 E+06	5.16 E+06	7.69 E+06	4.30 E+06	6.15 E+06	6.15 E+06	7.99 E+06	4.49 E+06	1.18 E+07	7.27 E+06	9.09 E+06
PC(36:0)	3.86 E+05	3.33 E+05	3.58 E+05	6.83 E+05	3.82 E+05	5.46 E+05	6.14 E+05	7.98 E+05	4.48 E+05	2.54 E+06	1.56 E+06	1.95 E+06
PC(36:2)	8.98 E+05	6.95 E+05	8.69 E+05	1.60 E+06	8.95 E+05	1.28 E+06	9.97 E+05	1.30 E+06	7.28 E+05	2.36 E+06	1.45 E+06	1.82 E+06
PC(36:4)	5.02 E+06	6.10 E+06	5.37 E+06	5.76 E+06	3.22 E+06	4.61 E+06	4.28 E+06	5.56 E+06	3.12 E+06	8.80 E+06	5.42 E+06	6.77 E+06
PE (36:0)	2.50 E+05	3.11 E+05	2.41 E+05	3.22 E+05	1.80 E+05	2.57 E+05	2.87 E+05	3.73 E+05	2.09 E+05	2.05 E+05	1.26 E+05	1.58 E+05
PE(34:1)	5.83 E+05	9.52 E+05	1.00 E+06	1.57 E+06	8.79 E+05	1.26 E+06	9.17 E+05	1.19 E+06	6.69 E+05	8.84 E+05	5.44 E+05	6.80 E+05
PE(34:2)	5.60 E+04	1.15 E+05	8.02 E+04	6.13 E+04	3.43 E+04	4.91 E+04	1.06 E+05	1.38 E+05	7.72 E+04	6.93 E+04	4.27 E+04	5.33 E+04
PE(36:1)	7.19 E+04	1.09 E+05	1.29 E+05	1.30 E+05	7.28 E+04	1.04 E+05	7.57 E+04	9.84 E+04	5.53 E+04	2.12 E+05	1.30 E+05	1.63 E+05
PE(36:2)	5.91 E+05	5.18 E+05	5.37 E+05	9.54 E+05	5.34 E+05	7.63 E+05	7.58 E+05	9.85 E+05	5.53 E+05	6.50 E+05	4.00 E+05	5.00 E+05
PE(36:3)	5.13 E+06	6.70 E+06	1.19 E+05	6.50 E+06	3.64 E+06	5.20 E+06	5.53 E+06	7.19 E+06	4.04 E+06	6.41 E+06	3.94 E+06	4.93 E+06
PE(36:4)	2.84 E+04	8.11 E+04	4.12 E+04	4.81 E+04	2.69 E+04	3.85 E+04	1.12 E+05	1.46 E+05	8.18 E+04	2.15 E+05	1.32 E+05	1.65 E+05
PG(34:1)	1.47 E+07	1.35 E+07	1.54 E+07	2.13 E+07	1.19 E+07	1.70 E+07	1.43 E+07	1.86 E+07	1.04 E+07	3.20 E+07	1.97 E+07	2.46 E+07
PG(34:2)	2.28 E+05	1.05 E+05	1.95 E+05	5.68 E+05	3.18 E+05	4.54 E+05	1.04 E+05	1.35 E+05	7.56 E+04	7.13 E+05	4.39 E+05	5.49 E+05

PG(36:2)	1.39 E+05	1.24 E+05	1.29 E+05	1.01 E+05	5.65 E+04	8.07 E+04	5.69 E+04	7.40 E+04	4.15 E+04	1.42 E+05	8.71 E+04	1.09 E+05
phenyllactate	1.20 E+07	1.70 E+07	1.39 E+07	1.24 E+07	6.92 E+06	9.88 E+06	1.36 E+07	1.76 E+07	9.89 E+06	1.36 E+07	8.39 E+06	1.05 E+07
phosphoenolpyruvate	3.60 E+05	4.21 E+05	2.29 E+04	7.04 E+05	3.94 E+05	5.64 E+05	7.78 E+05	1.01 E+06	5.68 E+05	3.96 E+05	2.43 E+05	3.04 E+05
phosphorylcholine	1.10 E+08	1.85 E+08	1.15 E+08	1.55 E+08	8.69 E+07	1.24 E+08	1.37 E+08	1.78 E+08	1.00 E+08	1.18 E+08	7.26 E+07	9.08 E+07
PI(34:0)	8.00 E+04	1.33 E+05	9.14 E+04	2.57 E+05	1.44 E+05	2.05 E+05	1.03 E+05	1.33 E+05	7.49 E+04	3.86 E+05	2.37 E+05	2.97 E+05
PI(34:1)	5.11 E+05	7.40 E+05	5.39 E+05	1.40 E+06	7.84 E+05	1.12 E+06	5.60 E+05	7.28 E+05	4.09 E+05	9.84 E+05	6.05 E+05	7.57 E+05
PI(36:2)	5.67 E+04	3.76 E+04	3.85 E+04	1.10 E+05	6.16 E+04	8.79 E+04	7.04 E+04	9.15 E+04	5.14 E+04	1.34 E+05	8.25 E+04	1.03 E+05
pipecolate	1.02 E+08	1.22 E+08	1.05 E+08	9.10 E+07	5.09 E+07	7.28 E+07	6.15 E+07	7.99 E+07	4.49 E+07	4.22 E+07	2.60 E+07	3.24 E+07
purine	1.28 E+06	2.93 E+06	1.15 E+06	3.03 E+05	1.69 E+05	2.42 E+05	5.12 E+05	6.66 E+05	3.74 E+05	1.99 E+05	1.23 E+05	1.53 E+05
pyridoxine	2.29 E+06	2.23 E+06	1.67 E+06	2.41 E+06	1.35 E+06	1.93 E+06	1.59 E+06	2.07 E+06	1.16 E+06	2.41 E+06	1.48 E+06	1.85 E+06
pyrophosphate	5.23 E+06	8.83 E+06	2.61 E+06	3.57 E+06	2.00 E+06	2.85 E+06	4.48 E+06	5.82 E+06	3.27 E+06	4.82 E+06	2.97 E+06	3.71 E+06
quinolinate	7.32 E+05	6.11 E+05	6.33 E+05	1.19 E+06	6.67 E+05	9.53 E+05	9.75 E+05	1.27 E+06	7.12 E+05	8.34 E+05	5.13 E+05	6.41 E+05
riboflavin	3.48 E+05	3.61 E+05	2.68 E+05	5.34 E+05	2.99 E+05	4.28 E+05	1.57 E+06	2.04 E+06	1.15 E+06	4.91 E+05	3.02 E+05	3.78 E+05
ribose-5-P	1.48 E+06	1.22 E+06	1.19 E+06	4.81 E+06	2.69 E+06	3.85 E+06	7.80 E+05	1.01 E+06	5.69 E+05	3.17 E+06	1.95 E+06	2.44 E+06
S-adenosylhomocysteine	1.52 E+08	1.53 E+08	1.53 E+08	1.57 E+08	8.81 E+07	1.26 E+08	2.07 E+08	2.69 E+08	1.51 E+08	3.89 E+08	2.40 E+08	2.99 E+08

sarcosine	1.84 E+07	1.50 E+07	2.23 E+07	9.66 E+06	5.41 E+06	7.73 E+06	3.78 E+06	4.91 E+06	2.76 E+06	1.58 E+06	9.70 E+05	1.21 E+06
sedoheptulose-P	2.63 E+06	1.99 E+06	1.63 E+06	4.81 E+06	2.69 E+06	3.85 E+06	2.48 E+06	3.23 E+06	1.81 E+06	3.97 E+06	2.45 E+06	3.06 E+06
shikimate-3-P	4.71 E+07	5.64 E+07	5.38 E+07	3.40 E+07	1.90 E+07	2.72 E+07	1.23 E+08	1.60 E+08	8.98 E+07	1.50 E+08	9.24 E+07	1.16 E+08
shikimate	2.84 E+07	2.16 E+07	1.87 E+07	2.50 E+07	1.40 E+07	2.00 E+07	6.27 E+06	8.14 E+06	4.57 E+06	3.75 E+07	2.31 E+07	2.89 E+07
spermine	3.07 E+06	1.66 E+06	2.41 E+06	1.25 E+06	7.01 E+05	1.00 E+06	2.18 E+06	2.83 E+06	1.59 E+06	4.88 E+05	3.00 E+05	3.76 E+05
succinate	1.05 E+07	1.22 E+07	1.09 E+07	1.09 E+06	6.08 E+05	8.69 E+05	8.98 E+06	1.17 E+07	6.56 E+06	1.73 E+06	1.06 E+06	1.33 E+06
taurine	8.09 E+05	7.21 E+05	1.23 E+06	2.62 E+05	1.47 E+05	2.09 E+05	1.32 E+06	1.72 E+06	9.64 E+05	2.94 E+05	1.81 E+05	2.27 E+05
thiamine	4.33 E+07	7.09 E+07	5.26 E+07	4.60 E+07	2.58 E+07	3.68 E+07	1.08 E+08	1.41 E+08	7.91 E+07	7.37 E+07	4.54 E+07	5.67 E+07
thymidine	5.24 E+04	8.74 E+04	6.95 E+04	7.56 E+04	4.23 E+04	6.05 E+04	2.08 E+05	2.71 E+05	1.52 E+05	1.28 E+04	7.89 E+03	9.86 E+03
UDP	2.12 E+05	4.33 E+05	1.87 E+05	3.56 E+05	1.99 E+05	2.85 E+05	2.89 E+05	3.75 E+05	2.11 E+05	2.03 E+05	1.25 E+05	1.56 E+05
uracil	1.96 E+06	1.82 E+06	1.43 E+06	2.35 E+06	1.32 E+06	1.88 E+06	1.85 E+06	2.40 E+06	1.35 E+06	1.68 E+06	1.03 E+06	1.29 E+06
urea	3.24 E+05	2.74 E+05	3.83 E+05	9.52 E+04	5.33 E+04	7.62 E+04	1.31 E+05	1.71 E+05	9.58 E+04	1.02 E+05	6.27 E+04	7.84 E+04
ureidopropionate	1.61 E+06	3.52 E+06	1.99 E+06	1.99 E+06	1.11 E+06	1.59 E+06	4.62 E+06	6.00 E+06	3.37 E+06	3.19 E+05	1.96 E+05	2.46 E+05
uridine	1.54 E+06	5.98 E+05	9.86 E+05	4.61 E+05	2.58 E+05	3.69 E+05	8.67 E+05	1.13 E+06	6.33 E+05	4.41 E+05	2.72 E+05	3.40 E+05
uridine-5-P	7.45 E+05	1.12 E+06	6.12 E+05	1.00 E+06	5.62 E+05	8.03 E+05	5.25 E+05	6.82 E+05	3.83 E+05	6.13 E+05	3.77 E+05	4.71 E+05

UDP-glucose	1.29 E+06	1.62 E+06	1.05 E+06	1.99 E+06	1.12 E+06	1.59 E+06	1.08 E+06	1.41 E+06	7.89 E+05	1.17 E+06	7.17 E+05	8.97 E+05
UDP-N-acetylglucosamine	2.08 E+04	1.66 E+04	9.41 E+03	2.69 E+04	1.51 E+04	2.15 E+04	1.66 E+04	2.16 E+04	1.21 E+04	2.33 E+04	1.43 E+04	1.79 E+04
xanthine	3.75 E+05	5.55 E+05	2.90 E+05	3.50 E+05	1.96 E+05	2.80 E+05	2.92 E+05	3.80 E+05	2.13 E+05	6.47 E+04	3.98 E+04	4.97 E+04
xanthosine	1.72 E+05	3.56 E+05	2.49 E+05	1.88 E+05	1.05 E+05	1.51 E+05	9.21 E+04	1.20 E+05	6.72 E+04	2.30 E+05	1.42 E+05	1.77 E+05

Table B4: Comprehensive root metabolite data as area under the curve (AUC) collected by LC-MS/MS throughout the plant metabolomic analysis from Chapter 3.												
Metabolites	A7 R1	A7 R2	A7 R3	M7 R1	M7 R2	M7 R3	A56 R1	A56 R2	A56 R3	M56 R1	M56 R2	M56 R3
1-methyladenine	7.56 E+05	8.44 E+05	6.70 E+05	5.33 E+05	2.75 E+05	5.26 E+05	1.10 E+06	1.37 E+06	1.10 E+06	1.83 E+05	1.20 E+05	4.84 E+05
1-methyladenosine	1.04 E+07	7.91 E+06	1.40 E+07	1.12 E+07	1.36 E+07	1.24 E+07	1.83 E+06	2.33 E+06	1.83 E+06	1.62 E+06	1.05 E+06	1.15 E+06
1-methylhistidine	4.16 E+06	3.43 E+06	6.71 E+06	1.01 E+07	1.02 E+07	7.07 E+06	6.17 E+05	6.17 E+05	7.91 E+05	5.78 E+06	2.95 E+06	3.43 E+06
1-pyrroline-5-carboxylate	NA	4.81 E+05	7.66 E+07	8.60 E+06	1.20 E+07	7.81 E+07	7.25 E+06	7.25 E+06	1.22 E+07	1.12 E+07	4.84 E+07	8.57 E+07
dGMP	6.74 E+06	5.01 E+06	6.23 E+06	9.31 E+06	1.22 E+07	8.27 E+06	1.96 E+06	3.62 E+06	1.96 E+06	8.96 E+06	8.66 E+06	1.04 E+07
dITP	3.59 E+04	2.02 E+04	6.19 E+04	4.35 E+04	5.29 E+04	3.45 E+04	1.57 E+04	2.69 E+04	1.57 E+04	6.72 E+04	6.70 E+04	6.81 E+04
2-hydroxyglutarate	4.50 E+06	5.47 E+06	3.44 E+06	2.67 E+06	2.67 E+06	2.64 E+06	4.47 E+06	4.47 E+06	4.74 E+06	2.22 E+06	1.23 E+06	1.40 E+06
2-isopropylmalate	2.66 E+06	3.85 E+06	2.55 E+06	3.52 E+06	3.52 E+06	4.32 E+06	2.52 E+06	2.52 E+06	3.44 E+06	2.49 E+06	7.42 E+05	1.04 E+06
2-ketobutyrate	2.55 E+07	2.40 E+07	2.06 E+07	1.61 E+07	1.53 E+07	1.28 E+07	2.27 E+07	2.30 E+07	2.27 E+07	2.10 E+07	1.19 E+07	1.34 E+07
2-ketogluconate	1.72 E+06	1.19 E+06	1.10 E+06	3.93 E+06	4.21 E+06	1.41 E+06	1.16 E+05	4.13 E+06	1.16 E+05	5.54 E+06	1.52 E+06	2.21 E+06
2-methylcitrate	1.24 E+06	5.83 E+05	6.59 E+05	1.45 E+06	1.80 E+06	1.04 E+06	6.36 E+05	6.46 E+05	6.36 E+05	8.60 E+05	7.11 E+05	1.58 E+06
3-PG	9.86 E+03	2.20 E+04	2.24 E+04	8.56 E+04	1.83 E+05	1.21 E+05	7.62 E+03	7.62 E+03	4.21 E+04	3.25 E+04	2.77 E+04	5.56 E+04
4-hydroxybenzoate	6.08 E+06	3.84 E+06	5.01 E+06	8.98 E+06	8.85 E+06	4.11 E+06	2.94 E+05	6.38 E+05	2.94 E+05	3.49 E+06	3.34 E+06	4.20 E+06

4-pyridoxate	3.16 E+06	3.17 E+06	2.38 E+06	3.13 E+06	3.58 E+06	2.05 E+06	4.31 E+06	7.37 E+06	4.31 E+06	4.07 E+06	3.53 E+06	3.62 E+06
5-methylthioadenosine	3.98 E+05	1.04 E+05	8.75 E+05	7.04 E+06	7.23 E+06	5.01 E+06	3.59 E+04	1.16 E+05	3.59 E+04	3.23 E+06	3.22 E+06	3.22 E+06
7-methylguanine	1.52 E+05	6.72 E+03	1.36 E+05	3.30 E+05	4.78 E+05	3.81 E+05	6.72 E+03	6.72 E+03	9.99 E+04	2.02 E+05	2.58 E+03	3.67 E+04
7-methylguanosine	1.26 E+06	5.93 E+05	1.03 E+06	5.79 E+06	7.14 E+06	4.00 E+06	1.90 E+05	4.89 E+05	1.90 E+05	1.02 E+06	5.56 E+05	6.36 E+05
acetylcholine	1.02 E+07	7.66 E+06	1.22 E+07	1.86 E+07	1.64 E+07	1.72 E+07	1.76 E+06	4.07 E+06	1.76 E+06	1.35 E+07	1.29 E+07	1.64 E+07
adenine	7.46 E+06	9.34 E+06	9.46 E+06	6.95 E+06	1.00 E+07	1.25 E+07	2.72 E+06	5.25 E+06	2.72 E+06	4.49 E+06	4.46 E+06	4.46 E+06
adenosine	1.72 E+07	1.79 E+07	1.93 E+07	2.41 E+07	4.71 E+07	6.29 E+07	3.51 E+06	7.16 E+06	3.51 E+06	1.12 E+07	1.11 E+07	1.15 E+07
adenylsuccinate	1.73 E+05	1.60 E+05	1.62 E+05	1.50 E+05	1.50 E+05	1.97 E+05	8.78 E+04	9.01 E+04	8.78 E+04	5.92 E+04	4.63 E+04	1.21 E+05
ADP	7.31 E+04	3.23 E+04	4.93 E+04	4.79 E+05	5.03 E+05	2.73 E+05	1.34 E+04	4.88 E+04	1.34 E+04	5.56 E+04	5.45 E+04	6.09 E+04
alanine	2.52 E+07	1.13 E+07	1.14 E+07	2.05 E+07	1.55 E+07	2.12 E+07	1.54 E+06	6.80 E+06	1.54 E+06	2.36 E+07	1.32 E+07	2.73 E+06
AMP	2.78 E+04	4.66 E+04	9.95 E+04	3.44 E+05	5.08 E+05	3.09 E+05	1.88 E+04	1.88 E+04	1.97 E+04	1.55 E+05	1.53 E+05	1.65 E+05
arginine	1.64 E+07	6.40 E+06	2.85 E+07	5.55 E+07	2.61 E+07	1.38 E+08	2.82 E+06	6.44 E+06	2.82 E+06	3.02 E+07	1.67 E+07	1.90 E+07
argininosuccinate	7.26 E+04	7.93 E+04	7.17 E+04	6.99 E+04	5.33 E+04	1.44 E+05	3.14 E+04	5.29 E+04	3.14 E+04	2.69 E+04	2.63 E+04	2.96 E+04
azelaic acid	1.41 E+06	1.21 E+06	1.36 E+06	4.85 E+06	8.32 E+06	3.28 E+06	2.30 E+06	2.30 E+06	2.31 E+06	1.21 E+06	1.04 E+06	1.07 E+06
betaine	2.66 E+06	1.08 E+06	1.19 E+06	6.14 E+05	9.29 E+05	1.02 E+06	1.00 E+06	1.39 E+06	1.00 E+06	3.80 E+05	1.94 E+05	2.26 E+05

carbamoylphosphate	6.27 E+03	1.79 E+03	4.48 E+03	1.30 E+06	1.45 E+06	1.03 E+06	1.39 E+04	1.39 E+04	NA	3.37 E+05	2.62 E+05	6.95 E+05
CDP-choline	1.34 E+04	6.72 E+03	2.47 E+04	5.92 E+04	6.59 E+04	2.91 E+04	3.59 E+03	1.43 E+04	3.59 E+03	2.87 E+04	1.46 E+04	1.70 E+04
aconitate	2.59 E+07	2.82 E+07	1.80 E+07	1.52 E+08	1.84 E+08	9.35 E+07	1.39 E+07	2.23 E+07	1.39 E+07	3.24 E+07	3.21 E+07	3.35 E+07
citramalate	7.85 E+06	6.42 E+06	4.94 E+06	6.86 E+06	7.47 E+06	4.76 E+06	4.61 E+06	4.61 E+06	5.68 E+06	4.52 E+06	3.96 E+06	4.05 E+06
citrate	7.95 E+05	3.59 E+05	4.32 E+05	3.35 E+07	3.54 E+07	1.05 E+07	5.57 E+05	2.15 E+06	5.57 E+05	5.51 E+06	3.08 E+06	3.50 E+06
citrulline	2.34 E+05	7.44 E+04	3.91 E+05	7.56 E+05	4.09 E+05	3.04 E+06	2.78 E+04	7.22 E+04	2.78 E+04	4.50 E+05	2.01 E+05	2.44 E+05
cAMP	6.14 E+04	1.01 E+04	6.90 E+04	2.82 E+05	3.16 E+05	3.53 E+05	8.07 E+03	8.07 E+03	8.96 E+03	1.11 E+05	1.10 E+05	1.17 E+05
cytidine	3.36 E+06	1.68 E+06	2.53 E+06	2.53 E+06	3.34 E+06	4.52 E+06	3.67 E+05	7.39 E+05	3.67 E+05	8.52 E+05	7.55 E+05	7.72 E+05
cytosine	3.95 E+05	4.27 E+05	1.18 E+06	6.66 E+05	8.32 E+05	8.63 E+05	3.63 E+05	6.58 E+05	3.63 E+05	8.18 E+05	3.91 E+05	4.65 E+05
deoxyadenosine	1.78 E+06	3.79 E+05	1.64 E+06	1.32 E+06	1.23 E+06	3.79 E+06	2.90 E+05	3.41 E+05	2.90 E+05	1.03 E+06	9.73 E+05	1.32 E+06
dAMP	8.29 E+04	2.87 E+04	1.06 E+05	7.66 E+04	5.46 E+04	3.85 E+04	2.11 E+04	2.11 E+04	2.11 E+04	1.03 E+05	8.77 E+04	1.77 E+05
dCDP	8.96 E+03	2.69 E+03	4.03 E+03	1.68 E+05	2.30 E+05	1.55 E+05	3.59 E+03	3.59 E+03	NA	2.11 E+04	1.94 E+04	2.91 E+04
deoxyguanosine	2.03 E+05	3.36 E+04	7.13 E+04	1.37 E+05	1.16 E+05	1.63 E+05	3.72 E+04	6.01 E+04	3.72 E+04	1.40 E+05	1.32 E+05	1.34 E+05
dGDP	9.77 E+04	8.06 E+03	9.46 E+04	5.26 E+05	5.79 E+05	3.13 E+05	1.70 E+04	1.70 E+04	4.03 E+04	7.26 E+04	7.07 E+04	8.16 E+04
dIMP	3.29 E+06	9.53 E+05	2.08 E+06	3.34 E+06	3.28 E+06	3.11 E+06	8.26 E+05	2.03 E+06	8.26 E+05	7.38 E+06	7.37 E+06	7.41 E+06

deoxyribose-P	2.96 E+04	2.29 E+04	1.66 E+04	6.00 E+04	4.80 E+04	7.26 E+04	2.78 E+04	2.78 E+04	2.78 E+04	1.57 E+04	9.19 E+03	2.69 E+03
G-3-P	9.01 E+04	3.77 E+05	1.29 E+05	1.22 E+05	6.75 E+04	3.72 E+04	1.16 E+05	1.16 E+05	2.19 E+05	1.44 E+05	7.86 E+04	1.30 E+04
DHAP	3.38 E+05	2.98 E+05	3.22 E+05	2.26 E+05	2.30 E+05	1.94 E+05	3.17 E+05	3.17 E+05	4.11 E+05	4.32 E+05	3.59 E+05	3.72 E+05
dimethylglycine	5.77 E+08	5.03 E+08	5.78 E+08	4.59 E+08	5.06 E+08	5.52 E+08	3.15 E+08	4.27 E+08	3.15 E+08	4.64 E+08	4.35 E+08	6.04 E+08
dimethylarginine	3.18 E+07	3.18 E+07	1.76 E+07	2.32 E+07	1.82 E+07	2.23 E+07	1.58 E+07	1.77 E+07	1.58 E+07	1.92 E+07	3.70 E+06	6.36 E+06
erythrose 4-P	4.92 E+05	4.63 E+05	3.59 E+05	4.20 E+05	5.36 E+05	1.88 E+05	2.76 E+05	2.76 E+05	4.38 E+05	1.73 E+05	1.57 E+05	2.49 E+05
ethanolamine	8.16 E+06	8.74 E+06	5.32 E+06	2.83 E+06	3.62 E+06	4.19 E+06	2.04 E+06	2.36 E+06	2.04 E+06	4.17 E+06	2.50 E+06	2.79 E+06
FAD	1.08 E+04	2.82 E+04	3.09 E+04	5.38 E+03	2.34 E+05	1.34 E+05	1.26 E+04	1.26 E+04	2.33 E+04	1.17 E+05	8.01 E+04	8.65 E+04
FMN	9.25 E+04	5.15 E+04	5.60 E+04	9.63 E+04	9.41 E+04	5.74 E+04	4.75 E+04	4.75 E+04	4.79 E+04	1.54 E+05	1.32 E+05	1.36 E+05
fumarate	4.39 E+06	9.37 E+06	4.39 E+06	6.31 E+07	7.77 E+07	4.14 E+07	4.96 E+06	6.06 E+06	4.96 E+06	8.44 E+07	7.87 E+07	7.97 E+07
γ -glutamylalanine	3.63 E+04	6.27 E+03	2.15 E+04	1.38 E+05	1.35 E+05	8.26 E+04	8.07 E+03	8.07 E+03	NA	3.50 E+04	2.01 E+04	5.15 E+03
gluconate	2.34 E+06	2.02 E+06	2.52 E+06	1.42 E+07	1.47 E+07	9.36 E+06	6.16 E+05	3.18 E+06	6.16 E+05	1.63 E+07	1.10 E+07	1.19 E+07
glucosamine	6.39 E+05	3.43 E+05	6.60 E+05	3.27 E+06	3.08 E+06	1.78 E+06	1.79 E+05	4.37 E+05	1.79 E+05	1.61 E+06	1.41 E+06	2.58 E+06
GSH	3.59 E+04	1.03 E+04	1.74 E+05	4.24 E+06	4.26 E+06	1.49 E+06	3.14 E+03	7.71 E+04	3.14 E+03	1.15 E+05	1.12 E+05	1.13 E+05
glycerate	1.22 E+06	7.90 E+05	1.30 E+06	1.95 E+06	2.42 E+06	2.41 E+06	3.62 E+05	7.69 E+05	3.62 E+05	2.09 E+06	1.49 E+06	1.59 E+06

glycerol	1.08 E+07	1.22 E+07	1.36 E+07	1.31 E+07	1.05 E+07	2.34 E+07	1.53 E+07	1.53 E+07	1.71 E+07	1.18 E+07	9.91 E+06	1.02 E+07
glycerol-3-P	8.11 E+04	5.74 E+04	1.57 E+05	2.54 E+06	3.57 E+06	1.58 E+06	4.66 E+04	5.11 E+04	4.66 E+04	7.24 E+05	6.82 E+05	9.26 E+05
glycerophosphocholine	6.39 E+07	4.64 E+07	7.52 E+07	8.13 E+07	7.98 E+07	1.29 E+08	3.60 E+07	3.60 E+07	5.78 E+07	2.82 E+07	1.88 E+07	2.04 E+07
glycine	2.92 E+05	1.10 E+05	2.04 E+05	2.86 E+05	3.42 E+05	2.92 E+05	3.05 E+04	4.48 E+04	3.05 E+04	1.87 E+05	1.33 E+05	1.42 E+05
glyoxylate	7.93 E+04	6.54 E+04	1.00 E+05	1.20 E+06	1.51 E+06	5.92 E+05	2.17 E+04	3.45 E+04	2.17 E+04	1.81 E+06	1.34 E+06	1.42 E+06
guanine	1.37 E+05	9.99 E+04	1.09 E+05	4.30 E+04	4.03 E+04	7.08 E+04	1.82 E+05	2.01 E+05	1.82 E+05	1.08 E+05	1.02 E+05	1.37 E+05
guanosine	1.74 E+06	8.87 E+05	1.56 E+06	2.02 E+06	2.03 E+06	2.67 E+06	1.93 E+05	3.58 E+05	1.93 E+05	5.57 E+05	5.53 E+05	5.71 E+05
hexose diphosphate pool	7.63 E+04	1.85 E+05	6.57 E+04	1.46 E+05	1.19 E+05	8.34 E+04	1.79 E+04	7.48 E+04	1.79 E+04	5.38 E+03	1.05 E+03	1.79 E+03
hexose disaccharide monophosphate pool	6.27 E+03	5.24 E+03	5.83 E+03	3.05 E+05	3.23 E+05	3.10 E+05	4.03 E+03	6.27 E+03	4.03 E+03	6.10 E+04	5.92 E+04	6.95 E+04
hexose disaccharide pool	2.48 E+08	1.80 E+08	2.57 E+08	4.24 E+08	4.55 E+08	4.29 E+08	1.46 E+08	1.78 E+08	1.46 E+08	3.01 E+08	2.96 E+08	3.22 E+08
hexose phosphate pool	8.10 E+05	2.94 E+05	9.41 E+05	2.67 E+07	3.23 E+07	1.64 E+07	1.20 E+06	1.20 E+06	1.41 E+06	6.81 E+06	6.49 E+06	8.35 E+06
hexose pool	2.62 E+07	8.99 E+06	1.75 E+07	1.44 E+07	2.13 E+07	1.45 E+07	1.23 E+06	3.91 E+06	1.23 E+06	5.86 E+07	5.73 E+07	6.48 E+07
hydroxyproline	6.94 E+05	7.09 E+05	4.90 E+05	2.89 E+05	4.04 E+05	4.72 E+05	3.56 E+05	3.56 E+05	4.66 E+05	4.62 E+05	2.72 E+05	3.04 E+05
hypoxanthine	4.80 E+04	4.48 E+03	4.48 E+03	7.13 E+04	9.23 E+04	2.69 E+04	9.86 E+04	1.36 E+05	9.86 E+04	5.02 E+04	4.53 E+04	7.39 E+04
IMP	9.41 E+03	NA	4.93 E+03	4.48 E+04	1.37 E+05	4.17 E+04	2.24 E+03	NA	2.24 E+03	2.82 E+04	1.73 E+03	6.28 E+03

inosine	1.02 E+05	6.14 E+04	1.71 E+05	3.96 E+05	7.05 E+05	2.19 E+05	5.96 E+04	9.37 E+04	5.96 E+04	2.66 E+05	1.78 E+05	1.93 E+05
isocitrate	2.00 E+07	7.68 E+06	1.02 E+07	3.30 E+08	3.32 E+08	1.75 E+08	1.38 E+07	4.18 E+07	1.38 E+07	1.06 E+08	6.20 E+07	6.95 E+07
ITP	1.38 E+06	7.32 E+05	1.19 E+06	5.71 E+05	1.02 E+06	8.44 E+05	3.48 E+05	1.21 E+06	3.48 E+05	4.76 E+05	4.50 E+05	6.03 E+05
ascorbate	5.26 E+06	7.61 E+06	3.48 E+06	4.30 E+06	3.99 E+06	5.09 E+06	3.83 E+06	4.37 E+06	3.83 E+06	4.00 E+06	6.83 E+05	1.25 E+06
asparagine	1.86 E+08	1.55 E+08	1.77 E+08	1.67 E+08	1.58 E+08	2.67 E+08	1.41 E+08	1.73 E+08	1.41 E+08	1.20 E+08	7.51 E+07	8.28 E+07
aspartate	2.04 E+07	9.15 E+06	2.77 E+07	7.29 E+07	8.61 E+07	9.49 E+07	9.73 E+05	1.61 E+06	9.73 E+05	1.37 E+07	5.85 E+06	7.19 E+06
carnitine	5.13 E+05	6.69 E+05	7.21 E+05	2.37 E+05	3.50 E+05	1.39 E+05	4.93 E+05	4.93 E+05	8.29 E+05	1.62 E+05	9.24 E+04	1.04 E+05
glutamate	1.21 E+07	8.16 E+06	1.83 E+07	5.51 E+07	5.89 E+07	4.65 E+07	1.97 E+06	2.23 E+06	1.97 E+06	1.40 E+07	9.04 E+06	9.90 E+06
glutamine	2.23 E+08	1.83 E+08	2.54 E+08	2.58 E+08	2.54 E+08	3.11 E+08	1.45 E+08	1.45 E+08	1.68 E+08	1.79 E+08	1.76 E+08	1.91 E+08
histidine	3.08 E+07	1.34 E+07	2.35 E+07	4.06 E+07	3.99 E+07	5.82 E+07	2.45 E+06	4.52 E+06	2.45 E+06	3.35 E+07	1.93 E+07	2.18 E+07
linoleate	3.46 E+05	8.24 E+05	2.43 E+05	6.41 E+05	5.66 E+05	1.58 E+05	6.18 E+05	8.26 E+05	6.18 E+05	3.86 E+05	1.35 E+05	1.78 E+05
isoleucine	1.62 E+08	1.35 E+08	1.59 E+08	1.40 E+08	1.46 E+08	1.45 E+08	9.08 E+07	1.05 E+08	9.08 E+07	1.32 E+08	8.93 E+07	9.66 E+07
leucine	1.60 E+08	1.34 E+08	1.55 E+08	1.39 E+08	1.44 E+08	1.43 E+08	9.09 E+07	1.06 E+08	9.09 E+07	1.30 E+08	8.35 E+07	9.16 E+07
lysine	3.51 E+07	NA	3.85 E+07	3.74 E+07	3.37 E+07	4.41 E+07	1.20 E+07	1.66 E+07	1.20 E+07	2.87 E+07	1.68 E+07	1.89 E+07
methionine	1.56 E+07	6.66 E+06	9.27 E+06	9.29 E+06	8.41 E+06	6.68 E+06	2.15 E+06	3.09 E+06	2.15 E+06	6.44 E+06	2.72 E+06	3.36 E+06

phenylalanine	5.15 E+07	2.78 E+07	3.23 E+07	2.75 E+07	2.34 E+07	2.94 E+07	5.41 E+06	6.60 E+06	5.41 E+06	9.19 E+06	3.97 E+06	4.87 E+06
proline	3.29 E+07	7.50 E+06	2.93 E+07	8.47 E+06	6.97 E+06	6.03 E+06	2.43 E+06	8.22 E+06	2.43 E+06	3.41 E+06	3.12 E+06	4.80 E+06
serine	3.56 E+07	1.67 E+07	3.77 E+07	2.44 E+07	2.66 E+07	3.41 E+07	3.11 E+06	5.02 E+06	3.11 E+06	1.29 E+07	6.76 E+06	7.80 E+06
threonine	1.16 E+07	5.43 E+06	9.41 E+06	1.06 E+07	1.29 E+07	1.63 E+07	3.03 E+06	3.33 E+06	3.03 E+06	3.97 E+06	3.19 E+06	3.32 E+06
tryptophan	4.68 E+07	3.53 E+07	6.29 E+07	2.44 E+07	1.79 E+07	3.32 E+07	1.67 E+07	1.67 E+07	1.84 E+07	4.49 E+07	3.06 E+07	3.30 E+07
tyrosine	2.47 E+06	1.64 E+06	1.46 E+06	2.81 E+06	2.54 E+06	2.03 E+06	5.51 E+05	7.91 E+05	5.51 E+05	1.24 E+06	7.48 E+05	8.32 E+05
valine	3.33 E+05	3.24 E+05	4.90 E+05	5.10 E+05	4.88 E+05	6.40 E+05	2.41 E+05	3.20 E+05	2.41 E+05	5.11 E+05	4.92 E+05	6.05 E+05
lysoPC(16:0)	9.46 E+04	9.77 E+04	1.04 E+05	5.78 E+04	1.92 E+05	2.54 E+05	3.95 E+04	4.48 E+04	3.95 E+04	5.69 E+04	2.88 E+04	3.36 E+04
lysoPC(18:0)	1.26 E+06	1.25 E+06	1.85 E+06	3.64 E+05	5.80 E+05	4.74 E+05	1.31 E+06	2.14 E+06	1.31 E+06	3.16 E+05	1.34 E+05	1.65 E+05
malate	6.34 E+07	5.34 E+07	8.25 E+07	3.84 E+08	4.57 E+08	2.99 E+08	3.72 E+07	5.72 E+07	3.72 E+07	4.81 E+08	4.31 E+08	4.40 E+08
malonate	1.60 E+07	1.94 E+07	1.10 E+07	1.13 E+07	1.46 E+07	1.20 E+07	1.46 E+07	1.60 E+07	1.46 E+07	1.46 E+07	7.87 E+06	9.02 E+06
metSO	3.20 E+06	2.02 E+06	1.83 E+06	3.39 E+06	2.87 E+06	3.10 E+06	4.03 E+05	1.20 E+06	4.03 E+05	3.14 E+06	1.53 E+06	1.81 E+06
methylsuccinate	1.56 E+06	1.90 E+06	1.18 E+06	1.44 E+06	1.52 E+06	1.11 E+06	1.42 E+06	1.42 E+06	1.69 E+06	1.47 E+06	6.81 E+05	8.16 E+05
mevalonate	6.37 E+05	5.23 E+05	1.16 E+06	4.78 E+05	7.09 E+05	1.57 E+06	5.70 E+05	5.70 E+05	6.55 E+05	4.47 E+05	3.22 E+05	1.05 E+06
myoinositol	1.89 E+06	1.14 E+06	1.94 E+06	6.59 E+06	6.64 E+06	5.92 E+06	8.77 E+05	1.48 E+06	8.77 E+05	1.36 E+07	8.08 E+06	9.03 E+06

N-acetylglucosamine-1-P	2.47 E+04	2.78 E+04	5.15 E+04	6.51 E+05	9.69 E+05	5.46 E+05	8.07 E+03	8.07 E+03	1.61 E+04	2.01 E+05	1.39 E+05	1.49 E+05
N-acetylglutamate	2.27 E+05	1.13 E+05	1.49 E+05	1.52 E+06	1.04 E+06	3.18 E+05	1.01 E+05	1.50 E+05	1.01 E+05	2.22 E+05	6.21 E+04	8.96 E+04
N-acetylaspartate	2.42 E+06	1.43 E+06	1.95 E+06	2.39 E+06	1.95 E+06	1.44 E+06	2.31 E+05	2.77 E+05	2.31 E+05	1.37 E+06	6.07 E+05	7.38 E+05
N-acetylserine	1.22 E+06	9.80 E+05	1.38 E+06	2.05 E+06	2.33 E+06	1.89 E+06	3.36 E+05	5.96 E+05	3.36 E+05	9.40 E+05	8.57 E+05	8.71 E+05
NAD	1.82 E+05	9.95 E+04	3.10 E+05	1.45 E+06	1.50 E+06	1.18 E+06	2.38 E+04	3.81 E+04	2.38 E+04	5.73 E+05	3.76 E+05	4.10 E+05
N- α -acetylarginine	3.52 E+05	3.80 E+05	3.64 E+05	4.64 E+05	3.89 E+05	3.92 E+05	2.84 E+05	2.29 E+06	2.84 E+05	5.25 E+05	3.46 E+05	3.76 E+05
nicotinate	1.54 E+05	1.64 E+05	1.04 E+05	1.41 E+05	1.11 E+05	1.08 E+05	1.56 E+05	1.56 E+05	2.24 E+05	1.78 E+05	9.39 E+04	1.08 E+05
octulose-P	5.78 E+05	4.54 E+05	1.84 E+05	1.01 E+06	8.08 E+05	5.09 E+05	3.99 E+04	2.47 E+05	3.99 E+04	1.72 E+05	1.01 E+05	2.94 E+04
oleate	6.46 E+05	4.87 E+05	5.44 E+05	7.32 E+05	8.44 E+05	4.19 E+05	3.46 E+05	5.46 E+05	3.46 E+05	3.83 E+05	3.76 E+05	4.17 E+05
O-phosphoethanolamine	1.68 E+07	1.82 E+07	1.19 E+07	1.80 E+07	1.80 E+07	9.62 E+06	1.57 E+07	1.97 E+07	1.57 E+07	2.10 E+07	1.03 E+07	1.21 E+07
O-phosphotyrosine	6.27 E+03	3.59 E+03	1.70 E+04	7.51 E+05	8.89 E+05	5.36 E+05	2.69 E+03	2.69 E+03	9.41 E+03	1.85 E+05	1.83 E+05	1.97 E+05
ornithine	3.15 E+06	2.57 E+06	2.82 E+06	2.90 E+06	2.48 E+06	7.50 E+06	1.49 E+06	2.78 E+06	1.49 E+06	1.17 E+06	3.93 E+05	5.26 E+05
oxalate	8.69 E+04	9.55 E+04	8.51 E+04	9.01 E+04	1.20 E+05	9.46 E+04	7.39 E+04	7.39 E+04	1.33 E+05	5.38 E+04	5.11 E+04	6.68 E+04
OAA	7.62 E+05	4.16 E+05	7.58 E+05	4.67 E+05	4.99 E+05	1.55 E+06	2.46 E+05	6.44 E+05	2.46 E+05	1.64 E+05	1.15 E+05	1.24 E+05
GSSG	2.51 E+04	8.07 E+03	7.13 E+04	1.13 E+05	1.99 E+05	9.28 E+04	6.28 E+03	6.28 E+03	NA	2.69 E+03	4.61 E+02	1.34 E+04

pantothenate	9.77 E+06	8.84 E+06	1.42 E+07	1.64 E+07	2.73 E+07	1.89 E+07	2.27 E+06	2.27 E+06	2.55 E+06	1.76 E+07	1.07 E+07	1.19 E+07
PC(34:1)	2.90 E+05	9.99 E+04	6.37 E+05	1.12 E+06	1.17 E+06	1.55 E+06	9.21 E+04	1.41 E+05	9.21 E+04	9.62 E+05	8.43 E+05	1.54 E+06
PC(34:2)	2.37 E+06	1.38 E+06	3.95 E+06	6.62 E+06	6.24 E+06	8.30 E+06	5.15 E+05	9.17 E+05	5.15 E+05	4.54 E+06	4.34 E+06	5.51 E+06
PC(36:0)	1.96 E+05	1.53 E+05	2.34 E+05	1.59 E+05	1.75 E+05	2.51 E+05	1.31 E+05	1.50 E+05	1.31 E+05	2.05 E+05	1.72 E+05	3.63 E+05
PC(36:2)	5.54 E+05	3.81 E+05	1.01 E+06	1.51 E+06	1.61 E+06	1.82 E+06	2.06 E+05	2.06 E+05	2.22 E+05	1.06 E+06	1.05 E+06	1.11 E+06
PC(36:4)	3.66 E+06	2.48 E+06	6.97 E+06	8.43 E+06	9.11 E+06	9.69 E+06	1.30 E+06	1.96 E+06	1.30 E+06	5.94 E+06	5.56 E+06	7.79 E+06
PE (36:0)	6.72 E+04	1.88 E+04	6.23 E+04	9.55 E+04	7.89 E+04	1.32 E+05	8.07 E+03	2.24 E+04	8.07 E+03	8.83 E+04	4.53 E+04	2.24 E+03
PE(34:1)	2.90 E+05	2.25 E+05	4.28 E+05	1.45 E+06	1.75 E+06	3.18 E+06	9.63 E+04	1.55 E+05	9.63 E+04	1.09 E+06	1.04 E+06	1.34 E+06
PE(34:2)	1.27 E+05	9.19 E+04	1.86 E+05	1.71 E+06	1.42 E+06	5.61 E+05	5.78 E+04	5.78 E+04	8.96 E+04	1.81 E+06	3.35 E+05	5.88 E+05
PE(36:1)	9.68 E+04	4.17 E+04	1.31 E+05	2.35 E+05	1.73 E+05	2.66 E+05	2.69 E+04	4.12 E+04	2.69 E+04	1.62 E+05	1.61 E+05	1.67 E+05
PE(36:2)	6.37 E+05	6.58 E+05	1.06 E+06	1.64 E+06	1.94 E+06	2.15 E+06	1.43 E+05	2.27 E+05	1.43 E+05	1.43 E+06	9.80 E+05	1.06 E+06
PE(36:3)	3.18 E+06	2.44 E+06	6.66 E+06	1.24 E+07	1.64 E+07	1.74 E+07	1.08 E+06	1.33 E+06	1.08 E+06	5.68 E+06	5.41 E+06	7.01 E+06
PE(36:4)	1.28 E+05	7.35 E+04	1.31 E+05	9.32 E+04	1.31 E+05	9.85 E+04	1.79 E+04	1.79 E+04	3.67 E+04	1.03 E+05	9.71 E+04	9.81 E+04
PG(34:1)	4.03 E+05	2.11 E+05	8.50 E+05	3.37 E+06	3.75 E+06	3.43 E+06	1.40 E+05	2.14 E+05	1.40 E+05	1.16 E+06	8.96 E+05	2.44 E+06
PG(34:2)	1.75 E+04	1.21 E+04	1.08 E+05	5.66 E+05	7.17 E+05	3.51 E+05	3.14 E+03	3.14 E+03	5.38 E+03	3.07 E+05	2.62 E+05	2.70 E+05

PG(36:2)	1.34 E+04	5.33 E+04	4.57 E+04	3.87 E+05	5.15 E+04	3.97 E+05	1.12 E+04	1.66 E+04	1.12 E+04	7.93 E+04	5.77 E+04	6.14 E+04
phenyllactate	1.41 E+06	7.83 E+05	1.38 E+06	9.10 E+06	9.60 E+06	5.09 E+06	3.93 E+05	1.01 E+06	3.93 E+05	9.56 E+06	5.08 E+06	5.85 E+06
phosphoenolpyruvate	1.03 E+04	6.72 E+03	2.38 E+04	2.56 E+05	3.24 E+05	2.73 E+05	3.58 E+03	8.07 E+03	3.58 E+03	3.14 E+03	2.29 E+04	4.26 E+04
phosphorylcholine	1.26 E+06	8.34 E+05	3.25 E+06	1.17 E+08	1.35 E+08	1.20 E+08	6.07 E+05	9.21 E+05	6.07 E+05	5.60 E+07	5.41 E+07	6.54 E+07
PI(34:0)	9.14 E+04	5.74 E+04	1.97 E+05	9.59 E+04	1.36 E+05	2.56 E+05	1.57 E+04	1.57 E+04	2.60 E+04	6.54 E+04	4.75 E+04	1.52 E+05
PI(34:1)	1.84 E+05	6.05 E+04	3.54 E+05	7.65 E+05	1.11 E+06	1.74 E+06	2.33 E+04	6.72 E+04	2.33 E+04	5.23 E+05	3.83 E+05	1.20 E+06
PI(36:2)	1.75 E+04	1.26 E+04	6.54 E+04	8.11 E+04	8.34 E+04	1.98 E+05	1.34 E+03	5.83 E+03	1.34 E+03	5.02 E+04	4.03 E+04	9.81 E+04
pipecolate	1.02 E+08	5.27 E+07	8.60 E+07	7.61 E+07	6.60 E+07	5.63 E+07	2.16 E+07	3.25 E+07	2.16 E+07	5.93 E+07	1.90 E+07	2.60 E+07
purine	1.09 E+06	5.07 E+05	6.62 E+05	5.60 E+05	4.29 E+05	5.80 E+05	1.55 E+05	1.74 E+05	1.55 E+05	1.77 E+05	3.47 E+04	5.92 E+04
pyridoxine	2.77 E+06	3.19 E+06	2.92 E+06	8.15 E+05	7.56 E+05	1.44 E+06	7.81 E+05	8.62 E+05	7.81 E+05	2.34 E+06	6.18 E+05	9.13 E+05
pyrophosphate	6.11 E+05	6.88 E+05	3.49 E+05	1.49 E+06	2.44 E+06	1.27 E+06	5.78 E+05	7.16 E+05	5.78 E+05	1.24 E+05	3.06 E+04	4.66 E+04
quinolate	1.10 E+06	1.03 E+06	6.87 E+05	1.11 E+06	1.02 E+06	1.46 E+06	8.33 E+05	8.33 E+05	8.43 E+05	6.17 E+05	6.13 E+05	6.37 E+05
riboflavin	5.79 E+06	3.26 E+06	4.32 E+06	4.33 E+06	4.37 E+06	3.41 E+06	1.30 E+06	2.02 E+06	1.30 E+06	5.59 E+06	4.85 E+06	4.98 E+06
ribose-5-P	4.08 E+04	1.98 E+05	1.70 E+05	1.92 E+06	3.40 E+06	1.01 E+06	1.29 E+05	1.29 E+05	2.33 E+05	5.42 E+05	4.96 E+05	7.65 E+05
S-adenosylhomocysteine	8.57 E+07	5.64 E+07	1.17 E+08	1.95 E+08	1.94 E+08	9.82 E+07	1.99 E+07	3.94 E+07	1.99 E+07	1.97 E+08	1.01 E+08	1.18 E+08

sarcosine	1.99 E+07	9.59 E+06	9.59 E+06	1.52 E+07	1.15 E+07	1.77 E+07	1.18 E+06	5.12 E+06	1.18 E+06	1.92 E+07	1.05 E+07	1.83 E+06
sedoheptulose-P	1.69 E+05	4.47 E+05	9.66 E+04	5.79 E+05	6.86 E+05	2.64 E+05	1.77 E+05	1.77 E+05	3.11 E+05	1.66 E+05	1.41 E+05	2.88 E+05
shikimate-3-P	1.25 E+06	1.48 E+06	1.27 E+06	1.03 E+06	8.89 E+05	7.40 E+05	2.81 E+06	4.12 E+06	2.81 E+06	1.17 E+06	5.01 E+05	4.42 E+06
shikimate	7.47 E+05	5.67 E+05	6.09 E+05	2.58 E+06	3.13 E+06	1.00 E+06	1.38 E+05	2.94 E+05	1.38 E+05	1.85 E+06	1.26 E+06	1.36 E+06
spermine	1.55 E+06	3.56 E+06	8.94 E+05	3.86 E+06	2.31 E+06	7.95 E+05	8.76 E+05	1.92 E+06	8.76 E+05	3.17 E+06	3.49 E+05	8.34 E+05
succinate	7.92 E+06	8.07 E+06	6.18 E+06	1.42 E+07	1.09 E+07	5.44 E+05	6.86 E+06	6.94 E+06	6.86 E+06	8.43 E+06	4.82 E+06	1.21 E+06
taurine	1.04 E+06	5.30 E+05	3.34 E+05	4.32 E+05	3.71 E+05	3.99 E+05	4.42 E+05	4.42 E+05	4.58 E+05	7.24 E+05	3.29 E+05	3.97 E+05
thiamine	2.80 E+07	2.94 E+07	1.84 E+07	1.98 E+07	1.88 E+07	3.77 E+07	1.56 E+07	1.56 E+07	2.09 E+07	4.15 E+07	3.76 E+07	5.99 E+07
thymidine	4.54 E+05	3.18 E+05	1.22 E+05	7.32 E+05	6.54 E+05	4.65 E+05	2.89 E+04	1.07 E+05	2.89 E+04	7.04 E+04	2.28 E+04	3.09 E+04
UDP	7.67 E+05	4.18 E+05	8.47 E+05	3.99 E+05	4.33 E+05	1.74 E+05	4.28 E+04	1.05 E+05	4.28 E+04	8.07 E+03	1.29 E+03	4.08 E+04
uracil	4.56 E+05	4.01 E+05	7.95 E+05	9.09 E+05	9.96 E+05	1.52 E+06	1.69 E+05	1.69 E+05	1.84 E+05	1.11 E+05	6.99 E+04	7.69 E+04
urea	3.72 E+05	4.47 E+05	2.59 E+05	3.75 E+05	3.63 E+05	2.55 E+05	1.35 E+04	1.39 E+05	1.35 E+04	1.26 E+05	4.29 E+04	5.71 E+04
ureidopropionate	5.25 E+05	3.36 E+05	6.42 E+05	7.78 E+05	7.97 E+05	1.85 E+06	1.56 E+05	4.21 E+05	1.56 E+05	2.21 E+05	2.11 E+05	2.72 E+05
uridine	6.57 E+05	2.94 E+05	3.51 E+05	6.98 E+05	5.95 E+05	6.65 E+05	2.55 E+05	6.20 E+05	2.55 E+05	2.90 E+05	9.17 E+04	1.25 E+06
uridine-5-P	3.71 E+05	1.36 E+05	5.70 E+05	1.06 E+06	1.52 E+06	5.42 E+04	1.52 E+05	1.92 E+05	1.52 E+05	4.50 E+05	2.03 E+05	2.45 E+05

UDP-glucose	2.18 E+05	1.53 E+05	3.46 E+05	2.71 E+06	3.94 E+06	1.58 E+06	1.43 E+05	1.43 E+05	1.78 E+05	8.95 E+05	6.09 E+05	6.58 E+05
UDP-N-acetylglucosamine	2.42 E+04	1.48 E+04	2.60 E+04	2.42 E+04	2.64 E+04	1.48 E+04	5.83 E+03	5.83 E+03	2.55 E+04	1.57 E+04	1.03 E+04	1.12 E+04
xanthine	1.30 E+06	1.17 E+06	6.44 E+05	6.67 E+05	8.96 E+05	3.45 E+05	2.32 E+05	5.40 E+05	2.32 E+05	3.24 E+05	1.71 E+05	1.97 E+05
xanthosine	1.03 E+06	4.43 E+05	6.27 E+05	7.51 E+05	1.07 E+06	5.96 E+05	6.59 E+04	1.10 E+05	6.59 E+04	4.24 E+05	2.72 E+05	2.98 E+05

Table B5: Comprehensive water metabolite data as area under the curve (AUC) collected by LC-MS/MS throughout the water metabolomic analysis from Chapter 3, including plant treated waters (A7P) and control waters (A7C) with a minimum threshold of ~3.00E+4 AUC.				
Metabolites	A7P1	A7P2	A7C1	A7C2
1-methyladenine	5.83E+03	4.48E+03	NA	NA
2-hydroxyglutarate	2.50E+06	1.90E+06	NA	NA
2-isopropylmalate	1.96E+06	1.23E+06	NA	NA
2-ketogluconate	1.19E+05	1.93E+04	3.97E+04	NA
2-Methylcitric acid	1.42E+05	4.54E+05	NA	NA
3-PG	4.30E+04	3.29E+04	2.15E+04	NA
4-hydroxybenzoate	3.45E+04	4.70E+04	1.93E+04	NA
4-pyridoxate	1.08E+04	1.97E+04	3.14E+04	NA
5-methylthioadenosine	1.79E+04	3.59E+03	1.30E+04	NA
7-methylguanine	1.17E+04	8.29E+03	8.51E+03	NA
7-methylguanosine	6.72E+03	4.48E+03	NA	3.99E+04
acetylcholine	4.39E+04	9.77E+04	1.26E+04	NA
aconitate	2.54E+06	3.28E+07	NA	NA
adenosine	3.31E+05	8.65E+04	4.84E+03	NA
adenylsuccinate	6.27E+03	8.06E+03	3.14E+03	NA
ADP	1.75E+04	1.43E+04	7.62E+03	NA
alanine	6.21E+04	3.51E+05	1.07E+05	1.27E+05
AMP	8.96E+03	9.41E+03	NA	7.62E+03
argininosuccinate	NA	3.14E+03	3.59E+03	NA
azelaic acid	1.83E+06	5.98E+05	2.21E+05	1.11E+05
betaine	3.59E+03	2.56E+03	NA	NA

carbamoylphosphate	7.62E+03	6.72E+03	NA	NA
carnitine	2.69E+03	2.24E+03	NA	NA
citramalate	3.48E+06	1.83E+06	NA	NA
citrate	2.00E+05	8.76E+04	3.94E+04	NA
citrulline	1.34E+03	4.48E+03	5.83E+03	NA
cytidine	2.11E+04	1.79E+04	NA	1.84E+04
cytosine	1.08E+05	4.35E+04	6.81E+04	NA
dAMP	4.48E+03	7.95E+03	NA	NA
dCDP	4.03E+03	NA	4.48E+03	NA
deoxyadenosine	2.24E+04	2.06E+04	3.23E+04	NA
deoxyguanosine	8.52E+03	4.93E+03	5.83E+03	NA
deoxyribose-P	9.41E+03	3.59E+03	NA	NA
dGDP	2.02E+04	1.52E+04	2.82E+04	NA
DHAP	2.06E+04	1.66E+04	NA	NA
dIMP	8.96E+03	4.21E+04	1.43E+04	NA
dITP	2.11E+04	9.86E+03	NA	4.48E+03
erythrose-P	5.33E+05	5.45E+05	4.75E+04	NA
ethanolamine	3.82E+05	4.93E+05	6.10E+04	NA
FAD	1.32E+03	NA	NA	NA
FMN	8.95E+02	8.95E+02	NA	NA
fumarate	2.75E+06	7.39E+06	4.09E+06	5.11E+06
gluconate	1.65E+05	6.33E+05	4.69E+05	1.69E+05
glucose	6.82E+05	1.07E+06	1.57E+05	1.27E+05
glutamate	1.26E+04	1.41E+04	1.35E+03	NA
glyceraldehyde-3-P	2.25E+05	1.66E+05	1.17E+05	4.17E+04
glycerate	3.30E+05	7.17E+05	3.12E+05	2.22E+05

glycerol	2.29E+06	1.90E+06	2.40E+05	1.70E+05
glycerol-3-P	4.62E+04	4.62E+04	4.57E+04	NA
glycerophosphocholine	9.41E+04	7.71E+04	3.94E+04	NA
glycine	2.06E+04	1.75E+04	9.86E+03	NA
glyoxylate	7.17E+03	2.06E+04	7.62E+03	NA
GSH	1.30E+04	2.20E+04	1.08E+04	NA
guanine	1.43E+04	3.59E+03	3.14E+03	NA
guanosine	3.59E+03	3.81E+03	NA	9.86E+03
hexose diphosphate pool	4.66E+04	1.17E+04	3.81E+04	NA
hexose disaccharide monophosphate pool	2.24E+03	2.24E+03	NA	NA
hexose monophosphate pool	9.16E+05	4.47E+05	NA	NA
hydroxyproline	9.41E+03	1.30E+05	1.30E+04	NA
hypoxanthine	1.17E+04	8.07E+03	NA	NA
IMP	5.83E+03	1.08E+04	NA	7.17E+03
inosine	3.59E+03	2.69E+03	NA	NA
isocitrate	5.29E+06	5.62E+06	1.74E+06	6.40E+05
ITP	8.51E+03	1.61E+04	NA	3.58E+03
linoleate	3.04E+06	1.26E+06	7.17E+03	NA
lysoPC(16:0)	4.27E+02	4.41E+02	NA	NA
lysoPC(18:0)	2.82E+04	2.69E+03	8.07E+03	NA
malate	1.67E+07	1.08E+07	1.72E+07	1.15E+07
methionine	3.27E+04	1.97E+04	NA	2.24E+04
methylsuccinate	1.29E+06	1.47E+06	NA	NA
metSO	2.69E+04	4.66E+04	5.56E+04	NA
N-acetylaspartate	5.15E+04	7.75E+04	9.41E+03	NA
N-acetylglucosamine-1-P	7.90E+02	1.79E+03	NA	NA

N-acetylglutamate	9.41E+03	2.15E+04	7.40E+03	NA
NAD	NA	2.24E+03	5.38E+03	NA
N- α -acetylarginine	1.03E+04	2.24E+04	1.86E+04	NA
octulose-P	7.62E+03	1.48E+04	2.47E+04	NA
O-phosphoethanolamine	2.29E+07	9.34E+06	NA	NA
O-phosphotyrosine	3.59E+03	4.93E+03	1.79E+04	NA
ornithine	7.17E+03	7.62E+03	4.03E+03	NA
oxalate	5.02E+04	3.14E+04	7.62E+04	NA
OAA	1.34E+04	3.00E+04	3.27E+04	NA
pantothenate	1.08E+04	1.03E+04	1.03E+04	NA
PC(34:1)	4.41E+02	4.03E+03	NA	NA
PC(34:2)	8.95E+02	2.60E+03	NA	NA
PC(36:0)	4.93E+03	2.18E+03	NA	NA
PC(36:2)	1.28E+03	3.14E+03	NA	NA
PC(36:4)	4.11E+02	2.69E+03	NA	NA
PE (36:0)	8.18E+02	3.58E+03	NA	NA
PE(34:1)	3.59E+03	2.69E+03	NA	NA
PE(34:2)	4.48E+03	8.52E+03	NA	NA
PE(36:1)	2.54E+03	NA	NA	NA
PE(36:2)	2.16E+03	8.07E+03	NA	NA
PE(36:3)	2.69E+03	1.52E+04	1.79E+03	NA
PE(36:4)	2.24E+03	3.32E+04	1.79E+03	NA
PG(34:1)	1.30E+04	5.11E+04	NA	NA
PG(34:2)	1.79E+03	1.73E+03	1.34E+03	NA
PG(36:2)	1.79E+03	1.34E+03	2.69E+03	NA
phenyllactate	2.15E+04	5.11E+04	1.39E+04	NA

phosphoenolpyruvate	3.59E+03	2.69E+03	4.03E+03	NA
PI(34:0)	4.48E+02	1.34E+03	NA	NA
PI(34:1)	2.24E+03	4.03E+03	NA	NA
PI(36:2)	4.48E+02	4.48E+02	4.48E+02	NA
proline	3.59E+05	7.12E+06	NA	2.06E+04
pyridoxine	6.05E+04	2.90E+05	NA	4.12E+04
pyrophosphate	6.03E+05	4.16E+05	5.38E+03	NA
quinolate	8.05E+05	5.47E+05	NA	NA
riboflavin	7.62E+03	7.62E+03	3.59E+03	NA
S-adenosylhomocysteine	3.18E+04	8.35E+05	4.66E+04	NA
sarcosine	4.88E+04	2.94E+05	1.82E+05	1.12E+05
sedoheptulose-P	1.03E+04	5.29E+04	3.38E+04	NA
shikimate	6.72E+03	2.69E+03	4.48E+03	NA
shikimate-3-P	4.08E+04	2.00E+05	NA	6.95E+03
spermine	1.47E+06	4.59E+05	1.55E+06	1.05E+06
succinate	7.66E+06	8.33E+06	NA	NA
thiamine	1.26E+04	4.75E+04	1.08E+04	NA
threonine	7.84E+04	4.83E+05	1.35E+05	1.85E+05
thymidine	3.59E+03	2.24E+03	8.07E+03	NA
tryptophan	1.57E+04	3.23E+04	NA	3.23E+04
UDP	8.96E+03	NA	NA	7.62E+03
UDP-glucose	2.15E+03	4.18E+02	NA	NA
UMP	4.93E+03	4.93E+03	1.17E+04	NA
urea	4.21E+04	1.08E+04	1.70E+04	NA
ureidopropionate	6.72E+03	4.93E+03	3.23E+04	NA
valine	4.03E+03	8.07E+05	NA	NA

xanthine	2.24E+04	5.18E+04	1.57E+04	NA
xanthosine	3.59E+03	3.59E+03	3.15E+03	NA
γ -glutamylalanine	NA	NA	5.38E+03	NA