SUSTAINABLE LAND TREATMENT OF FOOD PROCESSING WASTEWATER USING POPLAR PLANTATION: EVALUATION OF METAL AND NITRATE MOBILIZATION

By

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ABSTRACT

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Land application of food processing wastewater, a common practice, can cause mobilization of metals such as arsenic, manganese, iron, chromium and lead by creating continuously saturated and anaerobic soil conditions. Conversely, aerobic conditions may promote nitrification and leaching of nitrate into groundwater. Poplar trees have great potential to decrease metals and nitrate mobilization, allowing increased loadings of food processing wastewaters without negatively impacting soil treatment. Land application, unlike previous phytoremediation applications, utilizes uncontaminated sites and prolonged irrigation of fields with organic-carbon rich wastewater that is relatively low in other nutrients. Therefore, this research evaluated the ability of poplar plantation to reduce the problem of metals and nitrate mobilization to ground water and the phyto-processes that are expected to reduce metals and nitrate mobilization to groundwater during land treatment of food processing wastewaters. The research evaluated the *central hypothesis* that poplar trees reduce metal and nitrate mobilization that can occur when carbon-rich wastewaters are land applied through major processes (plant uptake and evapotranspiration) and minor processes (increased microbial activity in the rhizosphere and oxygenation of soils).

The research used i) laboratory-scale small soil columns, ii) pilot-scale large soil columns and iii) field experimentation to investigate the plant associated processes that influence leaching of metals and nitrate due to microbial mobilization at wastewater application sites. The small-scale columns utilized 15 cm diameter columns (total 15) to assess effects of wastewater on poplar

trees and effects of poplar trees on treatment of carbon, nitrogen and metals. The large-scale columns (56 cm diameter, total 15) were used to assess the evapotranspiration, redox dynamics, moisture dynamics and treatment of carbon, metals and nitrate during application of food processing wastewater utilizing a combination of laboratory analysis of water, soil and plant samples and data collected from oxidation-reduction, moisture and temperature sensors. Field experiment consisted of an acre of actual land application site where poplar trees were planted at 3.05 m spacing and the effects of poplar trees on drainage, evapotranspiration, carbon and nitrogen treatment and reduction in metal mobilization were assessed utilizing laboratory analysis of leachate water, soil and plant samples and data from draingage and moisture sensors.

Results varied with the scale of experimentation. At small-scale columns, poplar trees showed no signs of toxicity under food processing wastewater application, enhanced the soil moisture and reduced the mass removal of organic carbon, metals and nitrogen. In large-scale columns, plants enhanced the microbial biomass, reduced the soil moisture at 30 cm and 91 cm or 122 cm below depth by virtue of high evapotranspiration, increased the carbon removal in fine textured soils and contributed to nitrate reduction in leachate waters. However, oxygenation in the soils and reduction in metal mobilization was not observed despite the high uptake of metals in the plant tissues. Field studies corroborated the large column study as plants enhanced biomass in the rhizosphere and decreased soil moisture with high crop coefficient. However, poplar trees did not contribute to the decrease in metal mobilization and minimally to decreasing nitrate leaching despite metal and nitrogen uptake. These results demonstrate the benefits of poplar trees, but need further consideration when the trees grow to full maturity.

Dedicated to my family

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KEY TO ABBREVIATIONS

ANOVA	ANalysis Of Variance
BOD	Biochemical Oxygen Demand
COD	Chemical Oxygen Demand
CRP	Conservation Reserve Program
CVRWQCB	Central Valley Regional Water Quality Control Board
EDTA	Ethylenediaminetetraacetic Acid
ET	Evapotranspiration
FAO	Food and Agriculture Organization
HDPE	High Density Polyethylene
IBP	Industrial By-Products
ITRC	Interstate Technology & Regulatory Council
K_c	Crop Coefficient
MBEP	Michigan Biomass Energy Program
MDEQ	Michigan Department of Environmental Quality
MPCA	Minnesota Pollution Control Agency
NAICS	North American Industry Classification System
NPDES	National Pollutant Discharge Elimination System
ORP	Oxidation Reduction Potential
PLFA	Phospholipid Linked Fatty Acid
POTW	Publicly Owned Treatment Works
PPB	Part Per Billion

PPM	Part Per Million
SDS	State Disposal System
SMCL	Safe Maximum Contaminant Levels
TDR	Time Domain Reflectometry
TDS	Total Dissolved Solids
TKN	Total Kjehldahl Nitrogen
TOC	Total Organic Carbon
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
US EPA	United States Environmental Protection Agency
USDA	United States Department of Agriculture
WB	Water Bureau
WDR	Water Discharge Requirements
WSDOE	Washington State Department of Ecology

CHAPTER 1: INTRODUCTION

Over 273×10^9 kg of fruits and vegetables were produced in the United States in 2011 for processing operations, such as canning, freezing and pickling (USDA, 2012, USDA, 2012b). Processing fruits and vegetables produces large volumes of wastewater; for example, processing a kg of potato produces approximately 8-28 L of wastewater (Charmley et al., 2006). Among many wastewater management options, land application of food processing wastewater is a commonly used and low-cost. For example, 80% of food processing plants land-apply their wastewaters in California (CVRWQCB, 2006). During land application, the wastewater constituents are treated by physicochemical processes (e.g., filtration, sorption, precipitation, redox reactions), biological processes (e.g., microbial degradation, microbial assimilation, plant uptake) and water transport processes (e.g., evapotranspiration, percolation) (Wang, 2004, Miller et al., 2008), which are described in detail in Chapter 2: Literature Review.

Land application of wastewaters is a reliable technology when wastewater application rates are lower than the capacity of the plant-microorganism-soil ecosystem to treat the pollutant load (Charmley et al., 2006); however, there are several detrimental consequences of improper application of wastewaters. Under a surplus of electron donors (i.e. carbon from wastewater), electron acceptors, mainly oxygen, are limiting. Land application at high rates can create continuously saturated and anoxic or anaerobic soil conditions, causing surplus of electron donors. Native metals in soils, including manganese, iron and arsenic, are used by microbial communities as electron acceptors and reduced to more soluble forms increasing the potential for metal leaching and pollution of groundwater. Occurrence of metals, including arsenic, iron, manganese, cobalt and nickel, in wells near food processing facilities at higher concentrations than permitted by Environmental Protection Agency (EPA) have been reported (MDEQ, 2009a, b). The elevated concentrations of metals has been attributed to formation of anaerobic conditions during soil treatment of wastewaters, a result of wastewater in excess of the capacity of ecosystems to assimilate the pollutant load (CVRWQCB, 2005, Safferman et al., 2007).

When the conditions are not reducing, ammonium and organic nitrogen present in the wastewater are nitrified under aerobic conditions, increasing leaching of nitrate to ground water (Patrick & Reddy, 1976, CVRWQCB, 2005, St Luce et al., 2011). Therefore, the solution to groundwater pollution from land application of food processing wastewater requires optimization of soil redox dynamics in the plant-microorganism-soil ecosystem. Consequently, study is needed to study the dynamics of redox potential and the soil-plant-microbial function to prevent groundwater pollution by both nitrate and metals.

Poplar trees uptake metals and nitrate in their tissues, enhance microbial degradation, decrease soil saturation and oxygenate soils in different waste remediation applications (ITRC, 2009). These processes have great potential to decrease metals and nitrate mobilization at land treatment sites for food processing wastewater, allowing increased loadings of wastewaters without negatively impacting land treatment. However, phytoremediation studies utilize sites with extremely elevated metal concentrations and rarely use continuous irrigation. In contrast, land application sites contain natural concentrations of metal and are regularly saturated with wastewaters that have low concentrations of metals (Alloway, 2013). Previous studies that studied capability of poplar trees to treat wastewaters were mainly for concentrated waste (Kirchmann & Witter, 1992, Kjeldsen et al., 2002, Zalesny & Bauer, 2007), unlike food processing wastewater which has very high carbon but relatively low nutrients and metals (Tanemura et al., 1994, Cheong et al., 2007). Processes that occur in land application sites where poplar trees are grown and their effects on overall fate of redox sensitive pollutants such as metals and nutrients have not been studied. Therefore, this research evaluated the capability of poplar plantation to reduce problem of metals and nitrate mobilization to ground water under application of high-carbon and low-nutrients wastewater to the land and the mechanisms that occur during the treatment. This study, unlike previous studies, studied the redox conditions in the soil during soil treatment of wastewaters.

The research evaluated the *central hypothesis* that poplar trees will reduce metal and nitrate mobilization that occurs when carbon-rich wastewaters are land applied through phyto-processes (plant associated processes) such as plant uptake, evapotranspiration, microbial activity in the rhizosphere and oxygenation of soils. Phyto-processes were hypothesized as major and minor contributors to reduction of metal and nitrate mobilization:

1. *Plant uptake* of mobilized metals and nitrogen is a major process that decreases metal and nitrate leaching.
2. *Evapotranspiration* is a major process that results in decreased soil moisture and the formation of reducing condition and mobilization of metals.
3. *Rhizostimulation* only limitedly contributes to increased microbial diversity and biomass and carbon degradation.
4. *Oxygenation* of the root zone contributes to a significant increase in redox potential, contributing to increased degradation of carbon and shift from anaerobic to aerobic bacteria.

The sub-hypotheses (1) to (4) were evaluated for two soils: sandy loam and loam as soil may affect the phyto-processes. Therefore, the overall goal of the proposed research was to determine the mechanisms by which poplar trees affect the fate of metals and nitrate after application of food processing wastewater to soils and evaluate the poplar's ability to reduce the pollution of groundwater by metals and nitrate. The specific objectives under land application are listed in Table 1-1.

Table 1-1 Hypotheses and objectives of the research

Sub-hypothesis	Objectives
Plant uptake	Assess metal uptake by poplar trees and leaching to groundwater
	Study nitrate dynamics, more specifically, uptake by poplar trees and leaching into groundwater
Evapotranspiration	Evaluate the evapotranspiration and crop coefficient of poplar trees
	Measure soil moisture at different depths and potential of poplar trees to reduce soil moisture
Rhizostimulation	Compare the microbial diversity and biomass in root zone of poplars and control soils
	Evaluate carbon treatment by poplar plants
Oxygenation	Evaluate oxidation reduction potential of soil
	Relate effect of soil moisture to soil redox potential

The research results elucidate fundamental phyto-processes through which trees can be used to increase the waste assimilative capacity of soil ecosystems, focusing on emerging concern of metal mobilization during land application of carbon-rich wastewaters, furthering current knowledge on waste assimilative capacity of plant-based ecosystems. Understanding the fundamental processes under land application scenario is important to improve or design alternative ecological, effective and economical treatment technologies. Research will advance the understanding of the function of plants in treating metals, organic carbon and nitrogen during the land application of wastewater.

By potentially reducing metals and nitrate pollution of waters, the research protects water resources, human health and ecosystem health. Poplar plantation technology will potentially allow greater application of wastewater without polluting water with metals and nitrate while meeting regulations and boost the economic return of food processors through increased processing capacity.

The project was conducted at three different scales: small-scale columns, large-scale columns and field-scale. Common parameters measured in all experiments were COD treatment, evapotranspiration and concentration of metals and nitrate in leachate water. Unique parameters varied with the study. The small-scale column study was conducted at more controlled environmental conditions in a green house (i.e. no rainfall and with actual wastewater application). The unique parameter studied in the small-scale column experiment was the growth of poplar trees under the application of wastewater. The large-scale column study was conducted open to environmental conditions, but with synthetic wastewater application with added monitoring of soil moisture, microbial biomass and community and redox potential. Field-scale experiment was carried out under actual environmental conditions at a 1-acre land application site. Additional measurements included the microbial biomass and community in soils and soil moisture.

CHAPTER 2: LITERATURE REVIEW

The literature review provides background information and covers relevant literatures pertaining to the research and is the basis for the objectives and the rationale of the study. This chapter is divided into sections and subsections as described below.

2.1. Status of fruits and vegetable processing

The fruit and vegetable industry contributes trillions of dollars to US economy (USDA, 2012). In 2011, over 273×10^9 kg of fruits and vegetables were produced in the United States for processing operations such as canning, freezing and pickling (USDA 2012, USDA, 2012b). The annual processing quantity and percentage of total production are increasing over time. For example, processing of potato to make chips, french fries, frozen products, canned products and other processed products increased from 57% of the total produced 15,400,000 tons (14×10^9 kg) potato in 2002 (Charmley et al., 2006) to 63.4% of the total produced 24,060,000 tons (22×10^9 kg) potato in 2011 (USDA, 2012a). Vegetables that are processed in USA include asparagus, beets, cabbage, carrot, cauliflower, cucumber, peas, lima beans, mushroom, potatoes, snap beans, spinach, tomatoes, corn, peppers, pumpkins and squash (Safferman et al., 2007, 2012, USDA, 2012b, a). Whereas, fruits that are processed in USA include apples, apricot, blackberries, boysenberries, cherries, cranberries, grapefruit, grapes, guavas, kiwifruit, lemon, olives, oranges, papayas, peaches, pears, plums and prunes, raspberries, strawberries, tangelos, tangerines, dates and nectarines (USDA, 2012b).

Specialty crop processing is a crucial component of Michigan economy with an annual gross income of $496 million from fruits and vegetables alone (Safferman et al., 2007). There are 798 large processing facilities (> $25,000 annual gross sales) and 481 small processing facilities

(<$25,000 gross annual sales) [Table 2-1] that process about 241,100 tons (2.19×10^8 kg) of fruits and 623,350 tons (5.67×10^8 kg) of vegetables and produce 1,107 to 11,785 million gallon (4,190 to 44,611 million L) of wastewater annually (Safferman et al., 2007).

Table 2-1 Food processing operations and facilities in MI

Operation		**Number of facilities**	
		Large*	**Small***
Manufacturing	**Baking/Frying**	135	95
	Bottling	92	25
	Canning	54	22
	Additives	8	0
	Milling	7	1
	Minimal processing	233	233
	Other	2	1
	Pasteurizing	38	2
	Roasting	21	5
	Seed sprouting	5	2
Packaging		89	35
Preparation		18	26
Warehousing		96	34
Total		798	481

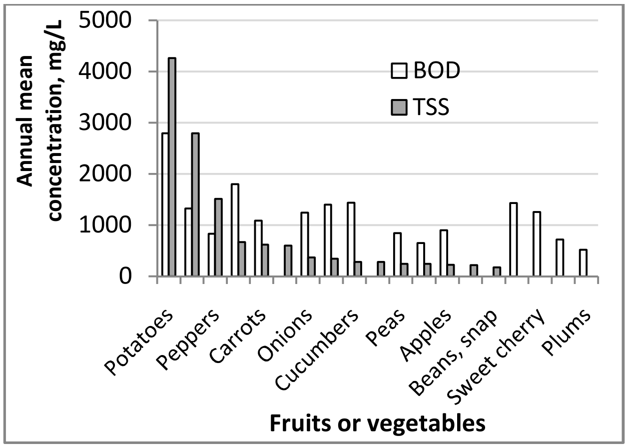
*Large have >$25,000 gross sales; small have <$25,000.

Derived from data provided by Michigan Department of Agriculture. Adopted from (Safferman et al., 2007).

2.2. Food processing wastewater production and characteristics of wastewater

Processing of large quantity of fruits and vegetables as mentioned above produces even larger quantity of wastewater. For example, about 2.1-7.4 gal (8-28 L) of wastewater is produced per kg of raw potato processed (Charmley et al., 2006). In total, trillions of gallons of wastewater are produced from fruit and vegetable processing industry annually in the USA.

Food processing wastewaters have variable characteristics depending on the processing operation, technology used, water supply quality, type of food [Figure 2-1] and is facility specific (Bruner & Burgard, 2003, MDEQ, 2007). Volume of wastewater fluctuates widely (Dennis, 1953); peaks with seasonal production peaks (Hunt et al., 1976). Generally, process wastewater contains organic carbon, suspended solids, scale removal chemicals, food additives, salts and equipment cleansers (Safferman et al., 2007). Containing grams per liter concentrations of pollutants, food processing wastewaters are classified as “high-strength” (Hunt et al., 1976, CVRWQCB, 2005, Safferman et al., 2007); concentrations can be as high as 8,537 mg/L biochemical oxygen demand (BOD), 12,230 mg/L chemical oxygen demand (COD), 9,993 mg/L total suspended solids, 1,514 mg/L total nitrogen and 1,277 mg/L total phosphorous (Church & Nash, 1970, Dornbush et al., 1976). A substantial portion of carbon in food processing wastewater can be recalcitrant, as BOD concentrations were 74% of COD for fruit processing wastes (Esvelt, 1970). Figure 2-2 shows the mean and range of concentrations of pollutants present in fruit and vegetable processing wastewaters.



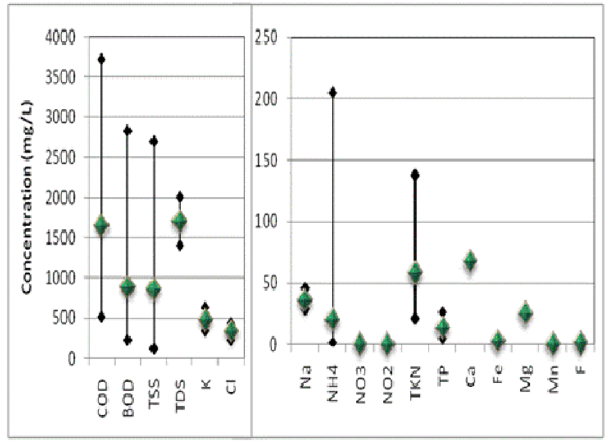


Figure 2-1 Annual mean concentration of TSS and BOD of food processing wastewaters (Safferman et al., 2007)

Figure 2-2 Characteristics of food processing wastewater [Source: An anonymous facility in MI]

2.3. Treatment of wastewaters and land application

Technologies that are used to treat food processing wastewater include conventional wastewater treatment, anaerobic digestion and land application or spray irrigation (Guttormsen & Carlson, 1970). All these methods use aerobic, anaerobic or facultative biological treatment. Conventional treatment include activated sludge (Esvelt, 1970), trickling filter (Guttormsen & Carlson, 1970), aerated lagoon (Dostal & Burm, 1970), stabilization or oxidation pond (Guttormsen & Carlson, 1970) and anaerobic ponds (Guttormsen & Carlson, 1970).

The treatment technology chosen for food processing wastewater depends on many factors such as volume and strength of wastewater, size, location and type of plant, management practices, availability of municipal treatment facilities, state and local legislations and economics (DDT, 1971). Though conventional treatment options treat wastewater effectively (e.g. greater than 90% removal of BOD by aerated lagoon (Dostal & Burm, 1970)), they are costly (e.g. the cost for removal of 1 lb BOD from fruit processing wastes for aerated lagoon and activated sludge were

$0.041 and $ 0.061 respectively (Esvelt, 1970)). Aerated lagoon, though cheaper, did not reduce suspended solids that contributed to effluent BOD (Esvelt, 1970). Moreover, wastes from food processing factories are deficient in nutrients for biological system; low in N and likely P (Rose, 1970) and require addition of nutrients to treat by aerated lagoon or by activated sludge to maintain N and P to BOD ratio (Esvelt, 1970). Though it is suited to anaerobic treatment system due to high BOD and just enough N and P, anaerobic treatment does not achieve P removal, often requires secondary or tertiary treatment to meet regulations, is slow in nature, takes significant start up and recovery time from washout or overloading and may produce odor (Szczegielniak, 2013). Alternatively, a common practice for management of the wastewater is land application. Land application dates back to centuries (Thomas, 1973), however, the first reported spray irrigation of food processing wastewater in the field for removal of high organic carbon content was in 1947 in Pennsylvania when lagoon and spray was combined to prevent stream pollution from food processing wastewater (Dennis, 1953).

Land application through spraying of wastewater for slow filtration is popular due to its simplicity, flexibility and low expense (Sirrine, 1978, Bruner & Burgard, 2003, CVRWQCB, 2005, Charmley et al., 2006, Safferman et al., 2007, Agency, 2008). Allowable because of the absence of hazardous substances in food processing wastewater, land application is simple (DDT, 1971) and costs 30 to 50% less than conventional wastewater treatment (Charmley et al., 2006, Zvomuya et al., 2006). Furthermore, the application of wastewater recharges the groundwater basin (CVRWQCB, 2005). Additionally, the inherent fluctuations in wastewater loading, the location of processing facilities, the capital and operation and maintenance costs of conventional wastewater treatment often increases the impracticality of constructing conventional wastewater treatment facilities at small and medium food processors (Hunt et al., 1976).

Food processing wastewaters are land applied throughout the United States at high rates including California (CVRWQCB, 2005, 2006, Miller et al., 2008), Minnesota (Zvomuya et al., 2006), Idaho (Bruner & Burgard, 2003), Michigan (Safferman et al., 2007), Wisconsin (Dennis, 1953, Resources), 2014) and Washington (Charmley et al., 2006). In the California valley, about 80% of 640 food processing plants which consume 7.9×10^{7} m^{3} of water per year, discharge the wastewater to land or water (CVRWQCB, 2006). In Michigan, wastewater from food processing facilities are land applied at the rate of 2.5 to15.4 mm/day (2,700-16,000 gal/acre/day) or up to 1800 lb BOD/acre/day (Mokma, 2006, Safferman et al., 2007). Currently, most of the 287 large and 255 small minimal processing and canning operations land apply their wastewaters (Safferman et al., 2007). In a facility at Minnesota, annual loadings were as high as 371.5 kg/ha and 110.9 kg/ha total phosphorus, 103 kg/ha and 49.4 kg/ha nitrate, 124.8 kg/ha and 77.8 kg/ha ammonium and 318.1 kg/ha and 260.8 kg/ha total nitrogen for growing season (Apr to Sep) and non-growing season respectively (Oct to Mar) (Zvomuya et al., 2006).

2.4. Regulations for land application

North American Industry Classification System categorizes fruit and vegetable preserving and specialty food manufacturing as a part of manufacturing sector with code of NAICS 3114 (United States Census Bureau, 2012). This sector includes industries that manufacture frozen foods and/or use preservation processes including pickling, canning and dehydrating from input of vegetable or animal origin. Direct application of food processing wastewater to water bodies is regulated by NPDES (National Pollution Discharge Elimination System) permit and indirect application to POTW (public owned treatment works) is regulated by POTW (in pretreatment). Land application, however, is regulated by states and differs by state based on hydraulic, nutrient, or salt loading rate.

Hydraulic loading rate is based on a maximum application rate that is equal to the sum of cover crop transpiration needs, evaporation rates and infiltration capacity of soils without exceeding field capacity of soil (DDT, 1971). Permissible hydraulic loading rates are calculated so that the field capacity of the soil is not exceeded after consideration of evapotranspiration and infiltration (DDT, 1971). For example, Eckenfelder developed following equation to predict wastewater application rate (DDT, 1971):

$$Q = (328 * 10^{-3})\, KS \qquad \text{Equation 1}$$

Where,

Q=steady rate for downward flow, gpm/acre

K= overall coefficient of permeability for soil for surface to ground water table, ft/min

S=degree of saturation, 1.0 at steady state conditions

The fact that hydraulic loading rate can be increased in crop growing period if not in winter, while not overloading the environment with pollutant, by selecting crop for optimal nutrient uptake and evapotranspiration has been overlooked.

The nutrient or salt based loading rate is calculated based on the capacity of the soil-plant-microbial system to remove the nutrient or salt so that nutrient or salt in the leachate water are under the regulatory limit. Excess saline or alkaline wastewater impacts soil permeability and crop productivity adversely. Sodium ion if present in excess in relation to Ca and Mg can disintegrate the soil structure of clays (DDT, 1971). Wilcox suggested sodium content of water be less than 80% of soluble mineral ions i.e. (DDT, 1971). In equation,

$$\frac{[\boldsymbol{Na^{+}}]\times\mathbf{100}}{\boldsymbol{Total\ cation\ concentration}} < 25\ \boldsymbol{epm} \qquad \textbf{Equation 2}$$

Where,

epm = equivalence per million

U.S. salinity laboratory suggested SAR (sodium adsorption ratio) and conductivity (C) µmho/cm;

$$\boldsymbol{SAR} = \frac{\boldsymbol{Na^{+}}}{(\frac{\boldsymbol{Ca^{++}+Mg^{++}}}{\mathbf{2}})^{\mathbf{0.5}}} \qquad \textbf{Equation 3}$$

For low salinity hazard of 250 µmho/cm,

SAR = 18.87-4.44 log C **Equation 4**

For medium salinity hazard of 750 µmho/cm

SAR= 31.31-6.66 log C **Equation 5**

For high salinity hazard of 2,250 µmho/cm

SAR = 43.75-8.87 log C **Equation 6**

Carbon loading rate can also be calculated using equations. Soil BOD for periodic loading and continuous loading are given by equations 7 and 8 respectively (DDT, 1971),

$$\boldsymbol{L} = \boldsymbol{L_0 e^{-knT}} + \boldsymbol{l}[\frac{\mathbf{1}-\boldsymbol{e^{-K(n+1)T}}}{\mathbf{1}-\boldsymbol{e^{-kT}}}] \qquad \textbf{Equation 7}$$

$$\boldsymbol{L} = \frac{\boldsymbol{l_0}}{\boldsymbol{k}} + (\boldsymbol{L_0} - \frac{\boldsymbol{l_0}}{\boldsymbol{k}})\boldsymbol{e^{-kt}} \qquad \textbf{Equation 8}$$

Where,

L_0=initial available BOD

l= BOD applied per loading

l_0 = BOD applied per unit time

T= time period for intermittent loading

n= number of periods

t=time for continuous loading

k=coefficient of rate of oxidation

Application of food processing wastewater to crop lands is prohibited for many reasons such as odors, runoff, ponding, drifting of aerosols and microorganisms, contamination of food and less crop productivity (DDT, 1971, Tarquin, 1976, Safferman et al., 2007). Regulations for land application of food processing wastewater in few states are discussed in subsequent paragraphs.

In Michigan, Water Bureau (WB) under Department of Environmental Quality (MDEQ) regulates wastewater discharges to surface water according to NPDES (National Pollutant Discharge Elimination System) (MDEQ, 2007). NPDES uses more stringent standard of treatment technology based effluent standards by EPA and water quality based effluent limitations. MDEQ requires that wastewater discharge not cause damage utility of water for drinking, agriculture, recreation, industry and other uses. In Michigan, discharging of fruit and vegetable process wastewater to land requires permit 2218. Wastewater characterization, design basis, alternatives to groundwater discharge, a hydro-geological survey, management plan and ground water monitoring are required as well. Michigan limitations for BOD is 45 mg/l daily maximum and 30 mg/L monthly average, for total inorganic nitrogen is 5 mg/L as nitrogen, for phosphorous is 1-5 mg/L and for pH is 6.5 to 9 (MDEQ, 2007). Total dissolved solids (TDS) discharge is not regulated in Michigan although TDS is a secondary contaminant in EPA classification with SMCL (Secondary Maximum Contaminant Level) of 500 mg/L. MDEQ strongly recommends not spraying irrigation in winter. MDEQ also requires processors to report the total wastewater volume applied annually.

Minnesota pollution control agency (MPCA) categorizes food processing wastewater under industrial byproduct (IBP) and regulates the land application (Agency, 2008). MPCA recommends the NPDES (National Pollutant Discharge Elimination System) and/or SDS (State Disposal System) to manage industrial by-product as the regulatory limit. MPCA requires permit to discharge more than 50,000 gal/year or 10 dry tons in land or to store large volumes of wastewater that could potentially harm environment. However, smaller amount is permitted to be land applied. MPCA requires the analysis of representative composite samples for nutrients and pollutants before licensure and yearly then after. Proper soil texture, slope (<6%), away from water bodies, deeper ground water than 3 m, crop and public restriction are other requirements for land application. Minnesota limit for hydraulic loading rates for coarse, medium and fine textured soils are 25,000, 15,000 and 10,000 gal/acre/day respectively; however, the rate in winter cannot exceed 15,000 gal/acre/day. No winter application is permitted in slope greater than 2%. Maximum allowable nitrogen loading is based on the crop need for the season. For example, 200 lb/acre/yr N or 50 lb N per ton yield is permitted for alfalfa. Maximum sodium load allowed is 170 lb/acre/yr. Additionally, land application is prohibited in flood prone areas, cropped or to be cropped areas, water ponded areas and runoff prone areas. Lastly, MPCA requires record keeping and reporting annually. Before the above regulation in 2008, Minnesota Pollution Control Agency (MPCA) warranted unlimited application of wastewater in summer (Apr to Sep) without exceeding agronomic N requirement of crop in fields and regulated application of treated food processing wastewater in winter such that $P \leq 6$ mg/L, $N \leq 10$ mg/L as NO_3-N and TKN ≤ 20 mg/L (Oct to Mar)(Zvomuya et al., 2006).

California requires complete degradation, transformation or immobilization of waste within the treatment zone of less than 1.5 m (5 ft) from the land surface (CVRWQCB, 2005). In Central Valley of California, CVRWQCB (Central Valley Regional Water Quality Control Board) regulated land application of food processing wastewater under Waste Discharge Requirement (WDR) program before 2003. The regulation exempted the food processing facilities from full containment, monitoring, financial assurance and corrective actions with the belief that processors ensure safe application of wastewater based on agronomic requirement of crop and microbial degradation and avoid odor or environmental degradation (CVRWQCB, 2005). However, after 2003, when all the WDRs were made to expire, a five year self-expiring permit was granted by public hearing and the permit required complete degradation or transformation or immobilization of waste within the treatment zone of less than 1.5 m from the land surface and monitoring of leachate (CVRWQCB, 2005).

Washington State Department of Ecology (WSDOE) grants permission to apply wastewater into land from food processing facilities by rigorous process that includes monitoring wastewater quantity and quality, crop harvest removals, application rates, testing soil and other conditions (Charmley et al., 2006).

Idaho embraces the 500 mg/L TDS while 16 other states have same or less stringent TDS regulation in groundwater (Bruner & Burgard, 2003).

Therefore, the recent changes in regulations, such as those discussed above in Minnesota and California, leave processors with options to either use conventional treatment to process more wastewater or produce less wastewater if the land is limited. Both options affect the profitability of food processing facilities. This further strengthens the rationale for this research.

2.5. Treatment of pollutants during land application

2.5.1. Processes during the land treatment

During land application, pollutants in food processing wastewater are treated by *physicochemical processes*, such as filtration of small particles through soil particles, adsorption on soil particles, mineral precipitation, ion exchange and redox reactions and by *biological processes*, such as biological degradation of entrapped organics and suspended particles in the soil by aerobic, facultative and anaerobic bacteria and uptake by plants and soil microorganisms; additionally, *water transport processes*, such as evapotranspiration and percolation, affect pollutant treatment (Wang, 2004, Miller et al., 2008). The effectiveness of each process depends on the soil characteristics, wastewater and cover crop grown on the field (Wang, 2004).

Fate of the metals in the soil treatment systems are dependent on the type and quantity of soil surfaces, pH, concentration and type of competing ions and ligands present and concentration of metals in the soil (Shammas, 2009). All these properties are soil specific. Metal adsorption increases with increase in soil pH, cation exchange capacity and organic carbon (Shammas, 2009). Degradation of organic matter can induce redox, pH and organic ligand concentration changes and drive the metal fluxes (Forstner, 1993). Clayey soils have higher surface area than sandy soils. As clay fraction increases in the soil, the sorption of metals to hydrous oxide of iron, aluminum or manganese increases. Sandy loam soil performed better in terms of removal efficiency of pollutants consistently as compared to loam and clay loam soil (Law et al., 1970).

2.5.2. Efficiency during the land treatment

Land application of nutrient rich wastewater can meet plants' nutrient and water demand and can be relied on if application rates are lower than the waste assimilative capacity of the plant-soil ecosystem (Charmley et al., 2006). Land application effectively removed pollutants such as total suspended solids (93.5%), total volatile solids (96.3%), total phosphorous (42.5%), ammonia nitrogen (50%), total Kjeldahl nitrogen (84.7%), COD (91.7%), TOC (90.8%) and BOD (98.4%) from wastewater (Law et al., 1970). Removal of carbon as high as 1800 lbs COD /acre/day or 900 lbs BOD/acre/day was observed in slow infiltration system (Tarquin, 1976). Land application of potato processing wastewater treated up to 96% of 3000-4000 mg/L COD after percolation through 6 feet of soil when the loading rate was 175 lb COD/acre/day, 5.8 lbs N/acre/day and 250 lb TOC /year (Sirrine, 1978). In another study, surface irrigation removed 99%, 98%, 90% and 45% of BOD, TSS, nitrogen and phosphorous respectively, however, careful operation increased the P removal to about 90% at the application rate of 0.5 inch per day (12.7 mm/d) in summer and 0.25 inch per day (6.35 mm/d) in winter (Glide, 1970).

2.6. Consequences of land application

Proper land application of wastewater is a reliable and approved technology for treatment of food processing wastewater as discussed above; however, there are several detrimental consequences of improper application of wastewaters. Soil conditions at land application sites will fluctuate between aerobic and anaerobic conditions based on waste application rates and soil type. Both oxidizing and reducing conditions during land application of wastewaters can cause contamination of groundwater with pollutants that present human health concerns. Redox sensitive metals and nitrate in the land application site can pollute groundwater during moderately reducing conditions and aerobic conditions, respectively. More discussions are presented below.

2.6.1. Metal mobilization

Soil redox potential, which characterizes the relative abundance of electron acceptors and electron donors, determines the movement, stability, availability and solubility of redox sensitive metal(loid)s and nutrients in land application systems (DeLaune & Reddy, 2005). Measuring oxidation reduction potential is the best available method to measure oxidation or reduction status in saturated soils because of

1. wider fluctuation of redox potential, 1000 mV as compared to 300 mV for unsaturated soils,
2. absence of oxygen in saturated soils that precludes use of oxygen or oxygen diffusion measurement and
3. indication of intensity of oxidation reduction (measurement of individual reduced species does not provide intensity of reduction) (Patrick et al., 1996).

Measuring redox potential can indicate the status of soil, redox condition, reactions in the soil and types of microorganisms present as shown in the Table 2-2 below.

Table 2-2 Redox condition, electron acceptors, microorganism type at different potential readings, adopted from (DeLaune & Reddy, 2005)

Soil condition	Anaerobic					Aerobic
Redox condition	Highly reduced	Reduced		Moderately reduced		Oxidized
Electron acceptor	CO_2	SO_4^{2-}	Fe^{3+}	Mn^4	NO_3^-	O_2
Microorganism	Anaerobic	Facultative				Aerobic
Eh reading, mV	-300 -200 -100 0 100 200 300					400-700

The following are common redox reactions in the order of aerobic (top) to anaerobic (bottom) conditions and the standard potential of redox changes at pH 7 (DeLaune & Reddy, 2005, Schink, 2006).

$O_2 + 4e^- + 4H^+ \rightarrow 2H_2O$ 810 mV **Equation 9**

$2NO_3^- + 10e^- + 12H^+ \rightarrow N_2 + 6H_2O$ 751 mV **Equation 10**

$MnO_2 + 2e^- + 4H^+ \rightarrow Mn^{2+} + 2H_2O$ 610 mV **Equation 11**

$Fe(OH)_3 + e^- + 3H^+ \rightarrow Fe^{2+} + 3H_2O$ 150 mV **Equation 12**

$SO_4^- + 6e^- + 8H^+ \rightarrow S^{2-} + 4H_2O$ -218 mV **Equation 13**

$CO_2 + 8e^- + 8H^+ \rightarrow CH_4 + 2H_2O$ -244 mV **Equation 14**

Electron donor is substrate or food for the microorganisms and provides energy in conjunction with electron acceptor (Rittmann & McCarty, 2001). Organic carbon in the food processing wastewater donates electron. High loading of organic carbon makes electron donor excess and the electron acceptor limiting condition since electron acceptors are quite few as compared to donors in the environment (Rittmann & McCarty, 2001). Electron acceptors, in order of most to least energetically favorable are oxygen, nitrate, manganese, iron, arsenic, sulfate and carbon dioxide (Marin et al., 1993, DeLaune & Reddy, 2005). The oxygen in the soil will be used up for the aerobic oxidation of carbon quickly.

The re-aeration of soil can supply oxygen through mass flow or diffusion (Troeh et al., 1982). While total pressure gradient or temperature gradient is driving force in mass flow, the partial pressure or concentration gradient is driving force in diffusion. The most important oxygen exchange mechanism between atmosphere and soil is gaseous diffusion (Troeh et al., 1982). Other processes for oxygen transfer to soil such as convective oxygen transport as a result of temperature, barometric pressure fluctuation and wind or rainfall effect are minor (Huesemann & Truex, 1996). Wind and rain can input oxygen into soil like piston driving air. Dissolved air in the percolating water or wastewater can transport oxygen to the soil. However, the amount of oxygen is negligible when dealing with concentrated waste (MacKay, 1997). The rate of diffusion is governed by pore space and degree of saturation because the diffusion coefficient of oxygen in water is 1/10,000 of that in air (MacKay, 1997). Therefore, re-aeration can only supply negligible oxygen under saturated condition.

Degradation of wastewater BOD consumes already limited oxygen in the soil, creating reducing, anaerobic conditions. The addition of 56 g BOD/m^2/day (499.6 lb BOD/acre/day) and higher organic loading to sand columns resulted in formation of reducing conditions (Safferman et al., 2010). As a result, subsequent utilization of metals as electron acceptors by microorganisms can reduce native metal species to more mobile forms (e.g., Mn^{4+} to Mn^{2+}, Fe^{3+} to Fe^{2+}, As^{5+} to As^{3+}) contributing to groundwater contamination with manganese, iron and arsenic. Co-existence of microorganisms that use different electron acceptors can occur if the favorable electron acceptor do not use up the electron donor (Rittmann & McCarty, 2001). Elevated metal concentrations in ground water due to metal mobilization at land application site threatens human health as more than 51% of the US population and 99% of rural residents rely on groundwater for drinking water source (Groundwater Foundation).

Manganese, iron, arsenic, lead, cobalt and/or nickel concentrations were higher than permitted for source drinking water in neighbor's wells and monitoring wells near food processing facilities at multiple sites in Michigan (MDEQ, 2009a, b). These metals are primary contaminants in the drinking water with the EPA approved maximum contaminant levels of: 10 µg/L As, 5 µg/L Cd, 100 µg/L total Cr and 15 µg/L Pb (US EPA). Harmful effects of these metals in drinking water are well known (US EPA). Arsenic threatens the skin and circulatory system and may increase cancer risk, while cadmium is believed to damage kidneys. Lead hinders mental and physical development in children and causes blood pressure and kidney problems in adults. Chromium is related to allergic dermatitis. Secondary contaminants such as iron and manganese pose undesirable color, taste and odor and increase water treatment cost (US EPA). National secondary level drinking water regulate Mn, Fe, Cu, Al, Zn with MCLs of 50 ppb, 300 ppb, 1000 ppb, 50-200 ppb, 5000 ppb, respectively (US EPA). Consequently, the multiple land application sites in Michigan were listed as Part 201 sites by Michigan Department of Environmental Quality (MDEQ) (Mokma, 2006, MDEQ, 2009a, b).

Elevated levels of metals in groundwater has been correlated with ethylenediaminetetraacetic acid (EDTA), which is used in food processing industry as direct additive to preserve color, flavor or as indirect additive to clean (MDEQ, 2009b). EDTA chelates metals and mobilizes them (MDEQ, 2007). When manganese and iron are already leaching, EDTA further leaches nickel, cobalt and other more toxic metals.

The reducing, anaerobic soil conditions created due to excessive carbon and hydraulic loading can also result in decrease of BOD removal and development of odor (Wang, 2004). Additionally, under reduced and moderately reduced conditions, iron and manganese (oxy)hydroxides formed in oxidizing conditions undergo dissolution, whereas precipitates of metallic sulfides or pyrites formed in anaerobic conditions undergo oxidation (Jacob & Otte, 2003, Cervantes et al., 2011), releasing manganese, iron and arsenic in both cases. Thus, suboxic conditions are more prone to metal mobilization and leaching as metals are neither bound to iron or manganese (oxy)hydroxides like in oxidizing conditions nor precipitated as metallic sulfides or co-precipitated with pyrites like in anoxic conditions (Jacob & Otte, 2003, Cervantes et al., 2011). As iron oxides and hydroxides provide sorbing sites to arsenic, dissolution of these oxides can release additional arsenic (Fitz & Wenzel, 2002).

When groundwater is hydraulically connected to streams, metal mobilization also threatens surface water sources (Safferman et al., 2007). Continued growth of food processing facilities in number and size will contribute to the problem of overloading the land, exceeding the capacity of plants and microorganisms to uptake or decompose the pollutant load resulting in environmental deterioration (CVRWQCB, 2005).

2.6.2. Nitrate leaching

Other consequences of over application of food processing wastewaters include elevated levels of nitrogen as nitrate shown in Figure 2-3 (Adriano et al., 1975, CVRWQCB, 2005), phosphorus (Zvomuya et al., 2006) and salts (CVRWQCB, 2005) in groundwater. Excessive application of nitrogen (greater than rate of plant uptake and denitrification) and phosphorus (than the rate of plant uptake) can cause nitrate and P movement in to groundwater and surface waters (Adriano et al., 1975, Zvomuya et al., 2006) potentially impacting ecosystem e.g. eutrophication and human health e.g. methemboglobinemia (blue baby syndrome). About 76% of total N input and 27% of total P input from fruit and vegetable processing wastewater in the sandy loam treatment system ended up in subsurface water (Adriano et al., 1975). Minealization of N, evapotranspiration and nitrification contributed to higher nitrate levels in surface soils (Adriano et al., 1975). At or near large food processing facilities in California Valley, the groundwater deterioration from salts and nitrate due to application of food processing wastewater to land has been the greatest concern and could potentially threaten to rule out the beneficial uses of water resources in the area (CVRWQCB, 2005). Of the 224 food processing facilities in Central valley California, 42 facilities out of 47 facilities that completed monitoring of groundwater degraded or polluted groundwater with nitrate and an additional 126 facilities were suspected of groundwater pollution (CVRWQCB, 2005) making 64% that potentially pollute ground water by salts, nitrogen compounds or high organic loading. Nitrogen and P contamination of groundwater from land application of wastewaters can be worse in cold-climates due to limited biological activity and no plant uptake and freezing in winter and thawing in early spring releasing large volumes of water (Zvomuya et al., 2006). The spring thawing contributes to ponding of water as winter application is not permitted in slope greater than 2% (Agency, 2008) and the thawing occurs over several days allowing water to infiltrate.

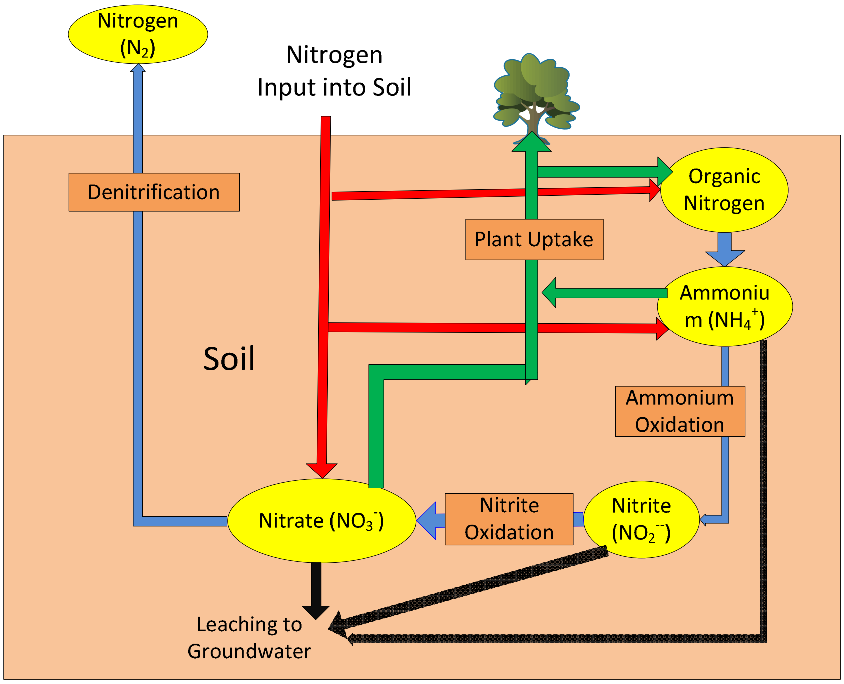


Figure 2-3 Simplified nitrogen cycle in the soil during the land treatment of food processing wastewater

Under anaerobic/reducing conditions, nitrate is denitrified to nitrogen gas and organic nitrogen can be converted to ammonium, minimizing the potential for groundwater contamination with nitrate. However, under aerobic/oxidizing conditions, ammonium and organic nitrogen in soil and in wastewater are converted to nitrate, a highly mobile pollutant.

The formation of aerobic or anaerobic conditions and removal of nitrogen are also associated with duration and interval of spraying. Frequent wetting and drying of land application sites (e.g., 9 hours spraying/15 hr drying (McMichael & McKee, 1966), 2-4 days wet /3-5 days dry (Bouwer & United States. Environmental Protection Agency. Office of Research and Monitoring., 1972)) resulted in treated water with highly oxidized condition wherein almost all N in influent water was converted to nitrate in the treated water exceeding the regulatory limit by 2-3 times (McMichael & McKee, 1966). However, 14-21 weeks of flooding alternated by 10-20 days of drying converted only about 20-50% of nitrogen to nitrate, with nitrate peaks at each flooding start period (Bouwer & United States. Environmental Protection Agency. Office of Research and Monitoring., 1972). Therefore, long drying and flooding operations can form ammonium in the system, an indication of reducing conditions.

Thus, both timing of wastewater application and rate of nitrogen uptake by plants grown in wastewater application fields are crucial factors in reducing nitrate contamination of groundwater. Critical to the science of this application is that crop selection can optimize nutrient uptake and evapotranspiration, increasing allowable hydraulic loading without overloading the environment.

2.7. Phytoremediation with poplars

Phytoremediation uses plants to remediate and/or contain pollutants from water, soil and air (ITRC, 2009). In soil-plant based treatment systems, the following plant-associated processes, combined with plant-independent processes such as adsorption, precipitation, settling/sedimentation, infiltration and oxidation/reduction (potentially affected by plants), affect the fate of metals and nitrogen (ITRC, 2009).

1. Phytosequestration - sequestration of pollutants in the rhizosphere through exudation of phytochemicals and on the root through transport proteins and cellular processes
2. Phytohydraulics - uptake and loss of water through transpiration
3. Phtoextraction - uptake of contaminants into the plant with the transpiration stream
4. Rhizostiumlation - increase of microbial diversity and biomass due to plant secretions

The use of poplar trees to remediate hazardous waste sites is a well-developed, low-cost technology (McCutcheon & Schnoor, 2003, ITRC, 2009). Phytoremediation with poplars has successfully remediated several types of wastes, including domestic wastewater effluents

(Tzanakakis et al., 2009), landfill leachate (Schnoor et al., 1995), brownfields (French et al., 2006), wood waste (Robinson et al., 2007), hazardous organic chemicals (Aitchison et al., 2000) and petroleum-contaminated aquifers (Cook et al., 2010). Poplars can degrade organic pollutants like dioxane (Aitchison et al., 2000) and PAHs (naphthalene and 3 ring PAHs) (Widdowson et al., 2005, Andersen et al., 2008).

Phytoremediation has the potential to help balance soil redox conditions under realistic wastewater loadings to simultaneously prevent nitrate and metal pollution of groundwater. However, processes that occur in land application sites where poplar trees are grown and their effects on overall fate of redox sensitive pollutants such as metals and nutrients have not been studied. Previous studies that studied capability of poplar trees to treat wastewaters were mainly for concentrated waste (Kirchmann & Witter, 1992, Kjeldsen et al., 2002, Zalesny & Bauer, 2007), unlike food processing wastewater which has very high carbon but relatively low nutrients and metals (Tanemura et al., 1994, Cheong et al., 2007). Moreover, prior phytoremediation research are carried out in contaminated soil with high metal concentrations and rarely use continuous irrigation, whereas, food processing wastewater is continuously and regularly applied over uncontaminated soil with normal concentration of metals (Alloway, 2013) over a period of time causing saturation of soils for prolonged periods. This study, unlike previous studies, studied the redox conditions in the soil during soil treatment of wastewaters. Therefore, this research evaluated the capability of poplar plantation to reduce problem of metals and nitrate mobilization to ground water under application of high-carbon and low-nutrients wastewater to the land and the mechanisms that occur during the treatment.

The ideal plant at the land where wastewater is applied should uptake high levels of nutrients, grow rapidly, tolerate moisture, evapotranspire extensively and be marketable with high revenue potential in order to sustain economically (Charmley et al., 2006). Dense grasses at land application sites, the status quo preference (Dennis, 1953), require time to establish each spring due to senescence in the winter from ice buildup (Zvomuya et al., 2006) and are frequently outcompeted by local grasses and weeds with shallower roots and less evapotranspiration, leading to decreased effectiveness (Smesrud et al., 2011a).

Poplar trees are superior to other trees with regards to growth rate, nutrient uptake, moisture tolerance, evapotranspiration, toxicity tolerance and revenue potential. Poplar trees can grow up to 2 m per year above ground even in temperate regions (Schnoor et al., 1995), with roots reaching 4.6 to 6.7 m below ground, often as deep as 12.2 m (ITRC, 2009). Additionally, phreatophytic plants such as poplars can withstand high soil moisture for prolonged period (Schnoor et al., 1995) and saline conditions up to 7.6 dS/m (Shannon et al., 1999) or as high as 200 mM NaCl (Chen et al., 2009). Poplars growth was not affected by irrigation of fertilized well-water (Zalesny et al., 2007) and municipal solid waste landfill leachate with concentration as high as 610 mg/L N (157 kgN/ha), 2.3 mg/L P (0.6 kgP/ha) and 450 mg/L K (115 kg K/ha). Poplar trees can also withstand high concentrations of chelating agents (e.g., EDTA) and salt concentrations greater than 2 g/L (Evangelou et al., 2007, Chen et al., 2009, Imada et al., 2009). These qualities of poplars make them one of the most used trees in phytoremediation.

2.7.1. Processes during phytoremediation using poplar trees

There are several processes associated with poplar plantation in the soil ecosystem that may help remediate the problem of metal and nitrate mobilization. As shown in Figure 2-4, the variables in the soil (in yellow rounded boxes) are affected by plants (in the rectangular boxes).

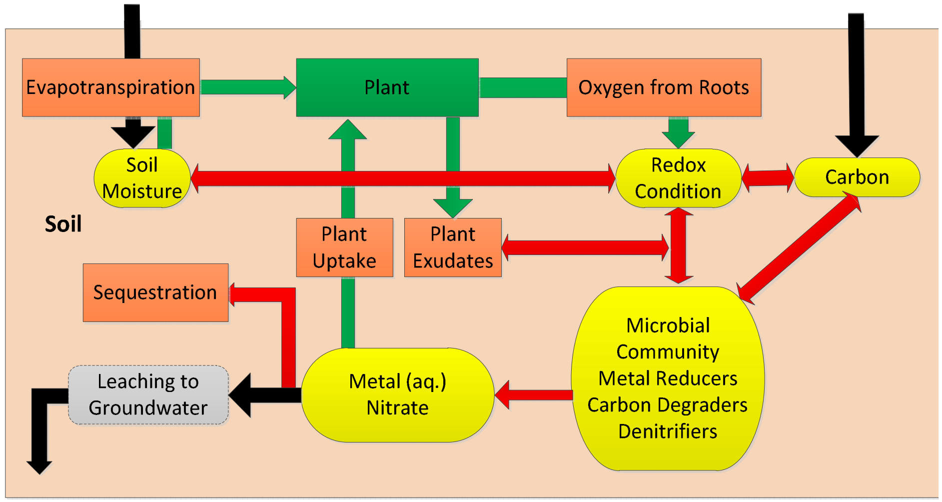


Figure 2-4 Processes and products that affect soil native metals in the soil treatment systems. Mainly, evapotranspiration, plant uptake, oxygenation and microbial processes may affect fate of nitrate and metals. These processes will be affected by plant exudates and oxygen from plants into the soil.

2.7.1.1. *Evapotranspiration*

By removing large volumes of water from soil to the atmosphere, poplar trees have the potential to decrease soil moisture thereby increasing oxygen diffusion in soils and reducing transport of metals and nitrogen to groundwater (ITRC, 2009). Consumptive use or evapotranspiration (ET) for 1-2 year poplar trees in Wisconsin from June through August was 4.4-4.8 mm/d (Hansen, 1988). Under ideal conditions, a 4-year old poplar tree can evapotranspire 81 - 91 cm water each year, with evapotranspiration coefficients as high as 4.2 (Shock et al., 2002, ITRC, 2009). Evapotranspiration by poplar trees removed 79% of 748 mm total precipitation and 84% of 996 mm total precipitation when used as evapotranspiration covers after one and two years of growth, respectively (Abichou et al., 2011). Poplars used for surface cover performed better than conventional covers by reducing percolation from 33% to 24% (Abichou et al., 2011). Of a total 173 inches of liquid, which included 133 inches cannery wastewater, applied at the rate of 14,195 gal/d/acre (assumed for 12 months) in Texas, approximately 18% was lost as evapotranspiration (10% in winter and 30% in summer), 61% as surface runoff and 21% as percolation (Law et al., 1970).

2.7.1.2. ***Phytoextraction***

Through uptake of metals and nitrogen, poplar plantations have the potential to decrease the mass of soluble metals and nitrogen available to contaminate groundwater. As poplar grew from second to third year, the nitrate leaching to ground water from fertilized land decreased (McLaughlin et al., 1985) indicating potential of poplar trees to do so in food processing wastewater applied land.

Poplars uptake substantially higher nitrogen than perennial herbaceous grasses (Adler et al., 2008) while also producing harvestable aboveground biomass. In addition, poplars uptake nitrogen and phosphorus at higher rate than grasses; poplar uptake efficiency (g plant inherent/g available) for N and P was higher than that of *Urtica dioica* (a herbaceous species) by 1.1 and 1.15 times (Adler et al., 2008). Average annual uptake of nitrogen by 5-year old hybrid poplars can reach 155 kg/ha/yr in the Great Lakes region and 300-400 kg/ha/yr in the Western U.S. (US EPA, 1981), as compared to 38.8 kg/ha/yr for reed canary grass and 125 kg/ha/yr for alfalfa (Zvomuya et al., 2006). Grasses reduced total N leaching by 19% and 57% of total N input from wastewater by plant uptake during non-growing season and growing season, respectively (Zvomuya et al., 2006). Hybrid poplars accumulated boron in its aerial parts and reduced B leaching in field experiments (Robinson et al., 2007). Mankin et al., 2010 concluded that poplar plantation reduced overall N and P losses from abandoned lagoon soils, however, the reduction may have been from sorption of ammonium, predominant form of N in lagoon abandoned soil (Douglas-Mankin et al., 2010).

Moreover, poplars can affect fate of metal by taking up high concentrations, including arsenic, lead, copper, aluminum, selenium and zinc through roots and translocating them into shoots (Banuelos et al., 1999, Fischerova et al., 2006, Evangelou et al., 2007, Robinson et al., 2007, Brunner et al., 2008, Migeon et al., 2009, Wang et al., 2011). Phytoextraction showed potential to decrease the mobility of metals in Brownfield contaminated soils (French et al., 2006). Poplar roots accumulated as high as 10,000 mg/kg and 27 mg/kg aluminum and chromium, respectively (Brunner et al., 2008). Hybrid poplars, such as *P. deltoides* × *P. nigra,* accumulated higher concentrations of lead and cadmium in leaf tissues than present in the growth media (soil) and qualified as accumulators (Fischerova et al., 2006).

Metal uptake by plants depends on the soil pH, chemical speciation/redox state, metal solubility and plant species (Kim et al., 2010, Vithanage et al., 2012). The presence of chelating agents like EDTA, common in food processing wastewaters (Hunt et al., 1976, CVRWQCB, 2005, Safferman et al., 2007), enhances phytoextraction of metals from contaminated soils (Evangelou et al., 2007). There is potential for increase of uptake through different strategy such as stimulation of rhizobacteriumm D14 strain for Arsenic (Wang et al., 2011).

Additionally, interactions between nitrogen and metals during land application of wastewater are expected to affect nitrogen and metal uptake. For example, presence of nitrate increased arsenic accumulation by increasing rhizosphere pH (Fitz & Wenzel, 2002). Net hydrogen ion released by N2 fixing symbionts favor lowering of pH. Consequently, in is essential to examine phytoextraction of metals concurrently with nitrate fate studies.

After uptake, poplars use different strategies to withstand toxicity of metals. Poplars produce organic acids, such as oxalate, malate, citrate and formate, at elevated metal concentrations of

Al, Cu and Zn to withstand toxicity and enhance phytoextraction (Qin et al., 2007). Poplar trees use enzymes to transport and detoxify metals (e.g., P-type heavy metal ATPases and phytochelatin synthases, respectively)(Adams et al., 2011). Poplar use enzymes like HMA 4 (1186-amino acid P-type heavy metal ATPase) to transports metals (Zn, Cu, Fe) and enzymes like PCS1 (phytochelatin synthases) to detoxify toxic effects of metals (Adams et al., 2011). Poplar plantations have been successfully grown on Brownfields contaminated with metals, with modeling indicating that phytoextraction could reduce contamination of Cd and Zn over a 30 year period. Consequently, extractability of Zn and Cd was reduced by phytoextraction during a short-rotation poplar plantation at a Brownfield site, indicating that the trees could stabilize these metals (French et al., 2006).

Through sorption, precipitation, complexation and redox reaction, poplar roots can stabilize metals in soils (Brunner et al., 2008, Shammas, 2009). Root exudates (such as pectins) have the potential to bind 1.6-6.3% of Cu and 0.4-6.1% of Zn (Brunner et al., 2008) in soils and decrease the mass of metals available to contaminate groundwater (ITRC, 2009). Phytosequestration may also occur by oxidation of metals by root-leaked oxygen, resulting in precipitation of metal oxyhydroxides (Jacob & Otte, 2003) and arsenic immobilizes in oxic conditions. Based on examining metal stabilization and uptake by poplar trees in uncontaminated and contaminated soils, poplar trees are expected to be the most effective at stabilizing metals at low concentrations (Brunner et al., 2008).

2.7.1.3. ***Rhizostimulation.***

Plant root-soil surface can have root exudates with significant inorganic and organic molecules affecting the number of microorganisms, aggregation and stability of soil particles and availability of metals (Shammas, 2009). Roots can exude as much as 10-20% of total photosynthesis in the forms of sugars, alcohols and acids (Schnoor et al., 1995). Hybrid poplar exudates contain 10-120 mg/L dissolved organic carbon and 1-10 mg/L acetic acid (Schnoor et al., 1995). In contrast, concentrations of BOD in food processing wastewater ranged from 300 – 2,700 mg/L (Safferman et al., 2007). The root exudates can enhance microbial diversity and biomass. Moreover, rapid decay of tiny roots and vegetation increases carbon source in soil thus increasing microbial activities. Populations of microorganisms including total heterotrophs, denitrifiers, pseudomonads were significantly higher in soil samples taken from rhizosphere of hybrid poplar trees than in adjacent agricultural soils (Jordahl et al., 1997). Moreover, genetically modified poplars may enhance microbial community structure to increase phytoremediation (Hur et al., 2011).

2.7.1.4. ***Oxygenation***

Plants can also affect the fate and mobility of metals by affecting pH and redox condition (by radial oxygen loss ROL) that may vary seasonally (Jacob & Otte, 2003). Plant roots can increase aerobic activity by leaking oxygen (Schnoor et al., 1995, Jordahl et al., 1997). Through provision on oxygen and carbon in the rhizosphere, poplar trees have the potential to stimulate microbial growth and activity (ITRC, 2009). Release of oxygen by poplar roots significantly enhanced aerobic biodegradation of naphthalene (Jordahl et al., 1997, Andersen et al., 2008). In wetland systems, the root aeration flux for emergent plants ranges from 0.5 – 12 $g/m^2/d$ (Kadlec & Knight, 1996). Of 5.86 $g/m^2/d$ oxygen influx into the bed substrate of soil based constructed reed bed, only 0.02 $g/m^2/d$ was released to surrounding soil after respiratory use (Brix & Schierup, 1990). In contrast, the BOD loading rate for food processing wastewaters is ~202 $g/m^2/d$, indicating that increased oxygen in the root zone would account for <5% of loaded BOD. The oxygenation was of no practical significance for aerobic biodegradation and microbial nitrification (Brix & Schierup, 1990). However, values for the plant aeration flux of poplars during growth in saturated, carbon-rich conditions are not known and the incremental addition of oxygen might be sufficient to decrease reducing conditions and thereby influence metal mobilization.

Thus, at land application sites, poplars has potential to aid land treatment by

1. uptake of metals and nutrients,
2. evapotranspiration, thus decreasing soil moisture,
3. increased oxygenation and change in soil moisture to influence soil oxidation reduction potential, or redox conditions of the soil and
4. enhanced microbial activity in root zone.

All these processes can be influenced by cultivar, planting density, mono or mixed culture, but screening and science need to be known before optimization can be done. Therefore, this research evaluated the hypothesis that poplar trees will reduce metal mobilization in food processing wastewater applied land by uptaking nitrate and metals, decreasing soil moisture, increasing microbial activity and increasing soil redox potential.

2.8. Secondary benefits of poplar plantation at land application sites

As discussed above, the poplar tree is the best candidate plant that can be grown at land application sites with numerous advantages over other plants or trees. In addition, growing poplar plants to treat food processing wastewater at land application sites has potential secondary benefits: a source of wood, biomass for biofuel and sustainability.

Poplar wood can be used to make varieties of products including pulp, paper and lumber (Balatinecz et al., 2001, Gasol et al., 2009, Guidi et al., 2009). Hybrid poplar can produce 10-15 times higher biomass than natural forests i.e. up to 10 dry dons per acre per year (Launder, 2002). Biomass yield of 12-15 Mg/ha, 13.1 Mg/ha and 12.5 Mg/ha was reported in Wisconsin, Pennsylvania and Romania, respectively (Hansen, 1988). Planting poplars at food processing wastewater application site could help meet the wood demand of the world which is growing at the rate of 60 million tons per year due to globalization and industrialization (Balatinecz et al., 2001) while being an additional source of income to food processors. Poplar biomass can also be used to produce electricity and has high potential to produce transportation fuel like ethanol at current biomass facilities and/or at coal co-firing plant(Launder, 2002). The Michigan biomass energy program (MBEP) identified short rotation woody crops like poplar as a promising biomass source with potential use for energy production (Launder, 2002). Benefits of short rotation woody crops for bioenergy production include fast growth (2.4-3.7 m per year), possible co-firing with coal and high productivity (Launder, 2002). The conservation reserve program (CRP) promotes plantation of short rotation woody plants like poplars and willows for biofuel in CRP lands instead of row crops (Dominguez-Faus et al., 2009). Maintaining suitable growth conditions, harvesting at proper time (after slow maturity, every 4 years), replanting and engineering poplar can even yield higher biomass (Balatinecz et al., 2001, Guidi et al., 2009, Smesrud et al., 2011b).

Poplars trees at wastewater application site contribute to water sustainability by using wastewater instead of freshwater; it requires 500-4000 L of water to produce enough crops such as sugarcane or corn for feedstock that produce 1 gallon of ethanol, equivalent to 50 gallon of water per mile of car driven (Dominguez-Faus et al., 2009). Additionally, substantial emission reductions could be achieved if poplar biomass is used for energy production, since carbon emission of poplars is 3,961 g/KWh, as compared to 49,618 g/KWh for natural gas and 88,758 g/KWh for coal (Launder, 2002). Moreover, poplars fix carbon at the rate of 2.5 $kg/m^2/yr$ (Schnoor et al., 1995) and may be used for carbon farming. Thus, poplar plantation has implications on reducing greenhouse gases while reducing pressure on food crops like corn to produce biofuel.

Hybrid poplars' other advantages include perennial life of about 20-25 years and easy propagation (Schnoor et al., 1995, Jordahl et al., 1997). In addition, poplars also work as wind break and prevent erosion (Schnoor et al., 1995). Moreover, young poplar tree stands provide suitable habitat for small mammals and migrating owls (Moser et al., 2002, Moser & Hilpp, 2004). Thus, poplar plantation produces additional source of income, domestic source of energy (energy security) and decreases dependence or pressure on food crops like corn and provides wild life habitat and environmental benefits.

2.9. Phospholipid fatty acid analysis for microbial community

PLFA (phospholipid linked fatty acid) analysis is an index of total viable microbial biomass and changes in overall composition of the community (Frostegard et al., 1993, Cavigelli et al., 1995, Calderon et al., 2001, Kaur et al., 2005, Frostegard et al., 2011). PLFA analysis for microbial biomass and community relies on the fact that phospholipids are found in all living cells and not in storage products or dead cells. PLFA is particularly effective at assessing temporal and spatial changes in community due to treatment effects such as soil types, environmental conditions, climate origins and different perturbations (Zelles, 1999, Steer & Harris, 2000, Hinojosa et al., 2005, Ramsey et al., 2006, Essandoh et al., 2013). PLFA analysis was able to differentiate treatment effects better than community level physiological profiling (LCPP) or PCR based molecular methods by 44% and 20% respectively (Ramsey et al., 2006). Lipid analysis does not require selection of sequences as in molecular methods and cultivation of microorganisms as in culture based methods to quantify the microbial community structure (Findlay & Dobbs, 1993). Combining fatty acid biomarker and the isotopic ration of 13 C/12 C can give insight into trophic interactions in soil ecology (Ruess & Chamberlain, 2010). The advantages of PLFA method include

1. ability to assess total microbial mass and community structure,
2. inclusion of the entire microbial community without truncation,
3. easy extraction of PLFA from samples,
4. suitable for sediments and soil extraction,
5. rapid, inexpensive and reproducible,
6. high precision and
7. possibility of using the extract for further biochemical characterization (Findlay & Dobbs, 1993, Cavigelli et al., 1995, Zelles, 1999, Frostegard et al., 2011).

On the other hand, the disadvantages include

1. no distinction on species or individual level and poorer resolution than molecular methods,
2. uncertainty in interpretation of PLFA for microbial communities,
3. need for complex equipment such as GC and
4. requirement of complex statistical methods (Findlay & Dobbs, 1993, Ramsey et al., 2006).

Particular attention is needed when liberating fatty acids as fatty acids related to non-living material may liberate as well (Zelles, 1999). FAME (fatty acid methyl esters) may liberate from cellular storage compounds or dead microbial and plant cells, whereas, PLFA (phospholipid linked fatty acid) liberate only from viable cell membrane (Hinojosa et al., 2005, Li et al., 2010).

CHAPTER 3: METHODOLOGY

This chapter covers the experimental plan and methods including data analysis used in each of the experiments.

3.1. Small-scale column experiment[1]

Experiments were conducted in a green house in East Lansing, MI from July to November 2012. Doors and a roof panel of the greenhouse were left open to minimize temperature and humidity differences between the greenhouse and the ambient environment. The experiment included ten planted columns and five control columns without plants.

3.1.1. Column construction

Columns were constructed using 15-cm diameter and 38-cm long PVC pipe. The bottoms of columns were sealed using PVC plates and roof sealants. A nozzle was fitted ½-inch from the bottom of each column for collection of leachate water. A hose connected to nozzle was used to collect leachate samples in plastic bottles or Erlenmeyer flasks. Columns were allowed to drain freely during and after wastewater application.

Soil was collected from a land application field in southwest Michigan. Soil was sieved through 3-mm sieves prior to use in columns. Columns were filled with pea gravel from the bottom to the level of the nozzle (1.3 cm) and with 31.7 cm of soil, leaving 5 cm of free board. The initial bulk density of soil in all columns was 1.3 g/cm^3. Soil analysis at the Plant and Soil laboratory at Michigan State University showed that the soil was a sandy loam with 61.2% sand, 22% silt and

[1] "Section 3.1 reprinted from Publication Ecological Engineering, Vol n/a, Niroj Aryal, Dawn M. Reinhold, **Reduction of metal leaching by poplars during soil treatment of wastewaters: Small-scale proof of concept studies**, Pages No. n/a, Copyright (2014), with permission from Elsevier.

16.8% clay and 1.3% organic matter. The soil had the following characteristics: pH 6.7, 167.5 mg/g phosphorus, 154.5 mg/g potassium, 127.5 mg/g magnesium, 4.9 meq/100 g cation exchange capacity, 25.4 mg/g nitrate-N, 6.2 mg/g zinc, 29.5 mg/g manganese, 7.15 mg/g copper and 208.5 mg/g iron.

Trees used for the study, *Populus deltoides* × *Populus nigra* DXN 34, were sourced from Cold Stream Farm, Free Soil, MI. The plants (6 – 11 inches tall) were planted in experimental columns using a completely randomized design and tap water was applied as required for tree establishment for 16 days. Tree height and root length prior to planting were recorded.

3.1.2. Wastewater application

Wastewater was applied to the five unplanted columns (soil-only control wastewater columns, CWW) and five of the ten planted columns (planted wastewater columns, PWW). Tap water was applied to the remaining five planted columns (planted water columns, PW). Wastewater was collected from a food processing facility in southwest Michigan. The facility processes seasonal fruits and vegetables for freezing and juicing. Process and wash water is collected and stored in an aerated lagoon prior to land application. Wastewater used in this experiment was collected from the lagoon and stored at 9°C, with the exception of the first batch which was stored at room temperature. Wastewater application started 16 days after planting and continued for approximately 4 months. Water or wastewater was applied at the rate of 16,000 gal/acre/day (1.54 mL/cm^2/d or 15.4 mm/d), which corresponds to the highest reported application rate in Michigan (Mokma, 2006), for the first four and last three weeks of the experiment. During the summer, negligible water leached from the columns. Therefore, the application rate was doubled to 3.08 mL/cm^2/d or 30.8 mm/d after 29 days of wastewater application. Toward the end of fall, temperatures decreased and evapotranspiration decreased substantially. Consequently, the application rate was reduced to the original loading rate after 99 days of wastewater application.

3.1.3. Sampling and analysis

Samples were collected to assess evapotranspiration, plant growth and leachate quality. Leachate water volume was measured daily. Evaporation or evapotranspiration for each column was calculated from subtracting the volume of leachate from the volume of applied wastewater or water. The number of leaves, height of plant and number and length of shoots were also recorded regularly. Wastewater and leachate samples were sampled weekly and assessed for pH, COD, anions, cations and transition metals. COD was measured by Hach method 8000. A Dionex Ion Chromatography ICS 5000 was used for cation, anion and transition metal analysis. Samples were filtered through 0.45 µm PTFE filter prior to ICS analysis. Anions, including nitrate, were separated on AG 22 and AS22 columns with a mobile phase of 4.5 mM sodium carbonate and 1.4 mM sodium bicarbonate at a flow rate of 1.2 mL/min and detected with a conductivity detector. Cations, including ammonium, were separated on CG 12A and CS 12A columns using 20 mM methanosulfonic acid at the flow rate of 1 ml/min and detected using a conductivity detector.

Iron and manganese were analyzed in leachate water samples to study the potential of poplars to reduce their mobilization under land application conditions. Iron and manganese were chosen due to relative ease and low cost of analysis and their relatively high solubility under moderately reducing conditions. Samples for iron and manganese were acidified with concentrated nitric acid to a pH<2 and stored at 4 ^{o}C until analysis. Transition metals were separated on a CG 5A and CS 5A columns using mobile phase of 7.0 mM PDCA (pyridine-2,6-dicarboxylic acid), 66 mM potassium hydroxide, 5.6 mM potassium sulfate and 74 mM formic acid at 1.2 ml/min. The separated metals were complexed using PAR (4-(2-pyridylazo) resorcinol) post column reagent consisting of 0.5 mM PAR, 1.0M dimethylaminoethanol, 0.5 M ammonium hydroxide and 0.3 M sodium carbonate and detected using UV/Vis detector.

Trees were sacrificed after 120 days of wastewater application and columns were deconstructed. Three soil replicates of each treatment were analyzed Trees from all columns were rinsed thoroughly, separated into roots, shoots and leaves and the mass was measured. Dry mass was obtained after drying at 104° C for 24 hours. Plant tissues and soils were analyzed by the Plant and Soil Laboratory at Michigan State University. Soil samples were analyzed for organic carbon, nitrogen concentrations and metal concentrations using widely accepted methods by the MSU Plant and Soil Laboratory. This specialized laboratory utilizes the following recommended methods (Brown, 1998) for soil analysis: pH (potentiometrically using a pH meter), Fe and Mn by acid digestion and atomic adsorption spectrometry and soil organic matter by loss-on ignition. Additionally, soils were analyzed for nitrate through cadmium reduction (Huffman & Barbarick, 1981), ammonium through the salicyclate method (Nelson, 1983) and total nitrogen through the micro-Kjeldahl digestion (Bradstreet, 1965). Plant tissues samples were analyzed for arsenic, iron, manganese, etc. using acid-digestion (EPA 3051 method (US EPA, 1994)) and analyzed on ICP-MS. Plant nitrogen was analyzed through micro-Kjeldahl digestion.

3.1.4. Statistical analysis

Statistical analysis, including one-way ANalysis Of VAriance (ANOVA) and one-tailed Student t-tests, was completed using SigmaPlot 12.5. All data that failed Shapiro-Wilk normality test were analyzed using Dunn's method for unequal sample sizes. A p-value of <0.05 was considered statistically significant. The reported values are mean±standard error of the mean.

3.2. Large-scale column experiment

Research was carried out in tree-scale columns in the open environment to represent actual ambient environmental conditions. The research approach was applying synthetic wastewater on poplar planted columns, measuring temperature, soil moisture and redox potential at different depths and collecting, measuring and analyzing leachate water for water quality. At the end of the experiment, plant and soil samples were analyzed for major nutrients and metals (iron, manganese and/or arsenic) and soil samples were analyzed for microbial biomass and diversity.

3.2.1. Column construction and setup

Experimental columns was designed and established in 2010 and experiment was conducted in summer 2011. However, due to leakage of columns and inability to assess the evapotranspiration, the columns were reconstructed again in fall 2011 and experiment was conducted in the summer 2012. Due to leakage again, the experimental set up was completely changed in spring 2013 and the experiment was conducted again from summer 2013 to fall 2014. The 2013-2014 set up enabled evapotranspiration assessment, while 2012 and 2011 experiments assessed all parameters but evapotranspiration. Thus, the experimental setup is described as 2011 setup, 2012 setup and 2013-2014 setup.

3.2.1.1. Experimental setup for 2011

Corrugated HDPE pipe of diameter 91 cm was cut into 147 cm long pieces and used as columns (Figure 3-1). Corrugated pipe was used to prevent short-circuiting of wastewater applied to the columns or movement of applied wastewater along the edges of the column. Three ports 5 cm by 10 cm were made in each column at 61 cm, 107 cm and 137 cm below top of the column. The ports were access points for sensors (Figure 3-1). 15 cm diameter drain fitting was fitted approx. 8 cm from the bottom of the column. From the drain fitting, 2 cm diameter flexible drain pipe was connected to the bucket placed at ground level to collect the leachate water.

Concrete blocks 61 cm×61 cm×244 cm were placed on the cemented parking lot (Figure 3-1). Plywood was placed over the concrete blocks and columns were placed over the plywood (Figure 3-1). All twelve columns were tied to at least one metal anchor anchored by the concrete base. The columns were filled with sand up to the height of the drain fitting, compacted and sloped down to the drain port. Pond liner used to cover the sand was attached and sealed to the walls of the column on all sides using silicones.

Figure 3-1 A single column with ports and drain shown (Left). Set up of 12 columns with concrete blocks, plywood, columns and plants (Right).

Columns were filled with soil leaving a head space of 16 cm. During filling of columns with soil, soil was gentle compacted and leveled with a tamper after each addition of approximately 0.1 m^3

of soil. As the experiment progressed, the columns leaked from the bottom and from the access ports. The access ports were sealed with varieties of sealants; however, no attempt was successful.

3.2.1.2. Experimental setup for 2012

Due to inability to assess evapotranspiration in 2011 experiment, the set up was deconstructed and reconstructed in October-December 2011. The 2012 experimental set up consisted 15 columns, 3 more than the 2011 experiment (Figure 3-2). A PVC (Poly vinyl chloride) plate with 107 cm diameter and 15 cm thickness was grooved by marking the edge of the base of the column. The HDPE pipe column base was inserted inside the groove and the sealants were used both from inside and outside. Similar to the columns in 2011, compacted sand was used at the base and covered by pond liner. Pond liner was attached to the column wall using sealants. In addition, ports for moisture sensors were closed from inside wall of the column and moisture sensors were placed from inside the column at the time of column construction.

Even with the modifications, moisture leaked from the bottom of the columns after one winter. Though different approaches including plastering the connection between PVC plate and HDPE, sealing with many sealants/silicones available in the local hardware store were applied, the moisture leaking could not be prevented in 2012 as well. The experimental set up in 2012 was completely given up in spring 2013 and new experimental set up was established.

Figure 3-2 Experimental set up for 2012 experiment

3.2.1.3. Experimental setup for 2013-2013

The new experimental set up contained tanks used as columns (Figure 3-3), obtained from U.S. Plastic Corporation. The tanks were open top 55 gal heavy weight white plastic tanks with an an inner diameter of 55 cm, height of 91 cm and wall thickness of 6.4 mm. Tanks had spigot at approximately 13 mm from the bottom to collect the leachate water. Columns were covered with black poly-wraps (not shown in Figure 3-3) to block light penetration into the soil. Ports with 12 to 25 mm diameter were made in the column wall to insert oxidation reduction probes. Columns were filled with soil as in 2011 and 2012 with 8 cm as freeboard at the top except no pond liner and sand was used.

Figure 3-3 Experimental set up for 2013 experiment. The tanks were wrapped with thick black polythene later.

3.2.2. Experimental design, plants and soil

3.2.2.1. Experimental design, plants and soil for 2011

The experimental design consisted of 12 columns which were randomly assigned as treatment or control (Figure 3-4). Treatment consisted of three varieties of plants: two types of poplar trees and a willow tree. Therefore, there were three replicates each of no-plant control, tall shade poplar (*Populus deltoides*× *Populus nigra* var OP 367*)*, shade poplar (*Populus deltoides)* and willow tree (*Salix nigra*).

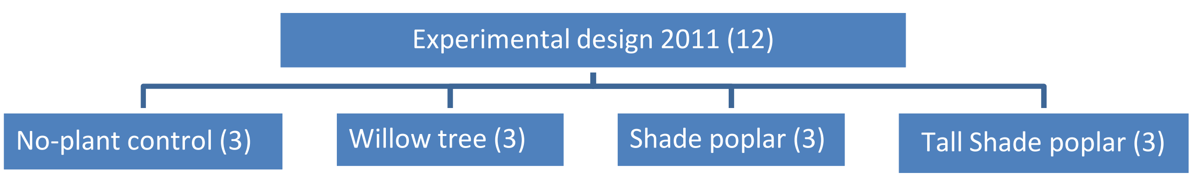


Figure 3-4 Experimental design for 2011 experiment with number of columns on the parentheses.

Poplar and willow were selected since both are phreatophytic species with high evapotranspiration coefficient that can withstand high soil moisture for prolonged periods. Poplar and willow are most used trees for phytoremediation (ITRC, 2009). Metal uptake, redial loss of oxygen and plant related processes can differ between varieties (Jacob & Otte, 2003, Kim et al., 2010, Vithanage et al., 2012). Therefore, two-poplar varieties were chosen to identify if treatment differs with the varieties.

A tree in each column except control was planted in August, 2010 [Figure 3-5]. Weeds were taken out occasionally in all columns including no-plant controls.

Figure 3-5 Columns with plants and buckets for leachate water collection during 2011.

The soil used for 2011 experiment was sandy loam (76.9% sand, 11% silt, 12.1% clay). The soil was provided by MSU physical plant (aka MSU infrastructure planning and facilities).

3.2.2.2. Experimental design, plants and soil for 2012

In 2012, only poplar trees were selected following the better vegetative growth and similar treatment performance to willow in 2011. The experimental design for experiment in 2012 is shown below [Figure 3-6].

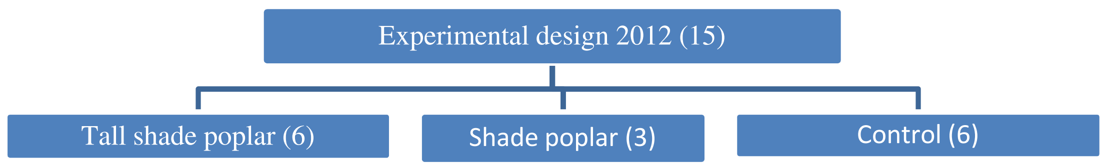


Figure 3-6 Experimental design for 2012 experiment. Number inside parentheses indicate number of columns for each treatment.

The poplar trees were not different than willow in pollutant treatment in 2011; however, poplars grew much better than willow. In addition, due to the higher economic revenue potential of poplars compared to willows, willows were not utilized in 2012. Therefore, two poplar plant varieties, tall shade poplar (*Populus deltoides*× *Populus nigra* var OP 367), and shade poplar (*Populus deltoides*) were chosen in 2012. The plants were taken out of columns in November 2011 and were re-planted in May 2012. In addition, two more plants (OP 367) obtained from Segal Ranch Nursery was planted in May 2012. Though the plants were different in size at the beginning of the experiment, the size difference disappeared due to growth of new plants and death of top portion of old plants.

Of the 15 columns, nine columns were filled with sandy loam textured soil from 2011 experiment. The other six columns were filled with new soil mixture provided by MSU physical plant. The new soil mixture was also categorized as sandy loam though it had lower sand content than the sandy loam soil used in 2011 [Table A-1 in Appendix]. The soil had 58.7 to 68.7% sand, 12.6 to 20.6% silt and 14.7 to 20.7% clay. The nutrients in the soil were either optimum or above optimum as shown in [Table A-3 in Appendix].

3.2.2.3. *Experimental design, plants and soil for 2013-2014*

In 2013, two soil textures, loam and sandy loam, were used. There were total of 15 columns, 9 for sandy loam and 6 for loam soil [Figure 3-7]. Due to unavailability of shade poplar trees, only tall shade poplars were planted in 5 columns with sandy loam and 3 columns with loam soil.

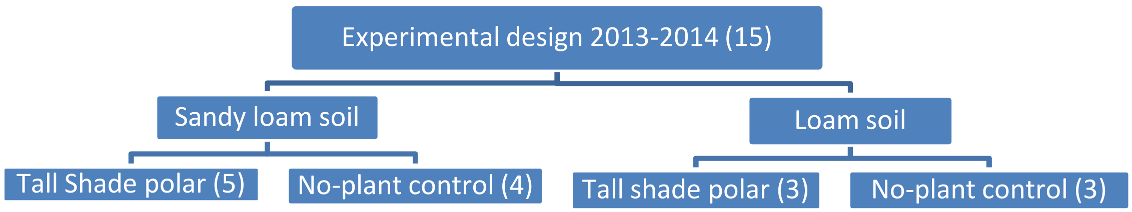


Figure 3-7 Experimental design for 2013-2014 experiment. Number inside parentheses indicate number of columns for each treatment.

Two varieties of poplar in 2012 experiment had no difference in growth and pollutant treatment. Therefore, only one species of poplar was utilized for 2013-2014. The most widely used poplar variety for remediation is the tall shade poplar (*Populus deltoides*× *Populus nigra* var OP 367). The plants, 183 cm tall were planted on June 2013 and 122 cm tall were planted on July 2013.

The soil used in 2013-2014 experiment was sandy loam for 9 columns and loam for 6 columns. The sandy loam soil was re-used from 2012 experiment. The loam soil was acquired from MSU infrastructure and planning facilities. The loam soil had 41.8% sand, 40.4% silt and 17.8% clay [Table A-5 in appendix]. Potassium and phosphorous were below optimum levels for growth of plants and TKN was 0.03%.

3.2.3. Instruments and sensors

Sensors (soil moisture, soil oxygen and temperature) can predict the onset of anaerobic conditions regardless of wastewater characteristics, soil and weather (Safferman et al., 2010). As measuring redox condition was important to study fate of nitrate and metals, this experiment used oxidation-reduction potential (redox potential). Redox potential was measured instead of soil oxygen because soil oxygen probe gives erroneous results under saturated and anoxic conditions (Patrick et al., 1996).

The experimental columns in all years were instrumented with sensors to measure soil moisture, oxidation-reduction potential and temperature at different depths. Continuous data from the sensors were collected using a CS 23X Datalogger and AM 16/32 Multiplexer, powered by a battery. The battery was continuously charged by a charger that converted A/C voltage to D/C voltage. The data was recorded in datalogger every 30 minute. Data was collected since November 2010 except for duration of reconstruction of the project. There was no instrumentation and data collection from September 2011 to April 2012 and from October 2013 to July 2013. Details of measurement of each parameter using sensors are described below.

3.2.3.1. Temperature

Temperature sensors (107 L-Campbell scientific) were used to measure the ambient and soil temperature at different column depths. The sensor uses thermistors whose resistance changes with temperature. Soil temperature measurement was important as it directly affects the treatment, especially the microbial growth and processes.

3.2.3.1.1 Temperature measurement for 2011

The temperature sensors were inserted from access ports at 46 cm, 91 cm and 122 cm below soil surface horizontally and placed at surface of the soil in the column. There were 12 temperature sensors in three different columns.

3.2.3.1.2 Temperature measurement for 2012

During filling of columns with soil, temperature sensors were horizontally placed at surface, 46 cm, 91 cm and 122 cm below soil surface. There were 16 temperature sensors at four different columns.

3.2.3.1.3 Temperature measurement for 2013-2014

During filling of columns with soil, temperature sensors were horizontally placed at surface, 30 cm, 61 cm and 76 cm below soil surface. There were 16 temperature sensors at four different columns.

3.2.3.2. Moisture

This experiment used CS 616 (soil water content time domain reflectometer or TDR) sensors obtained from Campbell Scientific to monitor moisture during the entire duration.

3.2.3.2.1 Moisture measurement for 2011

Total of 36 soil water content reflectometer (TDR) sensors, 3 in each column at the depths of 46, 91 and 122 cm below soil surface, were installed for monitoring moisture content of the soil in the columns. Soil moisture sensors were inserted horizontally from access port from outside of the column. The factory calibrated equation was used to calculate the moisture content. The equation accurately measures soil water content of mineral soil with bulk density less than 1.55 g/cm^3 and clay content less than 30%. The quadratic calibration equation used was

$$\mathbf{VWC = -0.0663\text{-}0.0063\times\tau+0.0007\times\tau^2} \qquad \textbf{Equation 15}$$

Where,

VMC= volumetric water content

τ =period of the signal, μS

3.2.3.2.2 Moisture measurement for 2012

Though experimental set up was different, three moisture sensors per column at 46, 91 and 122 cm below soil surface were installed in 2012 experimental columns. Total of 45 soil moisture sensors were used to monitor the soil moisture profile of the columns. Soil moisture sensors were placed at the intended depth horizontally while filling the soil in the column.

3.2.3.2.3 Moisture measurement for 2013-2014

In 2013, three soil moisture sensors per column, total of 45 moisture sensors, were used at 30, 61 and 76 cm from the soil surface. Soil moisture sensors were placed horizontally at the intended depth while filling the soil.

3.2.3.3. Oxidation-reduction potential

Soil oxidation-reduction potential or redox potential measures the availability of electrons for reactions. Soil redox potentials are used to characterize the soil activity in terms of intensity of oxidation or reduction occurring in the soil and to infer the biological activity in the soil (Patrick et al., 1996). Soil redox potential measurement can be an important tool to indicate onset of oxidizing conditions or reducing conditions, i.e. when does oxygen reenter the system and when does nitrate, ferric ion deplete in the system and to interpret processes (Patrick et al., 1996). The indirect method of measurement of redox by analyzing concentration of redox couples is tedious, expensive, lengthy and time consuming (Eshel & Banin, 2002). Because redox potential in soil is controlled by many redox species pairs and redox potential is not usually at equilibrium, mixed potential should be measured (Eshel & Banin, 2002). Patrick et al. (1996) state measuring soil redox potential to know the redox status of (un)saturated soil as best measure for four reasons as follows.

1. Wider fluctuation, 1000 mV for saturated and 300 mV for unsaturated soils
2. Better poised and reproducible results
3. Absence of oxygen in saturated soils precludes use of oxygen or oxygen diffusion measurement
4. Indication of intensity of oxidation reduction as measurement of individual reduced species like ferrous, sulfide sulfur, manganese may not indicate intensity of reduction and microbial characterization may not be always be applicable to every soil (Patrick et al., 1996) .

Some of the limitations of potential measurements of redox status using sensors are (Patrick et al., 1996, Fiedler et al., 2007)

1. irreversibility and permanent drift due to coatings, impurities, accumulations,
2. slow response of sensors due to slow reaction kinetics of many redox reactions and

3. measurement of mixed potentials as redox may be determined by composite of two or more processes (in the presence of complex inorganic and organic chemicals) and the thermodynamic calculation may be limited.

Soil redox potential is typically measured with the platinum electrodes as platinum readily transfers electrons and does not readily react (Patrick et al., 1996). Actual potential is measured by preventing the flow of electrons and putting suitable meters to read the electromotive force or potential by connecting the platinum half electrode to a suitable half-cell whose potential is known. In-situ measurement of soil redox potential is most efficient method of estimating reducing reactions in soil (Fiedler et al., 2007).

Eschel and Banin (2002) measured soil redox potential using platinum and silver electrodes and specialized interface for few days to few weeks in soil with wastewater recharge cycles and obtained reliable temporal information on soil redox in the soil profile by taking precautions to minimize atmospheric oxygen influx (Eshel & Banin, 2002). Precautions taken were waiting for equilibrium after disturbance and calibration. The authors observed that platinum electrode may measure mixed potential instead of true soil potential if not given enough time (at least few days) to equilibrate with the soil (Eshel & Banin, 2002). Platinum electrodes showed reliable results in measuring soil redox potential without much drift (<2 mV) even after weeks of insertion into soil (Eshel & Banin, 2002).

3.2.3.3.1 Construction of ORP probes

Platinum electrodes were constructed by modifying the method used by Patrick et al, 1996. Brass rod with a diameter of 3.175 mm was cut into 10 cm pieces each. A small hole (5 mm deep by 1.5 mm diameter) was pierced into each side. In one end, a 10 mm long and 1 mm diameter platinum rod was inserted, soldered and covered by marine epoxy. In the other end, braided copper wire was inserted and soldered to brass rod. Heat shrink tube was used to cover the brass rod and both ends (Figure 3-8). The whole sensor was inserted to an acrylic or pvc tube to protect it from damage due to moisture and abrasion in the soil and to provide ehough strength to push the probe into soil.

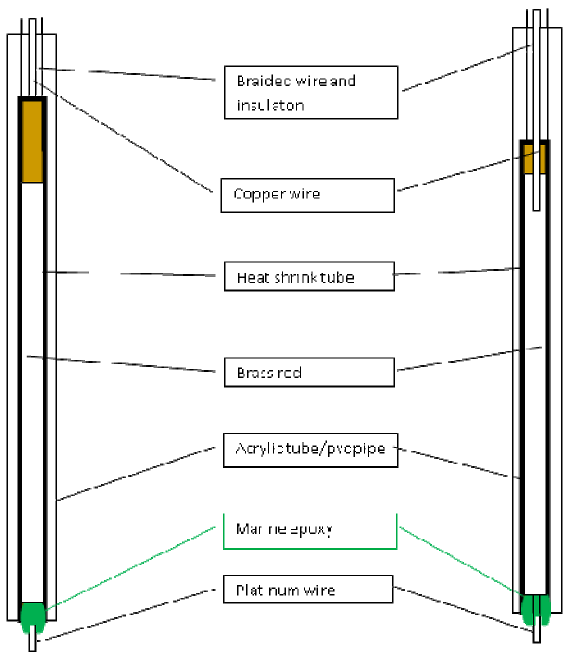


Figure 3-8 Schematic diagram of oxidation reduction probe

3.2.3.3.2 Calibration and Checks

Quantitative checks on the platinum probes and reference probes were done using several standard solutions as follows.

1. Light standard solution. The standard solution composed of 0.1 M (39.21g/L) of ferrous ammonium sulfate and 0.1 M (48.22 g/L) of ferric ammonium sulfate in 1M (56.2 ml/L) sulfuric acid (Light, 1972). The redox potential for standard solution at 25^{o}C for a

platinum electrode against an Ag/AgCl electrode with 4.0 M KCl electrolyte was +475 mV. The same value with SHE (standard hydrogen electode) is 675 mV. Hence a correction factor for silver electrode was 200 mV.

2. Orion standard solution. Standard ORP should be 220 mV against Ag/AgCl electrode with 4.0 M KCl reference probe at 25°C.
3. Qunihydrone solution buffered at pH 4 (Patrick et al., 1996). Standard ORP should be 462 mv against Ag/AgCl electrode with 4.0 M KCl reference probe at 25°C.
4. Quinhydrone solution buffered at pH7 (Patrick et al., 1996). Standard ORP should be 285 mV against Ag/AgCl electrode with 4.0 M KCl reference probe at 25°C.

In 2011, the sensors that met the minimum criteria of within ±10 mV of the standard value were used and those that did not meet the criteria were reconstructed or discarded. In 2012 and 2013, the sensors were re-checked. A few of the sensors that gave incorrect voltage reading in the standard solution were repaired.

3.2.3.3.3 Measurement

Research used platinum oxidation reduction potential probes (Patrick et al., 1996, Vepraskas & Cox, 2002) and reference probes (Ag/AgCl electode with 4M KCl electrolyte from Fisher Scientific) to measure redox potential of soil in the columns. Sensors were inserted at two different depths (46 cm and 91 cm), from the sides of the column into soil, in two of three replicates of each treatment in 2011 and 2012 experiment. In 2013, the depth of installation was 30 cm and 61 cm. A total of 16, 20 and 20 ORP sensors were installed in 8, 10 and 10 columns in 2011, 2012 and 2013, respectively. ORP sensors were inserted horizontally from outside the column/tank by making holes which were sealed with sealants including roof tar. A reference probe was placed at the top of each column that was shared by two platinum sensors in the column. The voltage was continuously recorded in a datalogger powered by battery.

3.2.4. Wastewater preparation

Synthetic wastewater was designed to mimic characteristics of a representative average sample of a food processing facility [Table 3-1]. COD represented approximately 74% of BOD in food processing wastewater (Esvelt, 1970) and 1g glucose equivalent to 1g ultimate BOD has been used previously (Safferman et al., 2010). COD concentration was obtained by adding sucrose (87.5%) and starch (12.5%). Starch was added as a recalcitrant source of COD. The percentage of starch used was limited to 12.5% due to low solubility of starch in the water. Salts were weighed in the required mass to make a concentrated salt mixture [Table 3-1].

Table 3-1 Synthetic wastewater characteristics and composition

	Characteristics	Wastewater strength, mg/L	Compound used
1	COD	1438.5	Sucrose, $C_{12}H_{22}O_{11}$
		205.5	Starch, $C_6H_{10}O_5$
2	Ca	66.8	Calcium chloride, $CaCl_2$ or $CaCl_2.2H_2O$
3	Mg	24.9	Magnesium sulfate, $MgSO_4$ or $MgSO_4.7H_2O$
4	K	476.9	Potassium sulfate, K_2SO_4 Potassium carbonate, K_2CO_3
5	Na	35.3	Sodium bicarbonate, $NaHCO_3$
6	Fe	2.4	Ferric chloride, $FeCl_3.6H_2O$
7	Mn	0.1	Manganese sulfate, $MnSO_4.H_2O$
8	NH4-N	19.7	Ammonium sulfate, $(NH4)_2SO_4$
9	Zn	0.2	Zinc sulfate, $ZnSO_4.7H_2O$
10	Cu	0.1	Cupric chloride, $CuCl_2.2H_2O$
11	Co	0.02	Cobalt chloride, $CoCl_2$
12	B	0.01	Sodium borate, $Na_2B_4O_7.10H_2O$
13	Mo	0.01	Sodium molybdate, $Na_2MoO_4.2H_2O$
14	Ni	0.07	Nickel nitrate, $NiNO_3.6H_2O$

Wastewater was prepared by diluting the concentrated salt mixture with tap water (not dechlorinated) in a PVC tank. Dilution was done once every three days in 2011-2013 and once every other day in 2014. Wastewater was mixed before application.

3.2.5. Wastewater application

3.2.5.1. Wastewater application for 2011

Wastewater application was made using the irrigation set up consisting of the timer switch enabled sump pump housed inside the source tank. Irrigation set up consisted of main and distributor lines made up of 12 mm diameter PVC pipe, valves to control the flow, flexible tubing and drip nozzles. Around each plant, three drip nozzles were fitted. The wastewater application rate was adjusted using the controller in the drip nozzles and control valves.

Synthetic wastewater was applied at the highest current rate of application in Michigan (Mokma, 2006, Safferman et al., 2007) i.e. at the rate of 15 mm/day or 16,128 mg/day/column COD. The application rate was 32.7 ml/min for 5 hours each day (9.81 L/day/column) with 19 hours rest. However, microorganisms grew inside the pipes and the flow rate varied. Therefore, the irrigation set-up was used to pump the wastewater in the tank to the top of the column and 9.81 L wastewater was collected in buckets and manually applied to each column every day. Synthetic wastewater was applied daily from 2/17/2011 until 7/20/2011.

3.2.5.2. Wastewater application for 2012

The 2012 experiment manually applied the wastewater at the same rate as 2011 (9.81 L/column/day) as the column size was equal. Wastewater addition was started from 6/12/2012 and ended on 8/19/2012.

3.1.1.1. Wastewater application for 2013-2014

In 2013, there was no irrigation set up except for the tank. The wastewater was applied manually at the same rate as in 2011 and 2012 (15 mm/d). As the column had smaller area, 3.6 L/column/day was applied daily. In 2014, the application rate in 2013 was halved and wastewater was applied at the rate of 3.6 L/column/day every alternate day. The application rate was halved due to frequent ponding of columns in 2013.

3.1.2. Sampling and analysis

A daily log of wastewater applied and leachate water collected was maintained. As the columns in the 2011 and 2012 experiment leaked, the log of water data in only 2013 was used for assessment of evapotranspiration.

Leachate water collected in the bucket and the influent water were sampled every other week for analysis of water quality. The water quality measurement included pH, chemical oxygen demand, anions (nitrate, phosphate and total nitrogen), cations (sodium, calcium, magnesium, potassium and ammonium) and transition metals (total iron and manganese). All sample analysis except the transition-metal analysis was done immediately after sampling. For transition metals, the samples were filtered using a 0.45 µm filter into a glass container, acidified to pH below 2 using nitric acid and stored in refrigerator at 4 ^{o}C until analysis at the end of the season.

Soil samples at the end of the experiment were taken from triplicate columns at each depth where moisture sensors were located. Soil samples were analyzed for major nutrients (N, K, P, Ca, Mg), trace metals (Mn, Fe, As) and total nitrogen in October 2014.

Plants were sacrificed and separated into roots, stems and leaves and analyzed for major nutrients and trace metals (Mn, Fe, As) in October 2014. For phopholipid linked fatty acid analysis, soil samples from triplicate samples at 30 cm and 61 cm depth were analyzed in Octobe 2014.

All analytical methods are described in more detail in section 3.4.

3.1.3. Data and analysis

The difference of daily influent volume plus rainfall volume and leachate water volume was taken as the actual evapotranspiration (ET). ET was calculated on a weekly basis. Crop coefficient was calculated as the ratio of actual evapotranspiration of planted columns to that of respective control columns. Moreover, research compared the moisture inside columns at different depths between the planted columns and no-plant control columns. The moisture data was converted to daily data to compare patterns. Oxidation-reduction potential raw values were corrected for standard hydrogen electrode by adding 200 mv to the recorded values before comparing between treatments, especially planted vs. no-plant controls.

For each treatment, the efficiency of carbon treatment was calculated using the influent and leachate (effluent) COD data. The efficiency of treatment and/or effluent COD of planted columns was compared to that of control columns under different soils.

Total nitrogen in soil and plant and ammonium and nitrate in leachate water were used to assess mass balance on nitrogen in 2013-2014. Uptake of total nitrogen by poplar and its influence on nitrate mobilization and pollution of groundwater was then evaluated. With concentration of metals in the plants, soil and leachate water, mass accounting on those metals was assessed.

For statistical analysis of all chemical data, ANOVA was performed to evaluate the difference between treatments and control as appropriate. Whenever the data met normality requirements in Shapiro-Wilk test, one-way ANOVA on mean was performed. If the data was non-normal, Kruskal-Wallis one way ANOVA on ranks was performed. If the result of the one-way ANOVA (mean or rank) was significant, pair-wise comparison was performed using Tukey's test for equal sample sizes and Dunn's test for unequal sample sizes. For soil moisture and oxidation reduction potential in 2011 and 2012 data, two-way ANOVA was performed with treatments (different plants or control) as the first factor and depths as the second factor. For soil moisture and oxidation reduction potential data in 2013-2014, three-way ANOVA was performed with soil as the first factor, treatments (different plants or control) as the second factor and depths as the third factor. All pair-wise comparisons for each factor were considered in two-way and three-way ANOVA.

Total PLFAs were added to represent the total biomass present for each sample. For diversity, PLFAs were grouped together according to functional group biomarkers and the relative abundance of each type of microorganisms assessed. Interpretation of results was done by relating PLFA to organisms using phylogenetic relationships between organisms and their PLFAs because all organisms contains mixtures of fatty acid and sometimes the unique fatty acid qualifying it as "biomarker" (Findlay & Dobbs, 1993). The results of the PLFA analysis were used to conduct a cluster analysis using the standardized data and Euclidean distance in UPGMA (unweighted pair group method with arithmetic mean) method. The dendrogram was based on the relative presence of each type of PLFAs and the column soils were classified based on the type of microorganisms and their proportions.

3.2. Field experiment

The study was conducted in the actual land application field of a Michigan food processor to represent real case scenario.

3.2.1. Field set-up/field site

Approximately an acre (3,772 m^2) size plot of land application site without chemical application was made available for research purposes by a food processing facility in South Michigan. The food processing facility processes fruits and vegetables such as apples, tart cherries, blueberries, asparagus, plums, celery primarily for freezing and making juices and concentrates. The wastewater is applied by using center pivot covering a total of 67,180 m^2. The pivot was approximately 3.4 m in height and the sprinklers were hung to about 2.1-2.4 m from the height of pivot.

Vegetation primarily consisted of annul grasses with no maintenance except periodic mowing. The grasses die in the winter leaving the ground without any living cover. The site had test wells at several places for ground water sampling.

3.2.2. Experimental plots

In summer 2011, the experimental field was divided into four equal plots. Each plot was approximately 44.2 m in length (across pivot) and 21.3 m in breadth (along pivot) as shown in [Figure 3-9].

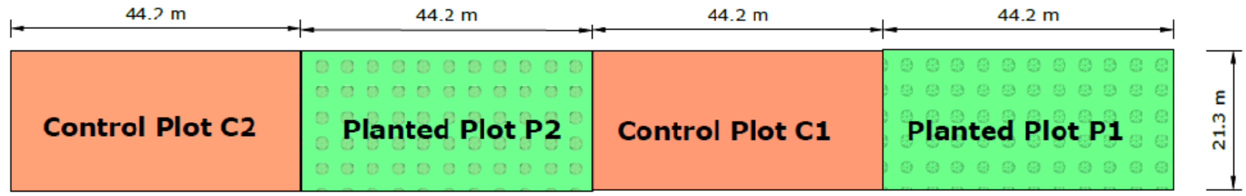


Figure 3-9 layout of plots in the field

The soil in the field was sandy loam with following characteristics (Table A-7 in Appendix). Top soil had higher metals including arsenic, iron, manganese, zinc, copper, higher nutrients including nitrogen, phosphorous, potassium, higher calcium and higher silt content. Nutrients were above optimum for plant growth.

3.2.3. Planting

Stakes were used to identify spots for plants. Glyphosate was sprayed around the spots to kill grasses. A total of 105 plants (15 columns ×7 rows) were planted per plot at the spacing of 3.05 m×3.05 m in the staggered pattern [Figure 3-10]. A general rule for preliminary phytoremediation design using trees is spacing of 3.05 m in staggered pattern that gives 7 m^2/tree (ITRC, 2009). On 8/26/2011, a total of 105 poplar trees obtained from Segal Ranch Nursery were planted in plot P1. On 9/1/2011, plot P2 was planted with 105 poplar trees obtained from Kelly Nursery Inc. In both plots, bare rooted poplar trees, *Populus deltoides*×*Populus nigra* var DXN OP367 were planted. The plants were approximately 1.22 m in height including roots, of which 0.61-0.91 m was above ground after planting.

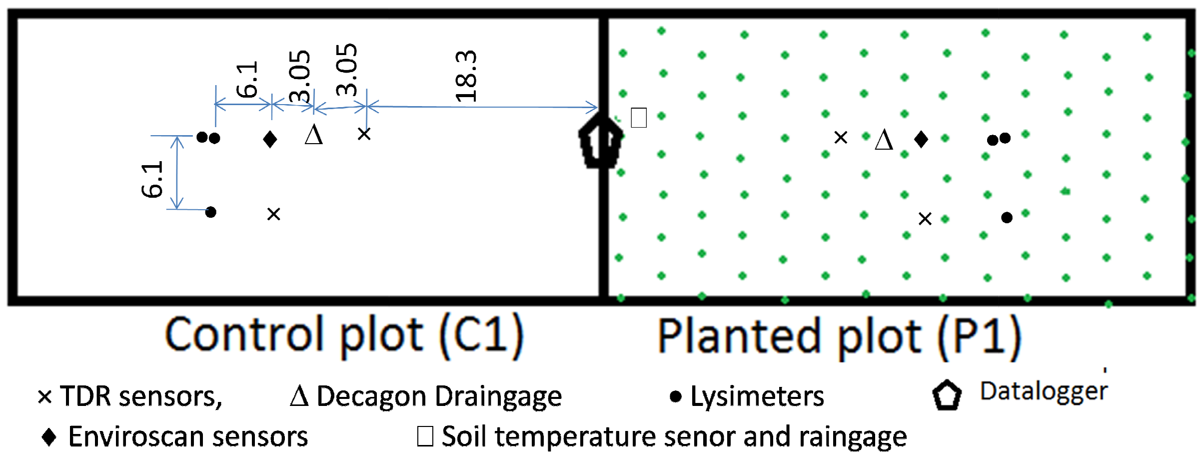


Figure 3-10 Layout of instrumentation and planting in the half of the field (2 plots). Green dots represent poplar trees at the spacing of 3.05 m×3.05 m. All dimensions are in m. The other half (control plot C2 and planted plot P2) are exact replicates of C1 and P1. The site is instrumented with 2 solar powered dataloggers, 4 drain gauges, 12 lysimeters, 4 enviroscan probes (each probe has 4 water content sensors), 24 time domain reflectrometry (TDR) sensors, 2 rain gauges and 2 temperature sensors.

3.2.4. Replanting

More than 95% of plants survived planting and grew in the fall 2011. However, most plants died during the next spring and summer 2012. The plants were attacked by rodents, who ate the bark of plants near ground level (Figure 3-11). Therefore, plants were re-planted on 7/13/2012. A total of 130 plants were planted in both plots and covered by tree wrap using Velcro to protect from rodent attach temporarily. As some plants did not survive and more plants continued to die, 32 more plants in plot P1 and 48 more plants in plot P2 were planted on 9/21/2012. On 11/2/2012, plastic spiral wraps were wrapped on the base of all trees to protect from rodents. Finally, the dying of plants from rodent attack was arrested. On 6/6/2013, about 85 plants in two fields (12 in P1 and 73 in P2) were planted to replace those that did not survive during the 2012 fall. The plants then did very well with survival rate of more than 95%.

Figure 3-11 Rodent attack to bottom of plant. Barks were eaten by rodents and plants ultimately died

3.2.5. Wastewater

The characteristics of the wastewater on the annual mean basis are given in Figure 3-12. The site had test wells at several places for ground water sampling. The application rate according to the processor was 20.3-38 cm per acre per year. In 2013, 458 mm (18 inches) wastewater was applied from April to November.

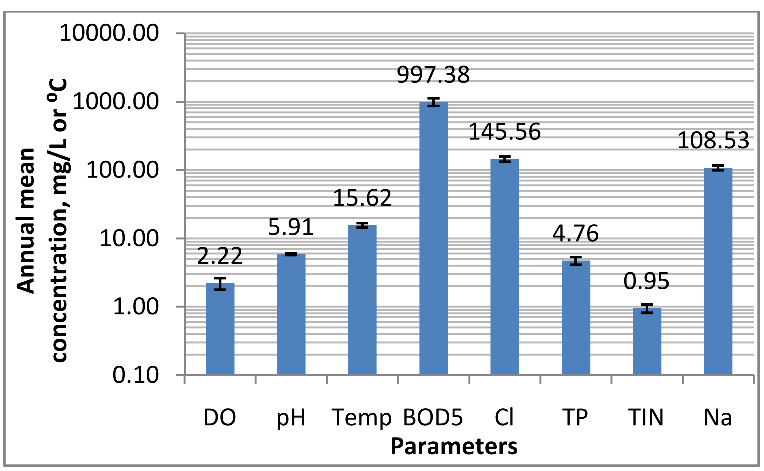


Figure 3-12 Mean annual wastewater characteristics of food processing wastewater in 2013

The synthetic wastewater used in the large column experiment had high potassium compared to high sodium in the actual food processing wastewater and had low nitrate but high ammonium compared to high inorganic nitrogen in the actual food processing wastewater.

3.2.6. Instrumentation

Layout of the instrumentation is shown in Figure 3-10. Each half of the field is instrumented with the following.

1. Solar powered CR 1000 datalogger with radiotransmitter located between boundary of P1 and C1 (or between boundary of P2 and C2) (Figure 3-13).

Figure 3-13 A station showing solar panel, datalogger, cables and battery.

2. Temperature sensor below 2.5 cm from surface of the soil and close to datalogger.
3. Raingage, 152 cm away from datalogger and 122 cm above ground surface.
4. Time domain reflectometry (CS616) sensors installed at 18.3 m away from the datalogger on both sides and 24.4 m away from the datalogger along the length of plot and 6.1 m towards center of pivot. At each location, these moisture sensors were installed at 46 cm, 91 cm and 122 cm below the surface. A total of 6 TDR moisture sensors were installed in each plot. Soil was dug with bore-hole digger and TDR

sensors were inserted vertically in staggered pattern as shown in Figures 3-14. After installation, the soil was carefully packed back in the same order as it was taken out.

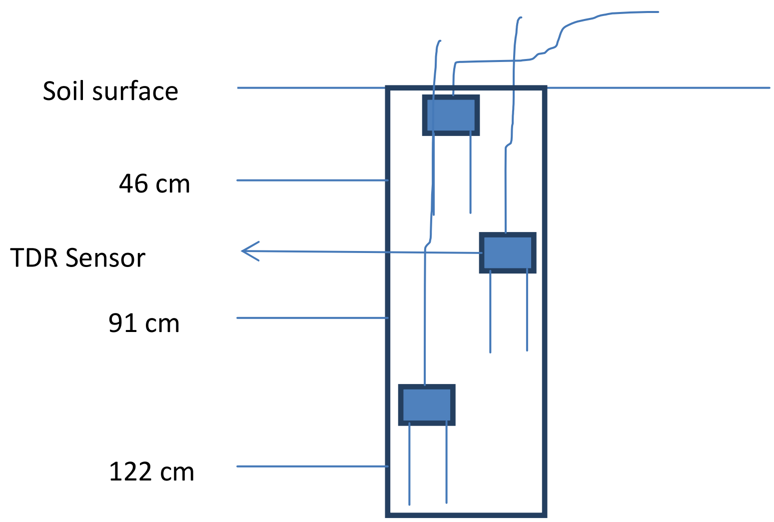


Figure 3-14 Layout of installation of TDR soil moisture sensors along the depth of the soil

5. Two enviroscan sensors at 24.3 m away on each side of the datalogger. Each enviroscan sensor consisted of probes placed at the depths of 46 cm, 91 cm, 122 cm and 213 cm below ground surface [Figure 3-15 to 3-16]. SenteK capacitance probes (Enviroscan sensors) have been used to monitor soil water continuously across the depths both in laboratory and field since more than a decade ago (Paltineanu & Starr, 1997, Starr & Paltineanu, 1998). For installation, a 5 cm diameter and 213 cm deep hole was dug using auger truck in the field.

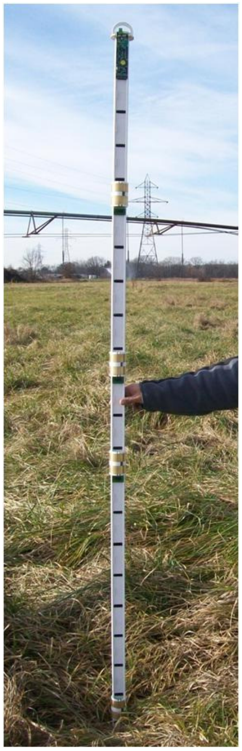


Figure 3-15 A 2m long enviroscan sensor ready to be installed. The sensor consisted of 4 probes positioned as shown.

Figure 3-16 Installed enviroscan sensor.

6. Two G2 draingages at 21.3 m away from datalogger along the length on either side installed below 213 cm from ground surface. The draingages measure drainage rate as

well as collect leachate samples. Approximately 4.27 m deep and 0.25 m wide hole was dug using well-truck. Draingages were installed on 12/23/2011 using the intact monolith method as described below [Figures 3-17 to 3-19].

1) About 1 m of top soil was removed so that vertical profile underneath was not changing.
2) Divergence control tube (DCT) was fully driven into the soil using sledge hammer by striking repeatedly.
3) Soil outside the DCT was removed using shovel to separate DCT.
4) Using rope, the DCT with intact soil inside was lifted up to the surface.
5) A 4.27 m deep hole with diameter of 0.25 m from surface to 3.05 m and diameter of 0.1 m from 3.05-4.27 m was dug using well truck.
6) The bottom 0.15-0.2 m of the hole was filled with gravel (3-5 mm size).
7) Wick section was covered by protective PVC pipe and diatomaceous earth on top. Then, DCT was carefully placed over wick section.
8) Using rope, the whole setup including the DCT and wick section was lowered until the wick section was at the bottom of the hole.
9) The soil was backfilled in the order it was dug out including grass layer at top.

Thus, the draingages were installed with undisturbed samples in the DCT. Minimal soil disturbance occurred while backfilling the top of DCT.

Figure 3-17 Driving the divergence control tube into the ground using sledge hammer and wood piece

Figure 3-18 Divergence control tubes containing undisturbed soil core of the subsurface.

Figure 3-19 The installed site after installation. Two tube, clear and blue, for water sampling and a cable for signal transmission come out of the draingage.

7. Six lysimeters installed at 91 cm (2 per plot) and 189 cm (1 per plot) below the ground surface. Lysimeters enable collection of leachate water from pores of unsaturated soil. A 5 cm diameter and 122 cm or 213 cm deep hole was dug using the auger. For deep lysimeters (183 cm below ground surface), lysimeter was inserted after filling the bottom with 5 cm deep layer of bentonite and 5 cm of silica. The sampling tubes of lysimeters were brought up to the surface using the pvc pipe of 12.7 mm diameter. After insertion of lysimeters, play sand and bentonite tablets were added to fill up 86.3 mm depth and 30.5 cm, respectively. The remaining depth (30.5-61 cm) was filled with sand and site soil. Similarly, shallow lysimeter (at 91 cm below ground surface) had 5 cm of bentonite, 5 cm of silica, lysimeter, 50 cm of sand slurry, 30.5 cm of bentonite and 30.5 cm of sand and site soil mix from bottom to top [Figure 3-20 to 3-21].

Figure 3-20 Installation of lysimeter. A layer of bentonite and silica sand was inserted in the hole before inserting lysimeter.

Figure 3-21 Installed lysimeters, one deep and one shallow. The clear and black tubes are for pulling sample out of ground.

The cables connecting sensors to the data logger were over the ground. On 6/23/2012, cables were buried in a trench about 15 cm deep using a trencher to protect from rodent attack. However, it was difficult to repair the buried cables and rodents still chewed the cables. Therefore, on 5/17/2013, they were inserted in PVC conduits.

Soil temperature, rainfall and drainage were measured using temperature probes, raingage and draingage, respectively. Soil moisture was monitored with enviroscan probes and TDR sensors.

The location of soil moisture sensors was such that variation of soil moisture along and across pivot measurement was possible. All the data were recorded in two solar-powered dataloggers at scan interval of 60 seconds, collection rate of 30 min and collected in computer or transmitted to MSU using radiotransmitters weekly.

The details of the sensors are given below in table 3-2.

Table 3-2 Detailed specifications of instruments and sensors used in the field. CS 616 and 107L were also used in large-scale column experiment.

Measurand/ Function	Sensor/ instrument	Vendor	Model	Specifications	Remarks
Soil Moisture	TDR sensor	Campbell Scientific	CS 616	Resolution= 0.1% VWC Precision>0.1% VWC Accuracy= ±2.5% VWC Power supply= 5 to 18 Vdc Operation temperature= 0 to 70 °C Probe to probe variability= ±0.5% VWC	Time Domain Reflectometry, change in dielectric constant/impedance
Soil Moisture	EnviroSCAN sensor	Sentek Technologies	Enviroscan-20-W-cq4-BC-EB-EA	Resolution= 0.1 mm soil moisture Reading range=0-65% Temperature effect = ±3% at 5 to 35 °C Operating temperature range= -20 °C to 35 °C Time to read one sensor = 1.1s Sphere of influence= 10 cm, covers 99% Probe length = 50 cm (20") Sensor diameter= 50.5 mm Access tube diameter= 56.5 mm	Principle based on high frequency capacitance
Rainfall	Texas electronics raingage	Campbell Scientific	TE525-L50	Temperature 0° to 50 ° C Resolution=1 tip Volume per tip=4.73 ml/tip or 0.01 in or 0.254 mm Accuracy= ±1% in 1 in/hr Funnel diameter= 15.4 cm, 6.06"	Tipping bucket/magnetic reed switch
Soil temperature	Temperature Sensor	Campbell Scientific	107 L	Range= -50 ° C to 100° Tolerance= ±0.2° C Linearization error = <0.5° C	Thermistor 100K6A1lA
Data recording	Datalogger	Campbell Scientific	CR 1000	Scan rate= 100 Hz Analog input = 8 differential, 16 single ended Digital port= 8 I/os or 4 RS-232 COM Input voltage range = ±5 Vdc Power requirement= 9.6 to 16 Vdc Temperature range = -25° C to 50° C Final storage = 4 MB	

Table 3-2 (cont'd)

Measurand/ Function	Sensor/ instrument	Vendor	Model	Specifications	Remarks
Drainage, water sample	Draingage	Decagon Devices	G2 Passive capillary lysimeter	Measurement time= 10 ms Diameter of DCT= 20 cm Drainage resolution= ± 0.1 mm Operating temperature= 0 to 50 ° C Volume per siphon event= 31 Cm3 or 1 mm of drainage Sample collection reservoir volume = 150 mL Output= mV Power required = 2.5 to 5 Vdc at 3 mA	Passive draingage
Water sample	Lysimeter	Soil Measurements Systems	SW 071	Porous steel length= 3.7 in Outside diameter= 2 in Storage volume=260 ml	Suction lysimeters, suction provided by vacuum pump

3.2.7. Sampling and analysis

Leachate water samples were collected from lysimeters and draingages every two weeks from May to November, 2013. Lysimeters yielded insufficient water on many occasions. Water samples could not be collected in multiple lysimeters due to low available moisture in the soil, which resulted from low rates of wastewater application and low rainfall. The sampling tube in the draingages either was clogged or was broken inside the soil and samples could not be collected from most draingages on most sampling events. On the hind sight, it would have been better to put the sampling tubes of draingages in the pvc tube or protective tubing. When water samples were available after analysis of COD, pH, anions and cations, water samples were stored at refrigerator for metals analysis after filtering and acidifying it to $pH<2$ using concentrated nitric acid.

pH was measured either immediately at site or immediately after bringing to laboratory (maximum of 4 hours). The water samples were carried in cooler at approximately $4^{o}C$ from the field to the laboratory. Water samples were analyzed for COD, anions (F, Br, Cl, SO_4, NO_3, PO_4), cations (Li, K, NH_4, Ca, Na, Mg) within few hours of collection.

In October 2014, triplicate samples of plants and soil from each plot were collected for analysis. Each plant sample was separated into root, leaves and stems and analyzed for arsenic, total nitrogen, iron and manganese. Soil samples were taken from 0-0.4 m at each sampling location as a composite sample. Soil samples were taken from rhizosphere of poplar trees in the planted plots approximately 0.3 m away from tree trunk. A soil sample from each plot was analyzed for major nutrients, duplicate sample from each plot for arsenic and triplicate samples for iron, manganese and total nitrogen. Triplicate soil samples from each plot were analyzed for phospholipid linked fatty acid.

The details of sample analyses are discussed in section 3.4.

3.2.8. Data collection and analysis

The data collected from the datalogger was processed by removing any zero or unreasonable values that were recorded due to power issues of the battery. Only months from May to November was considered due to unreliability of raingage during snow events and no-application of wastewater in the field during the winter. The data was compared between planted and control plots at different levels for each variable (moisture, drainage, temperature): mean daily data for whole duration and mean data for whole duration.

Research assessed evapotranspiration by the poplars by conducting a hydrologic balance on each plot on a monthly basis where the evapotranspiration volume was equivalent to the total volume of water added (irrigated + precipitation) less the volume leached, as runoff was zero in the leveled grassed field. Rainfall data was taken from a MSU Enviroweather station, located approximately 8 Km away from the field site and wastewater application was provided by the factory on the monthly basis. The precipitation data from raingage at the site was not used due to frequent clogging of raingage. The deep flow was measured by four decagon draingages placed below 183 cm from ground surface. The evapotranspiration in the planted field was divided by that in the control field to calculate crop factor for poplars.

For each treatment, the efficiency of treatment of carbon was calculated using the influent BOD data provided by the factory and the leachate COD data from the research. As COD is mostly greater than BOD, it under-predicts the efficiency of treatment. However, because COD to BOD ratio is close to 1 for food processing wastewater, the ratio of influent BOD to leachate COD can be used to approximate treatment efficiency.

Total nitrogen in soil and plant and ammonium and nitrate in leachate water were used to account nitrogen transformation processes. Uptake of total nitrogen by poplar and its influence on nitrate mobilization and pollution of groundwater was then evaluated.

The efficiency of treatment or effluent COD of planted plots were compared to that of control plots using one way ANOVA. Similar computation and analysis was done for other water quality parameters such as pH, nitrate, phosphate, sulfate, iron, manganese, ammonium and total nitrogen.

The sum of all fatty acids represented the total biomass of the soil that was compared between control and planted plots. The results of the PLFA analysis were also used to conduct agglomerative hierarchial clustering based on UPGMA (unweighted pair group averaging) method using euclidean distances of standardized data. Relationships among the treatments were explored. The dendrogram was based on the relative presence of each type of microorganisms in the planted and control soils.

3.3. Analytical methods

The following section contains the sample analysis methods used in the experiment in each year of the large-scale column experiment and field experiment.

3.3.1. pH

pH was measured using pH meter (Denver instruments UB-10 or Oakton pH/CON 300 series). The accuracy of the measurement was ±0.01 units. Before measurement, a three point calibration was done each time using pH standard of 4.0, 7.0 and 10.0. The reading was considered stable after the drift was slower than 0.01 per minute.

3.3.2. Chemical oxygen demand

Carbon treatment by poplars was evaluated by analyzing COD in the influent wastewater and leachate water on a 1-2 week basis. COD was measured by USEPA approved reactor digestion HACH method 8000 using the high range (0-1500 mg/L). The samples were analyzed within 2 hours of sampling. For quality assurance, at least a duplicate and standards were run on each batch of 16 samples. The standard (800 mg/L) within ±2% was considered acceptable.

3.3.3. Anions analysis

Anions, specifically nitrate, phosphate, fluoride, bromide, chloride and sulfate, analysis in the leachate water was performed using ion chromatography (Dionex ICS 5000). Research passed 4.5 mM sodium carbonate-1.4 mM sodium bicarbonate eluent at the flow rate of 1.2 mL/min through AG22 guard column and AS22 analytical column for separation of analytes, ASRS suppressor for enhancement of signals of analytes and conductivity detector for detection. The injection volume was 25 µL. External calibration standards with at least 5 levels were used to make linear calibration curve and quantify the amount of anions in the leachate water. The calibration curve had coefficient of correlation greater than 99.5% and relative standard deviation less than 10%. For quality analysis, each batch of 16 samples consisted of blank, replicates and standards.

3.3.4. Cations analysis

Cations such as sodium, lithium, ammonium, potassium, calcium and magnesium in samples were analyzed using ion chromatography (Dionex ICS 5000). The analytes were separated by pumping sample along 20 mM methanosulphonic acid eluent at the rate of 1 ml/min flow through CG12 guard column and CS12 analytical column, CSRS suppressor and detected using conductivity detector. The injection volume used was 25 µL. External calibration standards with at least 5 levels were used to make linear calibration curves and quantify the amount of cations in samples. Calibration curves had coefficient of correlation greater than 99.5% and relative standard deviations less than 10%. For quality analysis, each batch of 16 samples consisted of blank, replicates and standards.

3.3.5. Transition metal analysis

Transition metals, including manganese, iron II and iron III in samples were monitored throughout the experimental period every 2-week. These metals were analyzed using ion chromatography (Dionex ICS 5000). IC with UV-Vis and 50 µL sample injection had better detection limit for transition metals than AAS and ICP-MS (Fredrikson et al., 2002).

Before analysis, IC solutions and eluents were degassed using vacuum pump and sodium sulfite solution was passed through the column for ½ hour to remove oxygen that could oxidize metals. For analysis, analytes were separated using 2,4-pyridine dicarboxylic acid (PDCA) eluent at a flow rate of 1.2 mL/min, Ionpac CG5A guard column and Ionpac CS5A analytical column. The method used 8 mL autosampler syringe assembly to inject 4000 μL of sample and concentrator column TCC-LP1 to concentrate analytes and achieved as low as 1 μg/L detection limit on trace metals. The eluent with analytes was then mixed with 4-(2-pyridylazo) resorcinol (PAR) as a post column reagent, flowing at 0.6 ml/min, in knitted reaction coil and detected using a UV-Vis detector at a wavelength of 530 nm.

External linear calibration curves consisting of at least 5 calibration standards were used to quantify the metals in the samples. The calibration curve had coefficient of correlation greater than 99.0% and relative standard deviation less than 15%. For quality analysis, each batch consisted of blank, replicates and standards. Because the samples may have been oxidized during preparation and sampling due to exposure to oxygen, iron II and iron III were summed to get total iron. Straight calibration curves with high correlation coefficient and low relative standard deviation confirmed that the standards were not oxidized during preparation or running in IC.

3.3.6. Total nitrogen analysis

Total nitrogen was extracted using persulfate digestion method 4500-N C (Clesceri et al., 1998) and analyzed as nitrate in IC. The persulfate method determines total nitrogen by alkaline oxidation of all organic and inorganic nitrogenous compounds to nitrate at 100-110 ^{o} C. This method can also determine organic nitrogen if ammonia, nitrate and nitrite are determined individually.

Digestion reagent was prepared by dissolving 20.1 g low nitrogen potassium persulfate ($K_2S_2O_8$) and 3 g sodium hydroxide (NaOH) in 1000 mL e-pure water. Borate buffer was prepared by dissolving 61.8 g boric acid and 8 g NaOH in 1000 mL water. Digestion reagent (5 mL) was added to samples or standard solution (10 mL) in glass vials. Vials were capped tightly, mixed by inverting several times and heated at 110 ^{0}C in an autoclave for 30 min. The digested solution was allowed to cool and 1 mL of the borate buffer was added, mixed and filtered into a 10 mL IC vial using 45 uM cellulose acetate filter. At all times, the standard and sample followed the same dilution ratio (i.e. digestion reagent: sample: borate buffer = 10:5:1). The analysis of nitrate in the digested mixture followed nitrate analysis as described above in anion analysis.

3.3.7. Soil and plant analysis

Soil and plant analysis was performed by the Plant and Soil Nutrient Laboratory, MSU. Soil and plant samples were analyzed for nutrients and metal concentrations using the recommended methods for North Central Region for soil analysis (Brown, 1998).

1. pH and Lime Requirement: pp. 13-16.
2. Phosphorus: pp. 21-30. BrayP1 by ascorbic acid was the standard test used. On calcareous soils the Olsen P test was used.
3. Potassium and calcium by flame emission and magnesium colorimetrically: pp. 31-34.
4. Zinc, Manganese, Iron and Copper by atomic absorption spectrophotometry: pp. 41-44.
5. Soil Organic Matter by loss-on-ignition: pp. 57-58.
6. Soil Salinity by 1:1 method: pp. 59-60.

In addition, manganese and iron were analyzed by acid digestion and atomic adsorption spectrometry and soil nitrogen was analyzed through micro-Kjeldahl digestion. Moreover, soils were analyzed for nitrate through cadmium reduction (Huffman & Barbarick, 1981), ammonium through the salicyclate method (Nelson, 1983) and total nitrogen through the micro-Kjeldahl digestion (Bradstreet, 1965). Plant tissues samples were analyzed by the Missouri Science and

Technology laboratory for arsenic, iron and manganese using acid-digestion EPA 3051 method (EPA, 1994) and analyzed on ICPMS.

3.3.8. Microbial analysis

The microbial biomass and community structure at the end of the experiment was assessed by phospholipid linked fatty acid (PLFA) using gas chromatography (GC) and gas chromatograph-mass spectrometry (GC-MS). Extraction of lipid from soil was performed by using the mixture of chloroform:methanol:phosphate buffer solution at 1:2:0.8 v/v/v, silicic acid chromatography to separate neutral, glycol and phospholipids and alkaline methylation to produce phospholipid linked fatty acid (PLFA) before analyzing in gas chromatograph (Kaur et al., 2005).

The PLFA extraction was conducted using the method developed by Bligh and Dyer (1959) and modified by White et *al*. 1979 and Forstegard et *al*. 1991. Briefly, 5 g of moist sample was kept in a test tube with Teflon lined screw caps and 5 ml of chloroform, 10 ml of anhydrous methanol and 4 mL of 50 mM phosphate buffer were added. Another sub-sample of soil was used for moisture determination. The test tube was centrifuged at 2500 rpm for 20 min and rested for 2 hours. Then, 5 mL of chloroform and 5 mL of buffer were added, shaken well and kept overnight. The next day, the solution in the test tube was mixed and vacuum-filtered using Whatman filter paper (number 2) into culture tubes. The solution was allowed to separate and the top aqueous phase was decanted. The organic phase was dried under nitrogen gas. The dried lipid was re-dissolved in 4×150 µL of chloroform and transferred to silicic acid column (Bond Elut 500 mg SI cartridge-Agilent) that had been pre-conditioned with 3 mL of chloroform. The silicic acid column was eluted with 5 mL of chloroform to remove neutral lipid and 5 mL of acetone to remove glycolipids. Polar phospholipids were eluted with 5 mL of methanol, collected in another culture tube and dried under nitrogen gas. For alkaline methanolysis, 1 mL of methanol:toluene (1:1 v/v) mixture and 1 mL of 0.2 M KOH was added, the mixture heated at 37°C in a incubator for 15 min and allowed to cool to room temperature. Chloroform (2 mL) and water (2 mL) was added, mixed vigorously and dried under nitrogen. Finally, phospholipid linked FAMEs or PLFAs were re-suspended in 2×250 µL hexane, 40 µL internal standard (methal non-adecanoate, 500 µg/mL) was added and the solution was kept in 2 mL GC vials. The extraction consisted of blank and replicate for quality control.

The FAMEs were analyzed and quantified in Shimadzu gas chromatograph (GC2010) equipped with flame ionization detector (FID). Helium gas at 35 cm/sec velocity was used as carrier gas. The columns temperature program started at 150°C for 4 min, ramped up to 250°C at 4°C/min and remained at 250°C for 10 minutes. The injection of 1 µL sample was carried out in splitless mode. The column used was Supelco MXT-Wax column (30 m length, 0.53 m diameter).

Quantification of FAMEs was based on equal detector response i.e. 14-carbon saturated methyl ester and 20-carbon methyl ester are assumed to give the same integer count per nanogram (Ringelberg et al., 1989, Findlay & Dobbs, 1993). External calibration curve using methyl non-adecanoate standards was made with 9 calibration levels. The calibration curve was linear with intercept of zero and coefficient of determination of 0.998 and standard error of less than 10%. The peaks were identified by comparing the retention times of commercial standards, specifically, Bacterial acid methyl ester mix (BAME mix-Sigma Aldrich) and 37 component FAME mix (Supelco) (Zunino & Zygadlo, 2005, Li et al., 2010). Peaks identification was confirmed by running the selected samples and standards in 5973 MS connected to Agilent 6890 GC and verifying spectral signature of each fatty acid by comparing with National Institute of

Standards and Technology mass spectral library. GCMS used the same temperature program, a similar column (DB WAXETR column, 30 mm length and 0.25 mm diameter) and 70 eV energy.

The nomenclature of fatty acid followed pattern of Ca:bwd, where a= number of carbon atoms, b= number of double bonds and d=position of double bond from aliphatic end. The prefix i and a refer to iso and anteiso methyl branching. The prefix –OH refer to hydroxyl group. The suffix cy refer to cyclic fatty acids. The suffix c or t refer to cis or trans isomers.

CHAPTER 4: RESULTS AND DISCUSSION

4.1. Small-scale column experiment[2]

Following were the objectives of the small-scale column experiment.

1 Determine the inhibitory effects of food processing wastewater application on poplar growth

2 Quantify evapotranspiration of poplar trees under experimental conditions

3 Evaluate treatment of chemical oxygen demand (COD) in wastewater by poplar trees during food processing wastewater application

4 Investigate the effects of poplar trees on the fate of metals and nitrate during application of food processing wastewater in soils.

4.1.1. Vegetative growth

Phytoremediation relies on robust growth of plants; therefore, the vegetative growth of the poplars receiving food processing wastewater was evaluated. At the beginning of the experiment, the lengths of roots and shoots of the seedlings were not statistically different ($p=0.20$ for shoots and 0.31 for roots) for each treatment. Continuous application of wastewater did not hinder the above-ground growth and development of poplars; however, impacts were observed in root growth. Qualitatively, trees receiving wastewater appeared similar to control trees receiving water, with robust growth and green leaves.

Figure 4-1 shows number of leaves and tree height for all trees during wastewater or water application. At the conclusion of the experiment, trees receiving wastewater were quantitatively

[2] "Section 4.1 reprinted from Publication Ecological Engineering, Vol n/a, Niroj Aryal, Dawn M. Reinhold, **Reduction of metal leaching by poplars during soil treatment of wastewaters: Small-scale proof of concept studies**, Pages No. n/a, Copyright (2014), with permission from Elsevier

similar in height and number of leaves to trees receiving water (p=0.38 for height and 0.50 for leaves, Figure 4-1). Additionally, the measured increases in shoot lengths were statistically similar for both trees receiving wastewater (105.9±10.2 cm) and trees receiving water (107.8±7.9 cm) after four months of wastewater application. The numbers of shoots (3.4 per tree receiving water and 3.6 per tree receiving wastewater) were also independent of wastewater application.

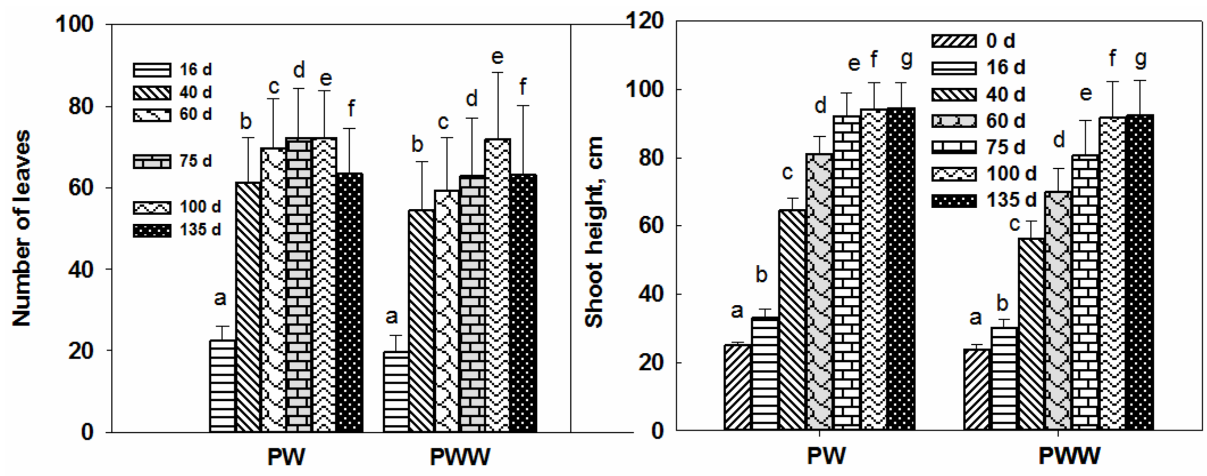


Figure 4-1 Comparison of number of leaves (left) and shoot height (right) with time. Wastewater application was started on day 16. Different letters indicate statistical difference between trees receiving water (PW) and trees receiving wastewater (PWW) on particular day. Error bars indicate standard error of the mean

The total above-ground biomass collected when the columns were sacrificed also indicated that wastewater application did not impede poplar tree growth. Above-ground biomass was equally divided into shoots and leaves; overall, above-ground dry biomass accounted for 74 – 96% of total biomass. Leaf biomass may have been underestimated as some leaves fell from the trees due to onset of winter during the final days of experimentation. While there was not a difference between dry masses of leaves and stems receiving water or wastewater (p=0.20 for leaves and 0.48 for stems), the observed root mass of trees receiving wastewater was significantly less than that of trees receiving water. This was consistent with a substantial, yet insignficant, decrease in root length for trees receiving wastewater (36.7±6.1 cm vs. 52.1±5.7 cm, p=0.10). Additionally, visual observations indicated that the roots were less developed in columns that received wastewater; this observation was verified by a signficant decrease in the number of branches in the root systems of trees that had received wastewater as compared to trees that had received only water. Root growth could have been hindered due to toxicity; however, as no toxicity was observed above ground, a more likely hypothesis is that the availability of surplus nutrients due to wastewater application decreased the need for extensive root structures.

4.1.2. Evaporation and crop coefficient

While a slight decrease in evapotranspiration was observed for trees receiving wastewater (as compared to water), evapotranspiration by poplar trees receiving wastewater still greatly exceeded evaporation observed in no-tree controls [Figure 4-2]. The small decrease in evapotranspiration by trees receiving wastewater was unexpected, as there was no decrease in number or mass of leaves due to wastewater application. While quantitative measurements were not taken, there was not a noticeable decrease in leaf size due to wastewater application; therefore, number or mass of leaves can serve as proxies for leaf area – a key predictor of evapotranspiration rate. Given no difference in number of leaves, the decrease in evapotranspiration due to wastewater application may relate to the decrease in root biomass observed for trees receiving wastewater. In trees receiving wastewater, wastewater could have bypassed a smaller rhizosphere, thereby decreasing the water available for evapotranspiration. The weekly evapotranspiration coefficients, calculated as the volume of water evapotranspired from planted columns divided by the volume of water evaporated from unplanted columns, for trees receiving water during the study months ranged from 1.24 – 6.70, while the weekly evapotranspiration coefficients for trees receiving wastewater ranged from 1.14 – 5.15. For the duration of the experiment, the average weekly evapotranspiration coefficients for poplars receiving water and wastewater were 4.04±0.89 and 3.25±0.31, respectively (p= 0.001). As expected, evapotranspiration coefficients peaked during summer months. The observed evapotranspiration coefficients were similar to the crop coefficients observed for poplar trees planted in Italy as part of a vegetative filter strip, which ranged from 1.06 to 4.25 (Guidi et al., 2008); however, the variability was substantially lower, which is expected for small-scale column studies.

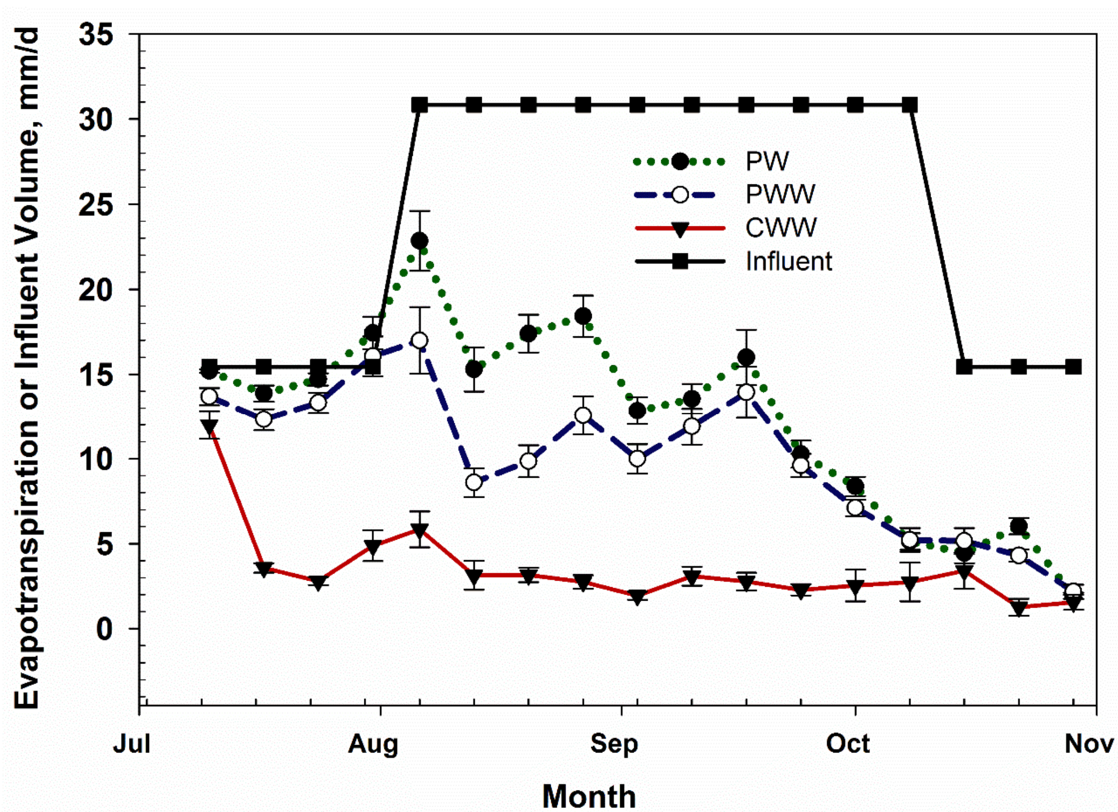


Figure 4-2 Weekly evaporation or evapotranspiration on a daily basis at two loading rates (15.4 mm/d and 30.8 mm/d). Influent volume of water is provided as a reference. Error bars indicate standard error of the mean.

4.1.3. pH

The wastewater that was applied to the columns was slightly acidic, with a pH of 5.34±0.96. Transport of the wastewater through the columns significantly increased pH; however, leachate produced from columns with poplar trees was slightly but significantly higher (7.85±0.50) than leachate produced from columns without trees (7.41±0.41) ($p<0.001$). Generally, metals form mineral phosphates and carbonates at high pHs and exist as ions or soluble organometals at low pHs (Twiss et al., 2001, Rensing & Maier, 2003). Consequently, metals such as iron and mangenese are more soluble at lower pHs (Cappuyns & Swennen, 2008). Therefore, the increase in pH due to poplar tree growth may have beneficially impacted mobility of iron and mangenese in the columns. Iron oxides are least soluble at pH of 7.5-8.5 (Colombo et al., 2014). Leachate pH-values were within the range specified by the EPA to have no impact on groundwater (pH 6.8-8.6). Therefore, while the pH results demonstrate the influence of trees on the system, they are in themselves not a reason to promote the use of poplar plantations for the treatment of food processing wastewaters. Continuous addition of wastewater to the soil for four months did not change soil pH considerably. Soil pH changed from 6.70±0.10 in the initial soil to 6.63±0.27 in columns with trees and 6.77±0.13 in columns without trees after four months of wastewater application.

4.1.4. Carbon

All columns efficiently removed COD from the influent wastewater [Figure 4-3]. High variability in COD was attributed to both the initial wastewater characteristics and the length of storage. A variety of vegetables and fruits were processed at the facility during the experiment; therefore, the initial wastewater COD varied. Additionally, length of wastewater storage in the lagoon and laboratory varied.   The observed variability was actually desired in this study, as it more closely represents field operation of land application sites. For example, wastewater may be stored in the lagoon for lengthy periods if weather is not conducive to land application.

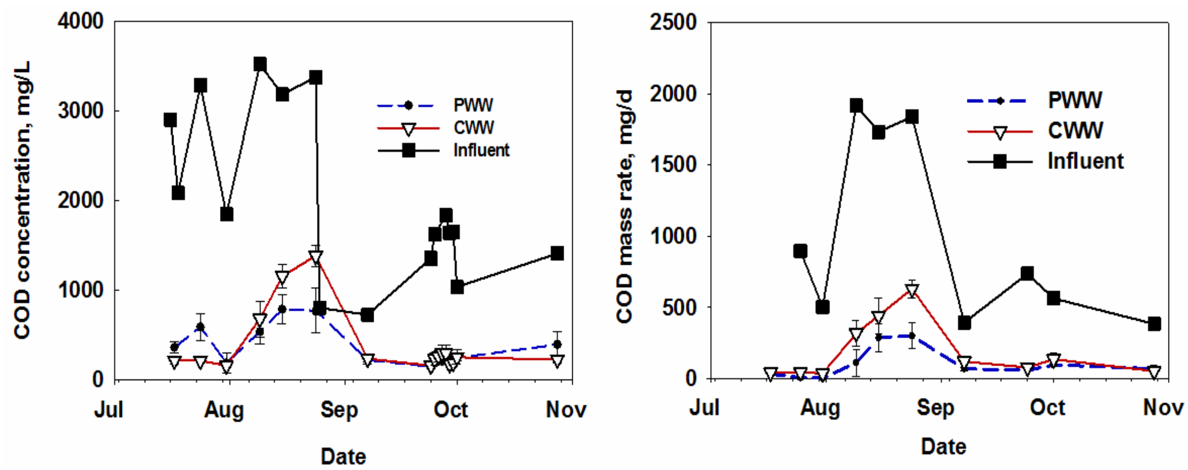


Figure 4-3 Concentration and mass rate of chemical oxygen demand (COD) in the influent and leachate. Error bars indicate standard error of the mean.

Concentrations of COD in the leachate were not significantly different for columns with and without poplar trees when the entire duration of the experiment is considered (p=0.24), but were significantly lower for columns with trees during the higher rate of application (p=0.04). On a concentration basis, 82.12±1.73% of the influent COD was removed in planted columns. Additionally, mass removal of COD was significantly higher in columns with trees (p=0.02) for the entire duration of the experiment. On mass rate basis, 88.93±2.48% of COD was removed in planted columns, as compared to 81.41±3.48% in soil-only columns. Decreased mass of COD in the leachate could be attributed to either increased carbon storage or increased microbial degradation of carbon. Soil organic matter at the end of the experiment was not significantly different for columns without trees (3.47±0.13 mg/g) and with trees (3.23±0.07 mg/g, p=0.07).

Therefore, the additional removal of 9.2% COD in the planted columns supports the hypothesis that poplar trees stimulated microbial degradation of carbon in the wastewater.

4.1.5. Nitrate

Initially, application of wastewater to columns without trees produced leachate with high concentrations of nitrate, while concentrations of nitrate in the leachate from columns with trees were similar to concentrations of nitrate in the influent wastewater [Figure 4-4]. Soil concentrations of nitrate and total nitrogen at the conclusion of the experiment were statistically similar for columns with and without poplar trees Production of nitrate in the unplanted columns corresponded with removal of ammonia from the influent wastewater. Conseqently, nitrate produced in unplanted columns was likely due to nitrification of ammonium [Figure 4-4] and possibly organic nitrogen, by nitrifying bacteria, which only occurs under aerobic condtions. Nitrate concentration in leachate from soil-only control columns decreased with time, while ammonium concentrations in leachate from control columns increased with time – two indications that the redox conditions in the columns were becoming more reducing, as high ammonium and low nitrate are typical for reduced systems (Dornbush et al., 1976).

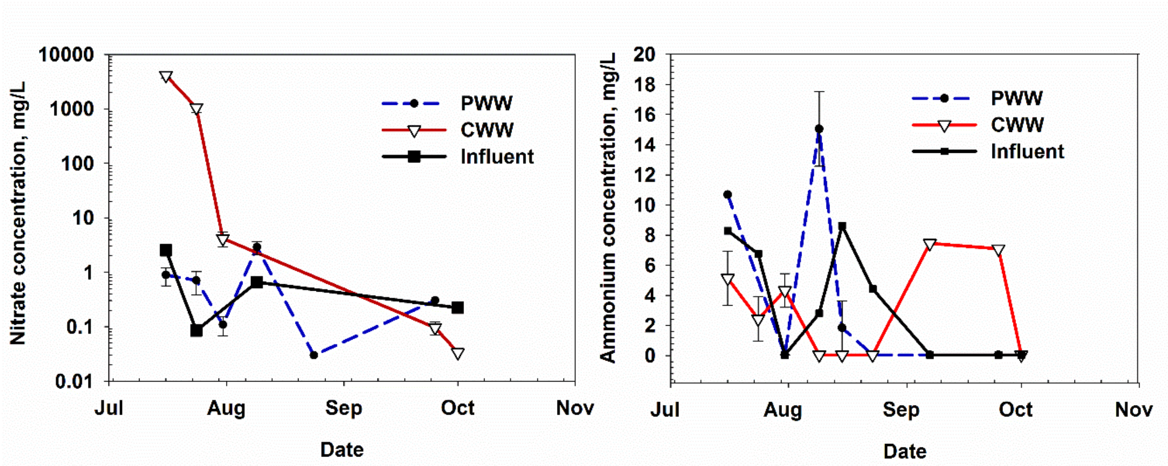


Figure 4-4 Nitrate (left) and ammonium (right) concentration in the leachate water and influent with time. Error bars represent the standard error of the mean.

In contrast to the control columns, similar concentrations of nitrate were measured in the influent and leachate samples collected from planted columns. Therefore, it is likely that either (1) uptake of nitrate by poplars likely prevented nitrate contamination of the leachate or (2) poplar trees affected the redox potential of the soil, preventing formation of denitrifying conditions. Observations of high ammonium concentrations in the leachate from planted columns (Figure 4) indicated that, for at least two time-points, ammonium was produced in the planted columns, suggesting the existence of reducing conditions. However, ammonium was not detected in the leachate of planted columns for much of the experiment. Consequently, both uptake of nitrate and the effects of poplar trees on soil redox may be responsible for the lower concentrations of nitrate observed in the planted columns. The nitrate concentrations in the leachate collected from planted columns was always less than the regulatory 10 mg/L limit set by EPA; in contrast, initial concentrations in leachate from unplanted columns exceeded the EPA limit by two orders of magnitude. Similar results were obtained by Mankin et al. 2010 who found that overall leaching of nitrogen was reduced by poplar growth on abandoned lagoon soils (Douglas-Mankin et al., 2010).

4.1.6. Leaching of metals

Iron and manganese are both important electron acceptors in soils systems. Even in the presence of nitrate, iron and manganese can be reduced (Charlatchka & Cambier, 2000, Dassonville & Renault, 2002, Cervantes et al., 2011). Manganese undergoes reduction from Mn^{+4} to Mn^{+2} under moderatelty reducing conditions or at the reduction potential of 100 to 300 mV (DeLaune & Reddy, 2005). In all experimental columns that received wastewater, manganese concentration increased at the onset of doubling the wastewater application and then decreased [Figure 4-5]. The observed trend was at least in part due to the variability in influent concentrations, as the peak in effluent concentrations for unplanted columns followed the peak in influent coentrations. Given this observation, it is difficult to determine the role of redox conditions in the effluent concentrations of managese. While manganese concentrations in the leachate from planted columns appear to be lower than those in the leachate from unplanted columns, the difference is not statistically significant ($p=0.07$). However, mass of manganese leached from the planted columns receiving wastewater was subsantially less than that leached from unplanted columns (0.37 vs. 2.55 mg, respectively). No difference in soil concentration of manganese was observed between columns with and without trees at the conclusion of the experiment ($p=0.48$); however, as the initial mass of manganese in the soil was roughly more than 180 g, differences due the manganese leached or uptake is likely within the standard error of the soil analysis. Most of the manganese concentrations observed in leachate from columns without trees exceeded 55 µg/L, EPA recommended maximum level in drinking water (EPA, 2007). In contrast, concentrations of manganese in columns with poplar trees were always less than 55 μg/L.

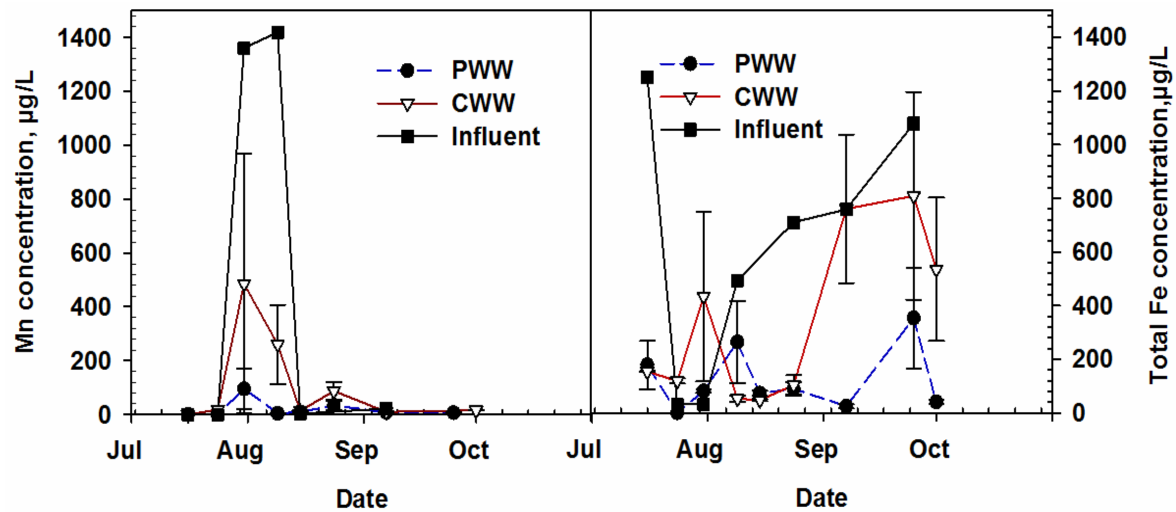


Figure 4-5 Manganese (left) and total iron (right) concentrations in the leachate water with time. Error bars indicate standard error of the mean.

Iron concentration in the influent wastewater generally increased with time (Figure 5). This likely reflected the variable characteristics of the wastewater. Concentrations of iron were generally less in the leachate from the columns than in the influent wastewater, with higher concentrations frequently observed in leachate from columns without trees. The concentrations of iron in the wastewater and leachate from unplanted columns frequently exceeded the EPA recommended level of total iron in drinking water (300 μg/L) (EPA, 2007). However, concentrations of iron in the leachate from planted columns only exceeded the recommended level once. No net soil loss of iron was observed in planted columns or unplanted columns, as indicated by statistically similar soil concentrations of iron at the conclusion of the experiment (p=0.88); however, as the initial mass of iron in the soil was roughly more than 1 kg, differences due the iron leached or uptaken is likely within the standard error of the soil analysis. The concentrations of total iron in the leachate of planted columns were significantly lower than the influent ($p=0.01$); however, the same was not true for control columns ($p=0.10$). Overall, approximately 2.28 mg of iron leached from the planted columns recieivng wastewater, as compared to approximately 12.28 mg from the unplanted columns. As with nitrate, the lower concentrations of iron in the leachate in planted columns could be due to (1) plant uptake of soluble iron or (2) poplar effects on the soil redox conditions.

4.1.7. Plant tissue

Tissue concentrations of macro and micronutrients in trees grown under land application of food processing wastewaters were within the range expected for healthy tree growth. Comparisons between tissue concentrations of nutrients indicated uptake of most macronutrients (nitrogen, potassium, calcium, magnesium and sulfur). Surprisingly, the shoot and leaf concentration of phosphorus was statistically higher in trees receiving water than in trees receiving wastewater [Figure 4-6]. Similarly, significantly lower concentrations of zinc, copper and boron were observed in leaves from trees that received wastewater than in leaves from trees that received water. Leaf concentrations of potassium, calcium, magnesium and sulfur also followed the same pattern (data not shown); however, for these elements, the difference was not significant. As decreased uptake of nutrients could eventually influence tree growth, more research is needed to verify this observation under a longer and larger scale and to determine what process limits uptake of phosphorus and some micronutrients in trees treating food processing wastewater. As a decrease in uptake of four macro and micronutrient was observed for trees receiving wastewaters, the most probable explanation is one of whole plant function, such as the observed decrease in root biomass and number of root branches.

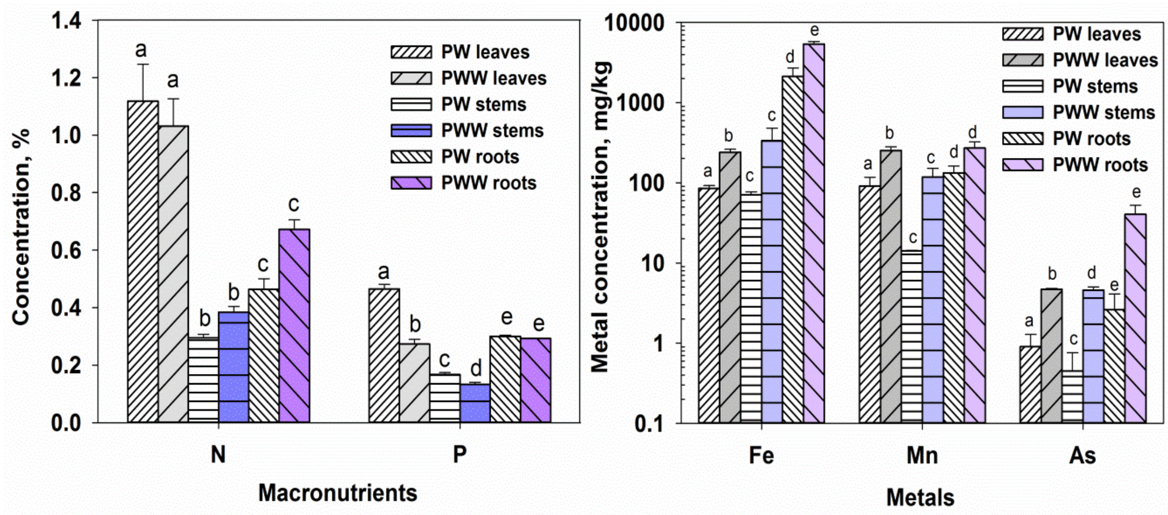


Figure 4-6 Macronutrients (N and P on left) and Metals (iron, manganese and arsenic on right) in plant tissues at the conclusion of the experiment. Different letters indicate statistical difference between trees receiving water (PW) and trees receiving wastewater (PWW). Error bars indicate standard error of the mean.

In contrast to the previously discussed elements, uptake of metals that function as electron acceptors (i.e., arsenic, iron and manganese) was greater in trees receiving wastewater than in trees receiving water [Figure 4-6]. The increased leaf and shoot concentrations of iron and manganese is consistent with the observed lower concentrations of iron and manganese in leachate water, further supporting the hypothesis that plant uptake of soluble metals can decrease mobilization of metals to groundwater at land application sites. Additionally, the mass of manganese and iron taken up by the poplar trees that received wastewater exceeded the mass that was added in the influent wastewater. Consequently, the plant tissue results provide evidence that redox conditions were low enough to mobilize manganese and iron for a portion of the experiment or within a portion of each column. Moreover, poplars are strategy I plants that uptake the more soluble form of iron (Mihucz et al., 2012), further supports the hypothesis that conditions conducive to iron mobilization were formed. The accumulated concentrations of iron in poplar shoots in this study ranged from 74 to 597 mg/kg, which was similar to or higher than the accumulation of 22 to 140 mg/kg of iron that was previously observed in poplar trees grown hydroponically in solutions of iron citrate or iron EDTA (Mihucz et al., 2012). However, concentrations of manganese in the shoot tissues of poplars in this study, which ranged from 60 to 290 mg/kg, exceeded those previously observed. Concentrations of manganese in poplar stems and leaves ranged were approximately 1.2 and 2.0 mg/kg when native poplar varieties were exposed to 1 mM Mn solution (Lei et al., 2007). Consequently, results indicate that accumulation of iron and manganese by poplars during land application of food processing wastewaters is likely similar to or greater than that observed in studies that expose poplars to greatly elevated concentrations of metals

The increase in uptake was most drastic for arsenic. Additionally, the presence of trees in columns receiving wastewater reduced the soil concentrations from 5.43 mg/kg in columns without trees to 4.86 mg/kg in columns with trees ($p=0.05$). Consequently, application of wastewater appeared to increase the bioavailability of soil arsenic for plant uptake, indicating that arsenic was in a more mobile form. Additionally, results indicate that poplar trees can uptake arsenic under conditions created by land application of food processing wastewaters. In four months, trees that received wastewater accumulated 0.46 mg arsenic – 0.37 mg more than trees that received water. The majority of arsenic was translocated to stems and leaves. Arsenic concentrations in the poplar stems and leaves in columns receiving wastewater ranged from 3.8 to 5.3 mg/kg. Shoot concentrations of arsenic were slightly lower than the leaf concentrations observed in poplars grown at a contaminated field site near Tuscany, Italy (e.g., 9 to 12 mg/kg after 12 months) (Ciurli et al., 2014). The small decrease in uptake was likely due to differences in initial soil arsenic concentrations (e.g., approximately 370 mg/kg at the contaminated site vs. 5 mg/kg in this study) and the duration of the experiment.

4.1.8. Conclusions

Plant-based treatment technologies, including phytoremediation, rely on a complex system of processes for treatment; consequently, many conditions or constraints can thwart success when proposing new plant-based treatment systems. This study evaluates the potential for poplar plantations to be used to enhance treatment of food processing wastewaters through land application.

First, land application of wastewater was not generally inhibitive to poplar growth. Production of above ground biomass was not affected by application of fruit and vegetable processing wastewater at the rate of one to two times the highest current application rate in Michigan. However, at the conclusion of the experiment, the root mass of poplar trees that received wastewater was significantly less than the root mass of control trees. Combined with reduced uptake of multiple nutrients into above-ground tissues, the decreased root mass strongly indicates that the rhizosphere was smaller in columns receiving wastewater than in columns receiving water. Consequently, current guidelines for the spacing of poplar trees at phytoremediation sites (ITRC, 2009), which were developed for nutrient-poor conditions, will likely need to be modified prior to use at land application sites. Specifically, more research on planting density may be required before poplar plantations can be used to treat food processing wastewater.

Second, poplar trees were able to withstand continuous saturation of soils while maintaining high evapotranspiration rates. Poplar trees evapotranspired 3.25 times more water than soil-only control columns, indicating that poplar trees can substantially reduce soil moisture. As saturation accelerates formation of reducing soil conditions that are conducive to metal mobilization, either by dissolution of iron and manganese oxyhydroxides and oxidation of sulfides, evapotranspiration is expected to reduce the formation of such reducing conditions. Additionally, decreasing percolation to the groundwater table will decrease pollutant transport. This trend was demonstrated for COD, as the mass of COD leached was significantly less when columns were planted. Ultimately, the high evapotranspiration of poplars would allow food processors to increase both their hydraulic and carbon loading of wastewater at land application sites, decreasing the land required for wastewater treatment.

Third, poplar trees decreased leaching of iron and manganese through evapotranspiration and uptake and translocation of metals into above-ground biomass. Reduction in leachate volume due to evapotranspiration meant that total mass of iron and manganese leached was substantially lower from planted columns than from unplanted columns, even when the concentrations of iron and manganese in the leachate were only slightly different. Uptake of manganese and iron likely contributed to the decrease in leaching of manganese and iron, while uptake of arsenic likely contributed to the observed decrease in soil concentration of arsenic at the conclusion of the experiment. The accumulation of manganese, iron and arsenic by poplars was greater than or similar to what has been previously observed in hydroponic and field studies with elevated metal concentrations, despite low concentrations of these metals in the influent wastewater. Consequently, native soils provide a substantial source of bioavailable metals for poplar uptake during land application of food processing wastewaters. Additionally, poplar growth prevented leaching of nitrate at the onset of wastewater application, further protecting groundwater. One solution that has been proposed for mobilizaton of metals from land application of wastewater is to require longer resting periods between applications, with the goal of maintaining more aerobic conditions (Bouwer, 1972). Under this scenario, the growth of poplar trees at land application sites could substantially decrease nitrate contamination of groundwater.

4.2. Large-scale column experiment

Following were the objectives of the large-scale column experiment under land application of food processing wastewater.

1. Evaluate treatment of chemical oxygen demand (COD) in wastewater
2. Quantify evapotranspiration and moisture reduction of soils by poplar trees
3. Investigate the effects of poplar trees on soil redox potential
4. Evaluate the effect of poplar trees on soil microbial biomass and community
5. Assess the decrease of metals and nitrates mobilization including plant uptake of nitrate and metals.

At East Lansing, April 15 to November 15 is considered growing period for trees. Poplar trees in the 2011, 2012 and 2013 grew well. In 2014, trees in the loam columns did survive harsh spring of 2014, but did not grow very well. The trees in sandy loam columns did not grow at all until August. Even after August, plants were small in size.

4.2.1. Soil moisture

One of our hypotheses was that planting poplar trees at land where food processing wastewater is applied will reduce soil moisture due to its high evapotranspiration. Figures 4-7 and 4-8 illustrate the variation of soil moisture for each treatment in each year.

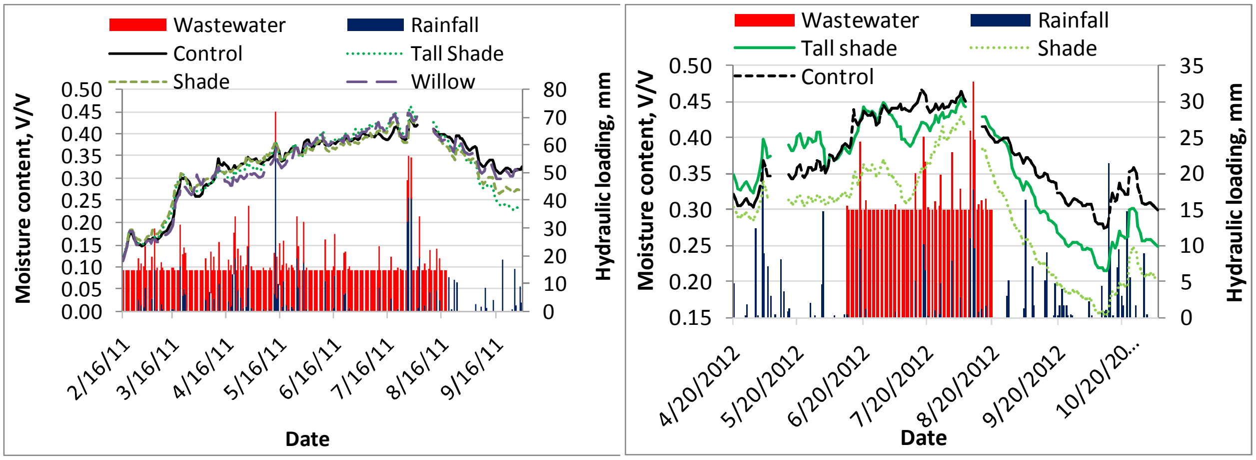


Figure 4-7 Overall mean soil moisture for each treatment in 2011 (left) and 2012 (right).

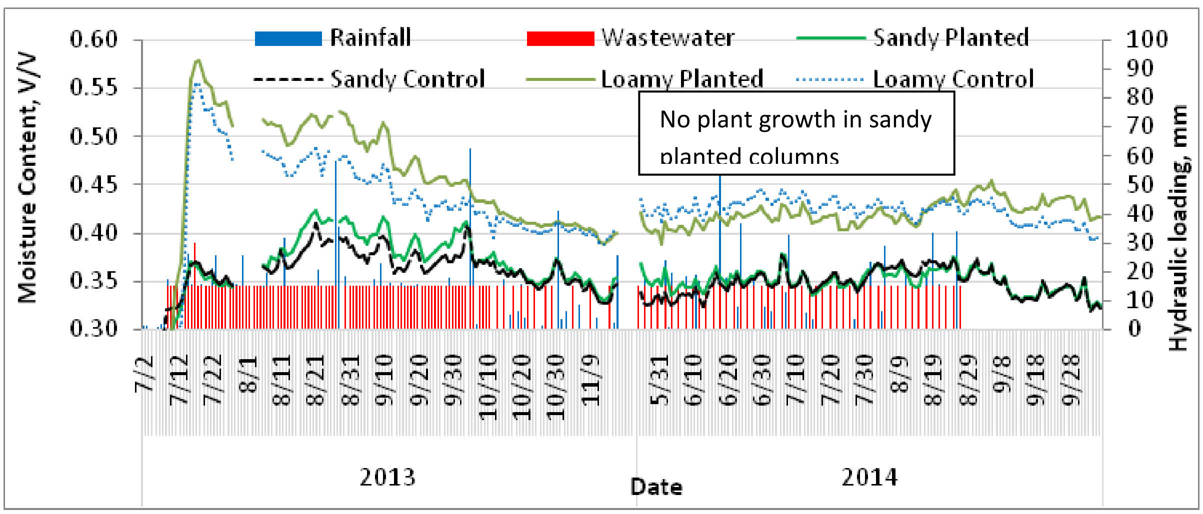


Figure 4-8 Comparison of overall mean soil moisture for different columns in 2013-2014. Trees in sandy planted columns did not grow until August 2014.

In 2011, the mean soil moisture content during the period of wastewater application for control, tall shade, shade and willow were 0.326±0.006, 0.330±0.006, 0.321±0.007 and 0.328±0.006 respectively. Two–way ANOVA with treatment groups and depth as factors found that treatments were not significant, however, depth and interaction were. Therefore, at any depth, treatment groups could have significant effect. Tall shade and control had similar moisture at 46 cm ($p=0.10$). At 91 cm, control had lower soil moisture than planted columns ($p<0.01$). At 122 cm level, planted columns had lower soil moisture than control columns ($p<0.001$). Therefore, in 2011, plants affected the treatment positively by reducing soil moisture at bottom depth. The roots of the poplars extended to bottom of soil where moisture was higher than at shallower depth. During deconstruction of columns, the roots of the poplar trees were found dense at the base of the columns. Therefore, the reduction of moisture at 122 cm was not surprising. However, the moisture of planted columns being higher than that of controls at 91 cm was surprising.

In 2012, the mean soil moisture during the period of wastewater application for tall shade, shade and control were 0.421±0.002, 0.362±0.004 and 0.435±0.002, respectively. The plants were growing very well in 2012 and may represent the best representative results. Control had higher moisture than tall shade and shade at all depths (Figure 4-9). Two-way ANOVA found that both factors (treatments and depth) as well as interaction were significant. Therefore, treatment as well as depth affected the moisture at columns. Between controls and planted columns, soil moisture were lower for planted columns at all levels ($p=<0.001$ to 0.02) except control and tall shade at 46 cm ($p=0.47$). Therefore, plant positively affected the moisture reduction of soils at different depths in 2012.

Moisture content was similar between controls and planted columns at the start of experiment in 2013. The control columns had lower moisture during July to October for loam soil and during August to September for sandy soils [Figure 4-10]. Moisture between controls and planted columns was similar for rest of the year in 2013. This observation, while unexpected, could be due to low temperature and lower plant growth rate starting October. In 2014, plants in the sandy columns died due to harsh 2013 spring, before sprouting from roots during August. Still, the plants were not big enough. Therefore, only data from 2013 was considered for statistical analysis in sandy columns. At 30 cm depth, the mean soil moisture was 0.318±0.004 for planted columns and 0.313±0.003 for control columns. At 61 cm level, soil moisture in control (0.366±0.003) was lower than that in planted columns (0.382±0.004) ($p=0.000$). Similar result was obtained in 2011 at 61 cm depth. While poplar roots can extend more than 2 m (Tufekcioglu et al., 1998, ITRC, 2009), >90% of poplar roots during phytoremediation do not extend beyond 1.5 m and 70-80% roots are shallower than 0.6 m (ITRC, 2009, Douglas et al., 2010). Therefore, more reduction of soil moisture at top 61 cm level is expected. In our columns during 2011-2012, we observed dense roots at the bottom of the columns indicating that roots can easily extend 122 cm. At 76 cm level, the moisture levels for control (0.400±0.003) was higher than for planted (0.396±0.004) ($p=0.07$). Therefore, on overall basis, no reduction of soil moisture by poplars in sandy columns was observed in 2013.

In loam columns, the mean soil moisture was 0.435±0.003 for planted columns and 0.398±0.003 for control columns at 30 cm depth in 2013-2014 [Figure 4-10]. The soil moisture for control columns was lower than that in planted columns at 30 cm level ($p=0.00$). At 61 cm level, soil moisture in control (0.426±0.004) was lower than that in planted columns (0.431±0.004) ($p=0.00$). Plant contribution in reducing soil moisture was not seen, likely due to poor growth of plants and inundation of wastewater in planted loam columns because of poor infiltration. Plants in loamy columns in 2014 were alive, grew well for few months before slowing growth due to lack of nutrients that was figured out late. As a result, clear difference was seen from May to Mid-August for loamy columns. From September onwards, the columns were saturated all the time as water stayed on the top due to poor infiltration. At 91 cm level, the moisture levels for planted (0.437±0.004) was lower than that for controls (0.443± .004) ($p=0.00$). As observed in columns in 2011 and 2012, the root density may have been higher at the bottom of columns.

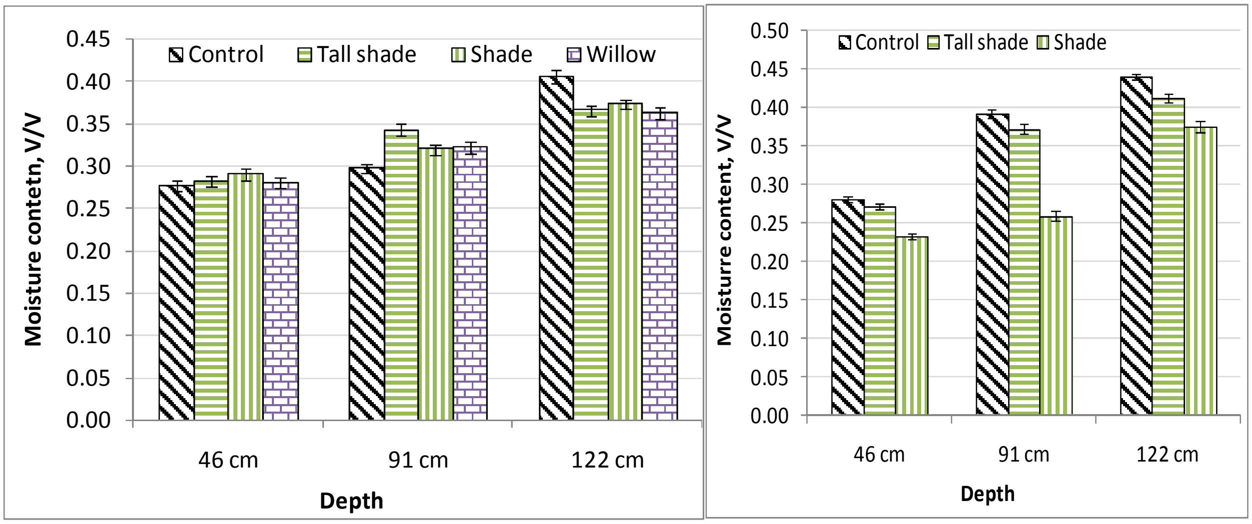


Figure 4-9 Mean moisture content of each treatment at different depths during the period of wastewater application for 2011 (left) and in 2012 (right)

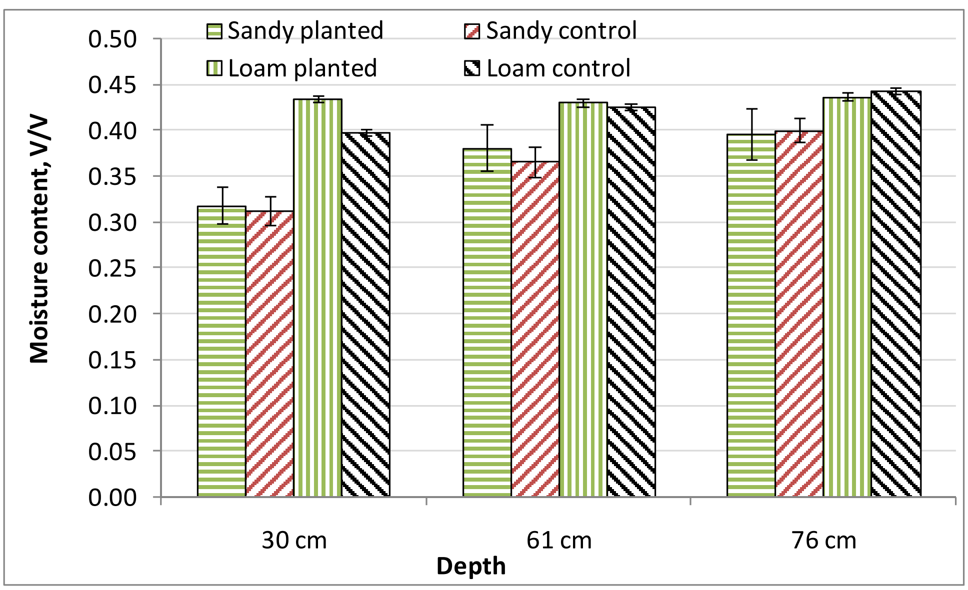


Figure 4-10 Mean moisture content of each treatment at different depths during the period of wastewater application for 2013-2014. Only 2013 data was considered for sandy soils due to plant death.

Three way ANOVA for 2013-2014 with soil, planted/control and depth of sensor in the soil as variables indicated that all interaction between two factors were significant ($p<0.001$). Tukey's pair-wise comparison for all pairs within a factor were significantly different, i.e. loam vs sandy loam, planted vs control and 30 cm vs 61 cm vs 76 cm were significant ($p<0.001$ to 0.009). For planted columns, all three soil levels had different soil moisture ($p<0001$ to 0.002). However, for control columns, 61 cm and 76 cm depth soil had similar soil moisture ($p=0.795$). These results indicate that plants did influence the soil moisture at 76 cm in planted columns, but not in control columns.

4.2.2. Evapotranspiration

Evapotanspiration was measured in 2013 and 2014 as the difference between total water applied including rainfall and leachate water as there was no other water loss from the system. Change in storage was considered negligible due to similar soil moisture at the start and the end of the experiment, small soil volume and long duration of the experiment.

The ratio of evapotranspiration of planted columns to that of control columns are plotted in Figure 4-11. The ratio is 1 for control columns. Loam planted columns had the ratio higher than 1 for all considered months except May 2014 when plants were still coming out of spring thaw. For Sandy planted columns, the ratio was higher in 2013 for all months.

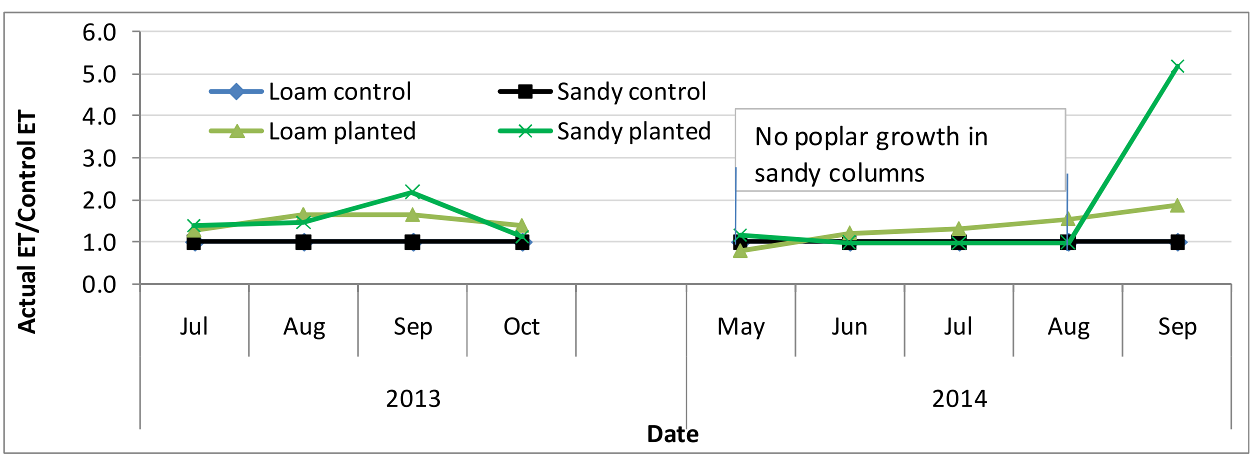


Figure 4-11 Ratio of evapotranspiration of planted columns to that of control columns. In 2014, Poplar trees in sandy columns started growing only after August.

As expected, loam planted columns had higher crop coefficient than controls both in 2013 and 2014. Poplar trees in the loam columns grew very well in 2013. However, in 2014, they did not grow well in height and number and size of leaves. Consequently, the crop coefficient in 2014 was less than that in 2013 for plants in loam soil. The lack of high growth was likely due to lack of nutrients in the soil. Soil nutrient analysis showed that the loam soil had below optimum levels of phosphorus, potassium and low nitrogen. Poor growth indicated that nutrients in the wastewater were sufficient for plants to survive but not enough for optimal plant growth in soils with low nutrients. Consequently, at land application sites, soil testing for nutrients is required before choosing to grow grasses or trees.

The crop coefficient, ratio of evapotranspiration for planted columns to that for control columns, calculated on monthly water balance data are shown in Table 4-1. By planting poplars in sandy and loam soils, the rate of moisture removal from soil can be expected to increase by 1.55 and 1.50 times based on 2013 data and by 1.55 and 1.42 based on overall data. These values are comparable to evapotranspiration of poplars (1.06-1.90) during first year in Italy (Guidi et al., 2008).

Table 4-1 Monthly evapotranspiration coefficient for 2013, 2014 and 2013-2014 calculated based on monthly water balance

Crop coefficient based on control evapotranspiration			
	2013	**2014**	**Overall**
Sandy planted	1.55±0.23		1.55±0.23
Loam planted	1.50±0.09	1.36±0.18	1.42±0.11

* indicate that the value was taken for only 2013 for sandy planted

These results indicate that poplar trees are beneficial at the land application sites to increase rate of moisture removal from soils as evapotranspiration is the only moisture loss mechanism other than leaching from soil columns/field (runoff is zero). Increase in moisture removal rate from the soil may increase the oxygen diffusion to soil and reduce mass leaching of pollutants. The oxygen diffusion is enhanced by 10,000 times when the pores are filled with air than water (Ponnamperuma, 1972). There is greater potential for reduction in soil moisture by increasing root density or planting density and having greater growth of poplar trees (Guidi et al., 2008).

One of the limitations of this study was that the plants were grown in the columns that did not have true representative temperature profile of the ground. The temperature of the columns reached sub-zero values at all depths which was different than that at the field site where soil barely went to 0°C at below 5 cm from the surface. Prolonged freezing of columns during 2013/14 winter caused the death of parts of the tall shade poplars in loam columns and whole plant in sandy columns. Thus, the column study did not have the normal growth and may have under-predicted the effects of poplar trees on moisture removal, evapotranspiration and likely associated biological activities.

4.2.3. Chemical Oxygen Demand

One of the objectives of the research was to evaluate the carbon treatment by land treatment and find the effects of poplar trees if any.

4.2.3.1. COD data summary

The COD of the effluent and influent samples with time are shown in figures 4-12to 4-14. COD of the influent wastewater varied due to microbial activities in the wastewater at the bottom of the source tank and possible incomplete dissolution of starch.

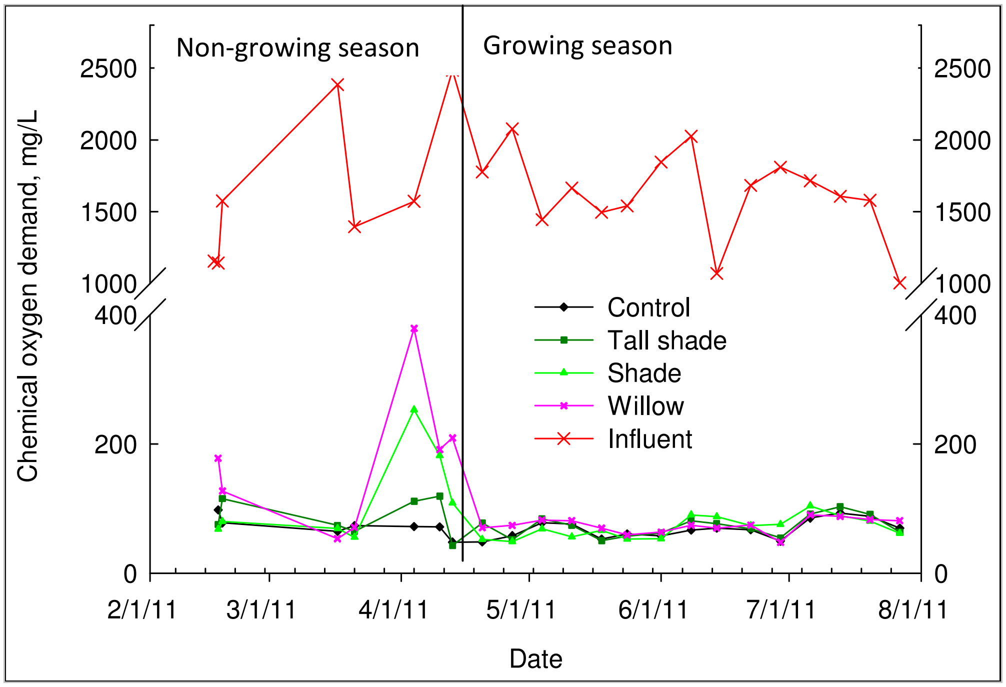


Figure 4-12 COD of leachate water and influent in 2011. COD concentration value for each treatment is mean of three replicates.

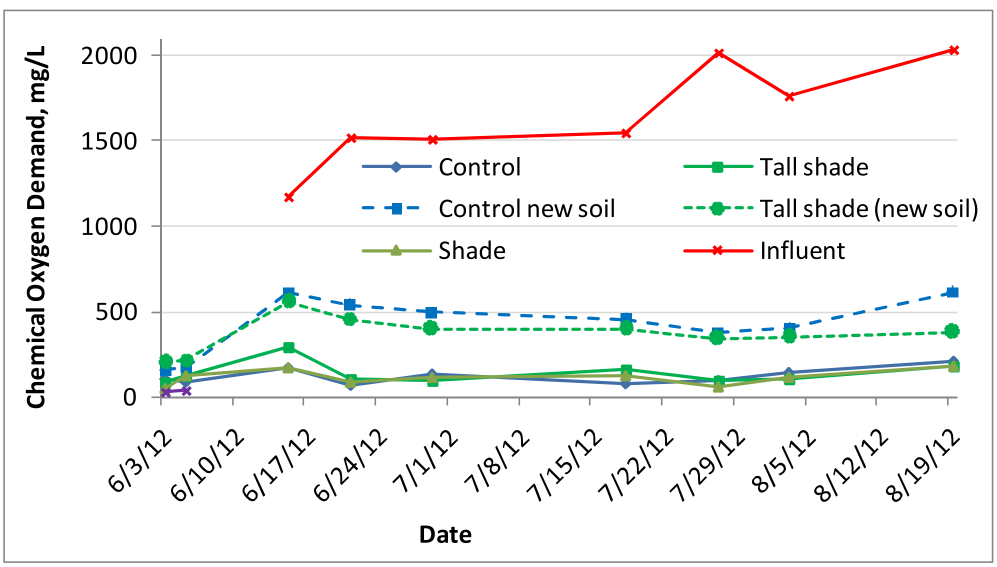


Figure 4-13 Chemical oxygen demand for influent and leachate samples for 2012. The newly constructed columns that had new soil have been separated from columns with old soil.

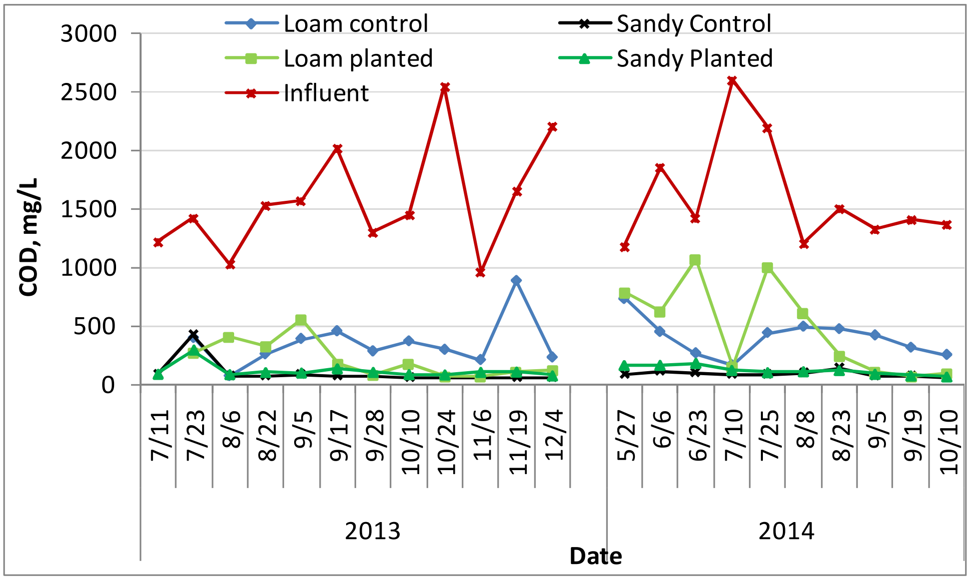


Figure 4-14 Influent and leachate water chemical oxygen demand over time for 2013-2014

In 2012, when water with COD of 28-42 mg/L was applied to the columns, the leachate water had COD of 49-167.5 mg/L, higher than the influent water, likely due to more dissolved carbon from soil or microorganisms in water. After wastewater with a mean COD of 1649.9±116.1 was added daily from 6/5/2012, the COD of the effluent leachate increased [Figure 4-12].

Effluent COD concentration was higher in year 2 than in year 1 for both sandy loam (between 2011 and 2012) and loam soil (between 2013 and 2014) [Table 4-2]. Consequently, lower treatment efficiency was obtained in 2012 than in 2011 for sandy loam soil and in 2014 than in 2013 for loam soil (Table 4-3).

Table 4-2 Summary of chemical oxygen demand concentration of leachate and influent for all years

Year	2011	2012		2013		2014	
Loading rate, mm/day	15	9	6	15	15	15	7.5
Soil	Sandy Loam	Sandy Loam	Sandy Loam*	Sandy Loam	Loam	Sandy Loam	Loam
Influent, mg/L	1661±81	1650±116	1650±116	1574±136	1574±136	1607±147	1607±147
Control, mg/L	78±5	121±16	424±56	103±30	350±62	93±7	40 ±50
Tall Shade, mg/L	78±5	137±22	366±37	113±17	212± 47	118±12	475±123
Shade, mg/L	85±10	113±15					
Willow, mg/l	105±17						

*indicate that the sandy loam soil that had lower sand content and higher silt content.

Table 4-3 Summary of COD treatment efficiency in percentage for all years

Columns	2011	2012		2013		2014	
	Sandy Loam	Sandy Loam	Sandy Loam*	Sandy Loam	Loam	Sandy Loam	Loam
Control	94.9 ± 0.5	92.0 ± 1.4	68.4±4.0	92.9 ± 2.1	77.8±3.7	93.9±0.6	72.3±4.8
Tall Shade	95.2± 0.4	90.3 ± 2.7	73.6±3.9	92.2±1.3	85.2±3.7	92.2±1.1	68.7±8.5
Shade	94.9 ± 0.6	82.1 ± 2.5					
Willow	93.6 ± 1.1						

*indicates that sandy loam soil that had lower sand content and higher silt content.

The treatment efficiency of all sandy columns was greater than 90% except of new-soil columns. The lowest COD treatment obtained was 68.4% in 2012 by control new soil (fine textured) and the highest obtained was 95.2% in 2011 by sandy tall shade columns. The efficiency was to some extent affected by the experimental set up, especially in 2013-2014, when the tanks did not have corrugation on the inside surface. The shallower soil depth in 2013-2014 columns and lack of corrugation on the inside surface of columns may have contributed to lower treatment efficiency. However, the observed efficiency were higher than 58.3% and 61.4% obtained in 1 m long soil columns with real and synthetic wastewater respectively (Ak & Gunduz, 2013).

4.2.3.2. *Effect of soil type*

Out of 15 columns in 2012, 9 columns had old soil from 2011 i.e. soil with 76.9% sand, 11% silt and 12.1% clay. The other 6 columns, 3 control and 3 tall shade, had new soil with lower sand and higher silt and clay i.e. 62.7% sand, 18.3% silt and 19.0% clay. The mean leachate COD of shade columns in old soil was 113.4±10.1 mg/L, of control in old soil was 120.9±16.0 mg/L, of control in new soil was 424.3±56.0 mg/L, of tall shade in old soil was 137.0±22.4 mg/L and of tall shade in new soil was 365.9±36.8 mg/L. The new, finer soil leached significantly higher COD than the coarser old soil ($p<0.005$). Mean COD treatment efficiency for control (old soil), control (new soil), tall shade (old soil), tall shade (new soil) and shade poplars (old soil) in 2012 were 91.46±1.4%, 68.38±4.03%, 90.34±2.67%, 73.56±3.93% and 92.26±1.36% respectively. Pair-wise comparison using Tukey's test indicated treatment efficiency by old soil columns was higher than that by new soil columns. Therefore, the decrease in sand content and increase in finer fraction in new soil resulted in higher COD of the leachate water in control and tall shade columns in 2012. Thus, the decision while selecting the land for application of food processing wastewater should base on the higher sand content soil, but supportive of plant growth.

Generally, columns containing loam soil leached water with higher COD concentrations in 2013-2014 [Figure 4-14]. Values of COD for some columns were very high likely due to some preferential flow at times. Whenever the preferential flow was noticed by qualitatively assessing leachate water collection rate, the soil at the top 15 cm was manipulated to seal the preferential flow. Another reason for higher COD in loam soil could be growth of microorganism when the water inundated the top of soil surface continuously for long time due to poor infiltration. The mean COD of leachate water for loam planted, loam control, sandy planted and sandy control were 337.7±68.6 mg/L, 374.7±40.0 mg/L, 115.3±10.7 mg/L and 98.1±16.2 mg/L respectively.

The treatment was significant as influent was significantly higher than the treatment groups ($p<0.001$). However, among Dunn's multiple group-wise comparisons, loam control had higher median than sandy control and loam planted had higher median than sandy control ($p<0.05$). It can be concluded that sandy soil had lower COD concentration or higher treatment than loam soils. The results are consistent with a previous study which obtained higher treatment of carbon by sandy loam soils than by loam or clay loam soils (Law et al., 1970).

4.2.3.3. Effect of plant growth

In 2011, the mean effluent COD was 77.6±4.7 mg/L for control, 77.9±4.6 mg/L for tall shade, 85.4±10.1 mg/L for shade and 105.4±16.7 mg/L for willow. One way ANOVA on ranks confirmed that the median of the influent COD was different than the median of each treatment group ($p<0.001$). However, no significant difference was obtained between the treatment groups (control, tall shade, shade and willow) ($p=0.78$).

There was significant treatment difference between planted and controls for new (finer) soil in 2012 ($p=0.03$). The COD concentration of leachate from control columns was 424.3±56.0 mg/L and from tall shade columns was 365.9±36.8 mg/L. Excellent tree growth was achieved in 2012 which might have resulted in better treatment of COD in finer soils. However, no difference in treatment was observed among treatments (shade, tall shade, control) with old sandy loam soil, consistent with the result in 2011 (i.e. no effects of plants in sandy loam soil). Similar result, no difference in dissolved organic carbon between planted and no-planted soils irrigated with nutrient enriched water, had been obtained before (Maria-Cervantes et al., 2010).

Poplar trees enhanced removal of COD in loam soil in 2013 from September onwards ($p=0.04$) and in 2014 from August onwards ($p=0.01$) compared to control, consistent with the treatment effect observed between planted and control columns in 2012 in finer textured sandy loam soil (new soil in 2012). No difference between controls in 2013 and 2014 ($p=0.3$) eliminated the effect of aging soil.

Thus, poplar trees enhanced COD removal in finer textured soil. For land application, if the selected or available site has lower sand content, planting poplar trees may enhance the carbon removal based on the results from this experiment.

4.2.3.4. Effect of loading rate

The effects of loading rate on COD removal rate are shown in Figure 4-15. While the wastewater was applied every day in 2013, it was applied every alternate day at the same rate in 2014. Control had almost the same rate of removal as the influent COD addition rate. Loam control and loam planted had lowest removal. The removal rate as well as influent rate decreased with the season due to lower loading when the columns were flooded by rainfall or wastewater addition. Columns flooded most of the latter part of 2013 and the loading frequency was decreased based on the standing water at the top. Moreover, the decrease in temperature and subsequent low rate of microbial degradation of carbon resulted in lower removal rate. There was no difference in mass removal rate when the loading rate was doubled ($p>0.1$).

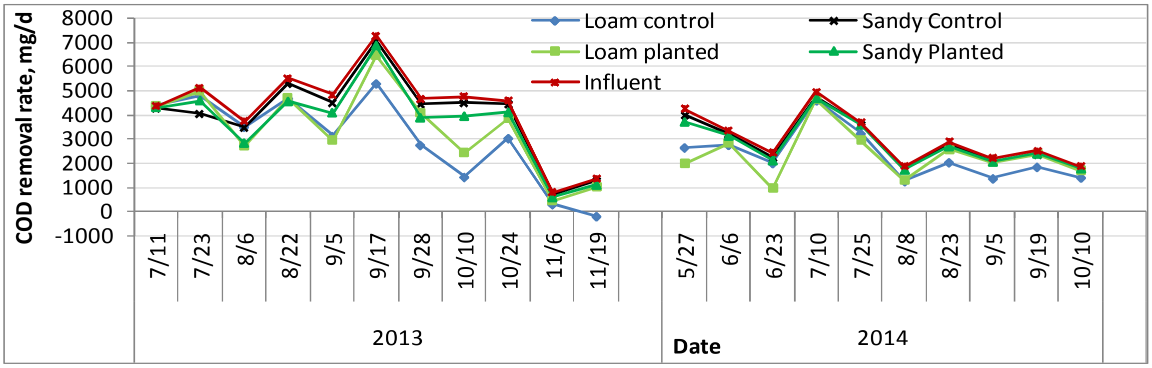


Figure 4-15 COD removal rate during 2013-2014. The application rate in 2014 was halved in 2013 by applying on alternated days only instead of every day.

4.2.4. pH

Summary of pH of influent and leachate water in all years is given in Table 4-4.

Table 4-4 Summary of pH of influent wastewater and leachate water for all years

Columns	**2011**	**2012**	**2013-2014**	
	Sandy Loam soil	Sandy Loam soil	Sandy Loam soil	Loam Soil
Influent	8.04±0.12	6.90±0.10	8.47±0.09	8.47±0.09
Control	7.80±0.05	8.15±0.05	7.69±0.05	7.69±0.04
Tall Shade	7.78±0.05	8.19±0.11	7.66±0.04	7.64±0.04
Shade	7.87±0.05	8.12±0.10		
Willow	7.88±0.12			

pH in the soil treatment system can fluctuate. pH increase in acidic soil can be from dissolution of metal oxides and hydroxides, whereas, pH decrease in basic soil can be from increased CO2 concentration and production of volatile fatty acids (Dassonville & Renault, 2002). pH affects hydroxide, carbonate, sulfide, phosphate and silicate equlibria and control the precipitation and dissolution of solids and the sorption and desorption of ions including iron and manganese (Ponnamperuma, 1972).

The variation in influent pH was due to use of different salts (for example, potassium carbonate instead of potassium sulfate), microbial activities at the bottom of wastewater tank and interaction of atmospheric carbon dioxide with water. The effluent water pH varied mostly from 7.3 to 8.5 in 2011, 7.5 to 8.5 in 2012 and 7.0 to 8.0 in 2013-2014. Most microorganisms grow in the range of 6-8 pH (Madigan, 2012). The bacterial composition in soil was positively correlated with pH from 4-8, whereas, bacterial diversity doubled from pH 4-8 (Rousk et al., 2010). Therefore, slightly basic pH obtained in this experiment was more favorable for diversity and composition of microorganisms.

The influent and leachate pH indicated that both acidic, as low as 6.4, and basic, as high as 9.1, wastewater were treated by soil buffering capacity, thus increasing pH for acidic wastewater and decreasing pH for basic wastewater. Kruskal-Wallis One way ANOVA on ranks did not find any difference among treatments and influent in 2011 ($p=0.22$). In 2012, group-wise comparison using Tukey's test confirmed that pH of the influent was lower than the other treatment groups ($p<0.001$). However, in 2013-2014, one-way ANOVA indicated that the pH of the influent was significantly higher than that of other treatments ($p<0.001$). In all years, treatment groups did not differ among each other in pH ($p=0.47$ to 1).

4.2.5. Soil Redox Potential

Soil redox potential combined with pH determines the availability, mobility and toxicity of metals in the soil systems. The daily mean across each treatment overall and with depth was further evaluated.

4.2.5.1. Soil redox potential variation in a column

Soil redox potential in a typical column in each year is shown in Figure 4-16 to 4-18.

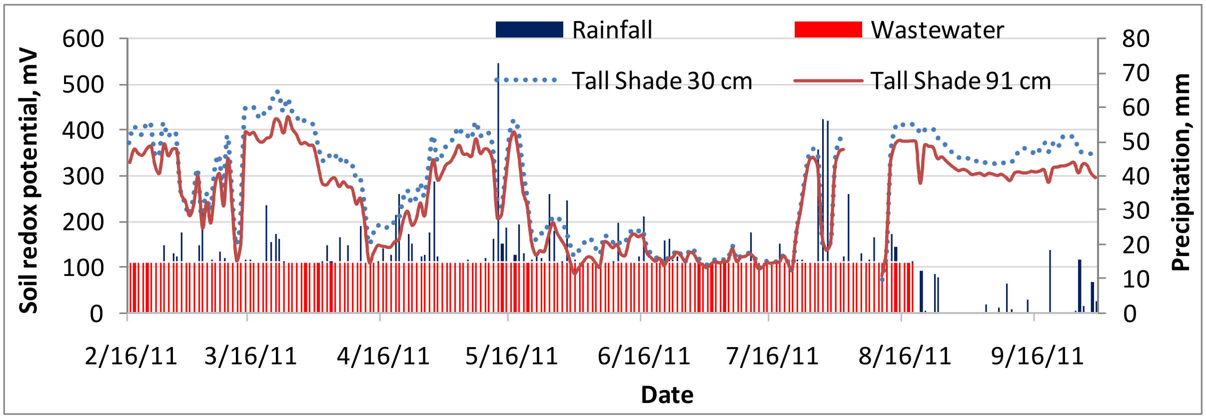


Figure 4-16 Typical soil redox potential curves for a column (Tall Shade) in 2011. Application of wastewater and rainfall are shown in the secondary y-axis on the right.

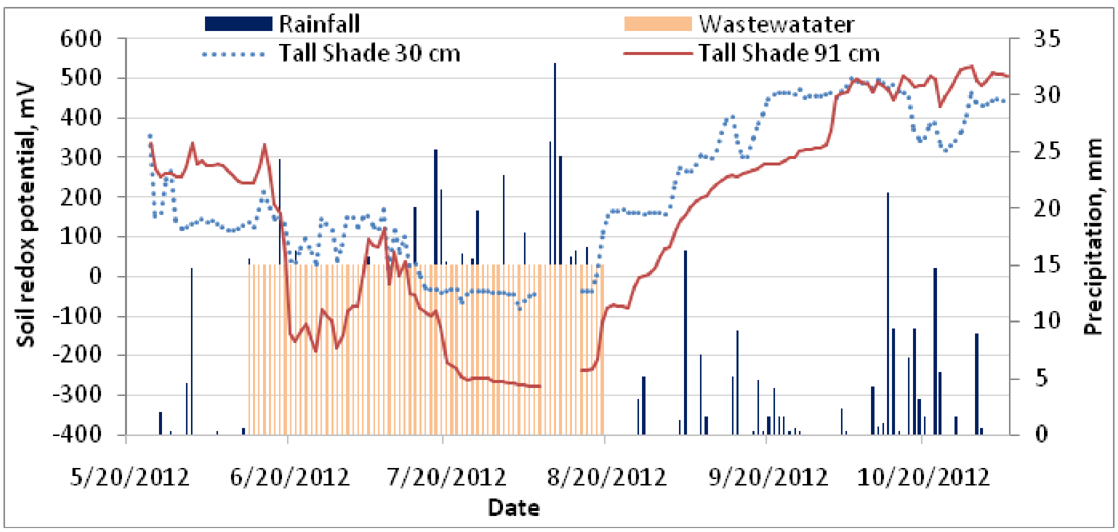


Figure 4-17 Typical soil redox potential curves for a column (Tall Shade) in 2012. Application of wastewater and rainfall are shown in the secondary y-axis on the right.

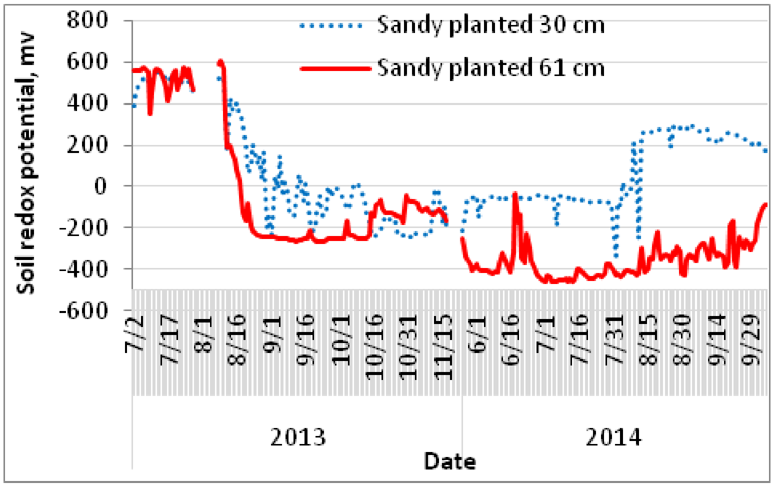


Figure 4-18 Typical soil redox potential curves for a column (sandy planted) in 2013-2014. Wastewater was applied throughout.

In all years, soil redox potential was greater than 350 mV at the start of the experiment. After application of wastewater, the addition of carbon to the system used up the oxygen and the redox potential decreased gradually to -270 mV to +100 mV depending on treatment and years. The redox potential value decreased with time as more and more carbon was loaded in the columns. As expected, the redox potential values at deeper depth were always lower than that at shallower depth in all years, consistent with increase in soil moisture with increase in depth. However, the redox potential values at two depths were highly correlated. The correlation coefficient between 46 cm and 91 cm data in 2011 was 0.99 indicating that the responses of the sensors at both levels were very similar. As less oxygen diffusion occurs from atmosphere and water gets deprived of oxygen when it travels down the column, the redox potential values are expected to decrease along depth. Moreover, redox potential under regular application of wastewater was affected by the rainfall events. Rainfall events were followed by dip in redox potential [Figure 4-16 and 4-17]. Redox potential recovered to some extent after few days without rainfall. The redox potential recovered to greater than 300 mV in all years after the application of wastewater was stopped. Thus, the calibrated sensors responded very well in the soil as evidenced by the decrease in redox potential with depth and loading of carbon and increase in redox potential with stoppage of carbon loading.

4.2.5.2. Effects of plants on soil redox potential

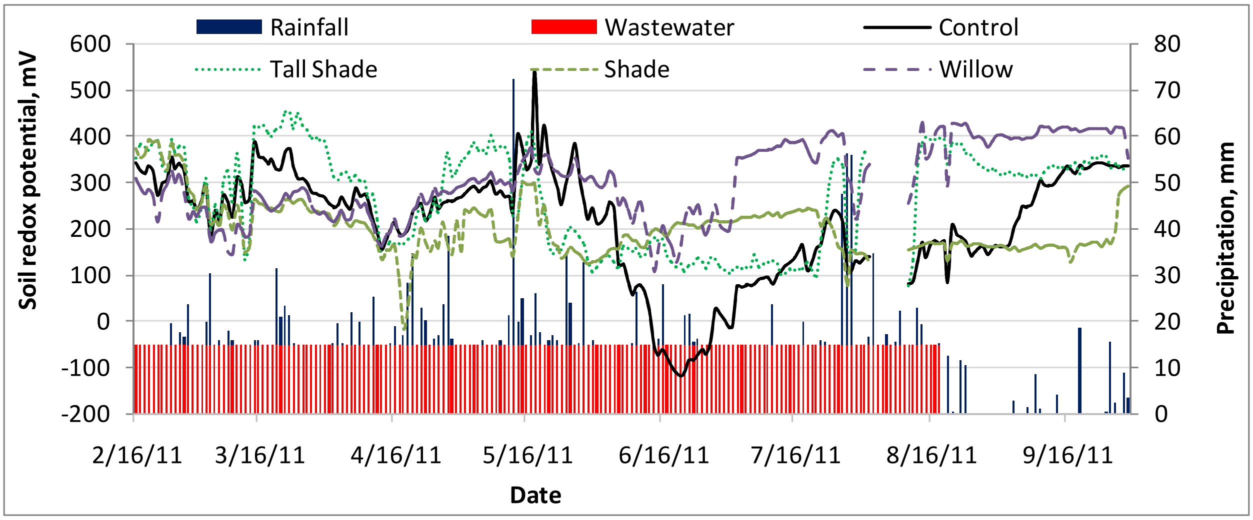


Figure 4-19 Mean soil redox potential for each treatment in 2011

One way ANOVA on ranks indicated that at least one treatment group was different from the other groups in 2011 ($p<0.001$). All pair-wise comparisons by Dunn's test were statistically significant ($p<0.05$). Willow, control, shade and tall shade had median of 297.2, 238.9, 244.1 and 197.7 respectively. Two-way ANOVA with treatment and depths as factors indicated that each factor and their interaction were statistically significant ($p<0.001$). Redox potential at 91 cm were lower than that at 46 cm for all columns ($p<0.001$) except control ($p=0.07$).

At 46 cm depth, all pair-wise comparison between treatments were significant ($p<0.001$)[Table 4-5]. However, willlow and tall shade had similar soil redox potential at 91 cm ($p=0.146$). Plant showed effect on redox as planted columns had higher redox potential than control columns except shade at 91 cm level. Oxygenation by roots (Kadlec & Knight, 1996) may have helped increase the redox potential in planted columns. These mean values of redox potential indicate that columns were mostly in nitrate reducing conditions except for shade at 91 cm depth.

Table 4-5 Comparison of redox potential for treatments at 46 cm and 91 cm for 2011

Depth	46 cm		91 cm	
Comparison	**LS Means vs Means, mV**	**P value**	**Means vs Means, mV**	**P value**
Willow vs. Control	335 vs 204	<0.001	270 vs 221	<0.001
Tall shade vs. Control	283 vs 204	<0.001	250 vs 221	<0.001
Shade vs. Control	282 vs 241	<0.001	163 vs 221	<0.001
Willow vs. Shade	335 vs 241	<0.001	270 vs 163	<0.001
Willow vs. Tall shade	335 vs 283	<0.001	270 vs 250	0.146
Tall shade vs. Shade	282 vs 241	<0.001	250 vs 163	0.014

In 2012, the redox potential at the start of the experiment was least for tall shade columns followed by controls and shade columns [Figure 4-20]. Redox potential of all columns decreased, bottoming out during July 20-August 20 and recovering gradually to oxic condition. All columns were mostly in manganese reducing zone before Aug 20, 2012. Two-way ANOVA showed that both factors and their interaction were significant ($p<0.002$). Redox potential at 91 cm was lower than that at 46 cm ($p<0.001$). At 46 cm, shade and control columns were not different [Table 4-6]. Overall, shade and control were not different ($p=0.7$) and tall shade was lower than others ($p<0.001$). In 2012, tall shade did not increase the redox potential compared to control, in contrast to what was found in 2011.

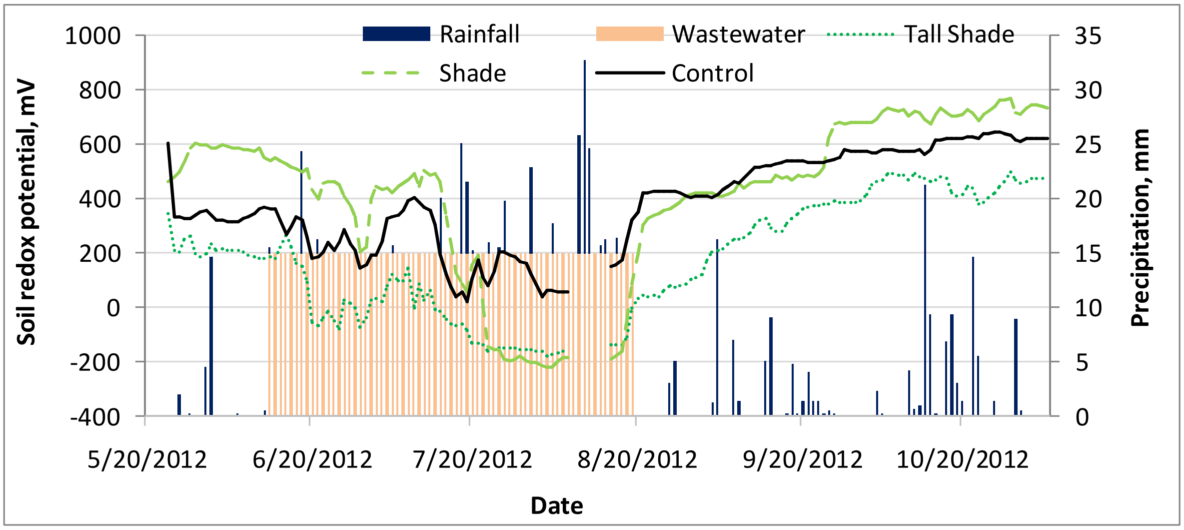


Figure 4-20 Mean soil redox potential for each treatment during 2012

Table 4-6 Comparison of redox potential for treatments at 46 cm and 91 cm for 2012

Depth	46 cm		91 cm	
Comparison	Means vs Means, mV	P value	Means vs Means, mV	P value
Tall shade vs. Control	214 vs 444	<0.001	147 vs 331	<0.001
Shade vs. Control	421 vs 444	0.663	440 vs 331	<0.001
Tall shade vs. Shade	214 vs 421	<0.001	147 vs 440	<0.001

The redox potential decreased as the season progressed in 2013 except for sandy control [Figure 4-21]. In 2014, the redox potential was expected to be higher for planted columns due to oxygenation of soil by plants. However, such effect of plants on soil redox was not observed except for Aug-Oct, 2014 in loamy columns. The lack of plant effect could be due to i) poor growth of plants, ii) less root density in the soil because the plants were first year plants that grew for few months only and iii) spatial variation in the redox potential.

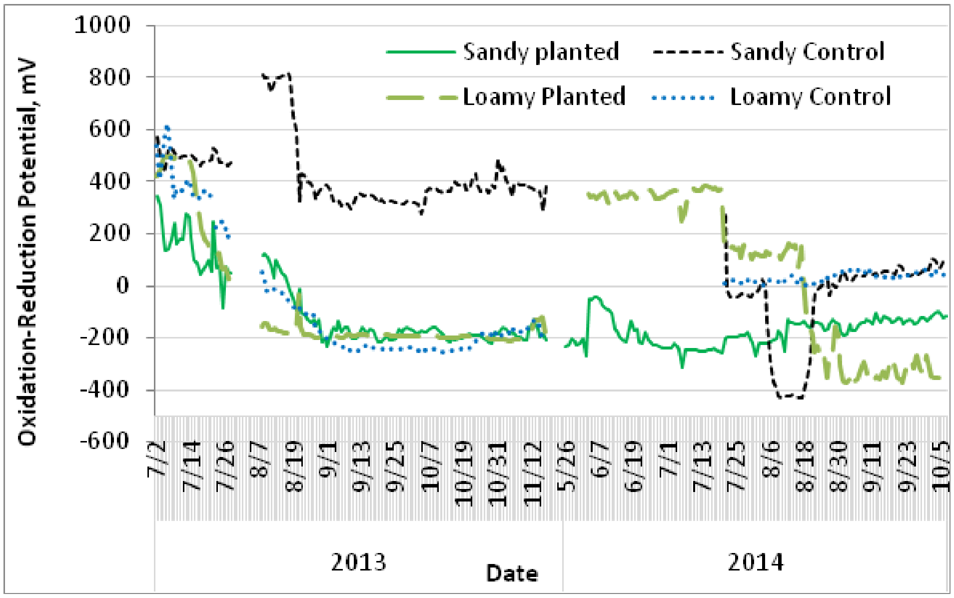


Figure 4-21 Mean soil redox potential for each treatment during 2013-2014

In 3-way ANOVA with soil type, treatments (planted/control) and depth (30 cm, 60 cm) as factors, all three factors and two-factor interactions were significant ($p<0.001$). Loam columns had lower redox potential than sandy loam columns ($p<0.001$). Similarly, 60 cm deep soil had lower soil redox potential than 30 cm soil ($p<0.001$) in both sandy loam and loam soils. As planted columns had similar redox potential to control columns in loam soils ($p=0.62$), no plant effect was observed.

4.2.5.3. Relationship between redox potential and soil moisture

In our experiment, soil redox potential was strongly negatively correlated to the moisture content [Figure 4-22] with correlation coefficients of -0.92 for tall shade, -0.80 for shade and -0.89 for control. The linear regression was statistically significant with negative slopes and R^2 of 0.84-0.62. The decrease in redox potential was due to less available oxygen in the void spaces of soil as void spaces were occupied by water and less oxygen diffusion occurred (MacKay, 1997). Similar results were obtained in 2011.

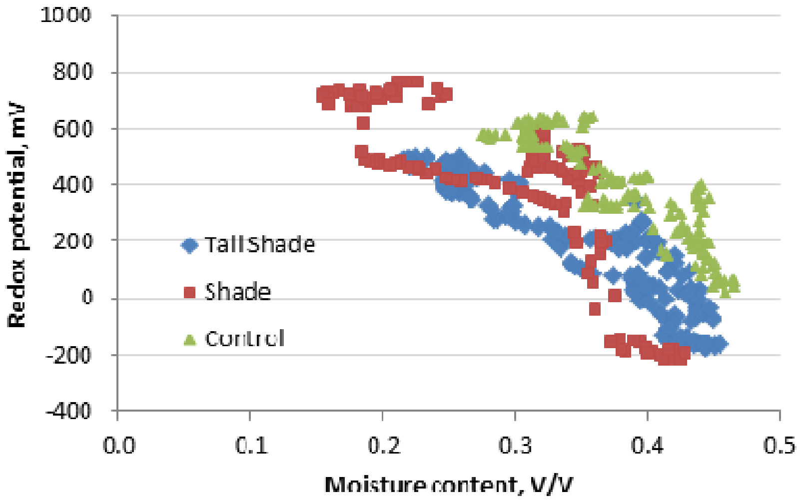


Figure 4-22 Relationship between soil redox potential and soil moisture. Data plotted were daily mean values of soil moisture and daily mean values of soil redox potential for 2012.

4.2.5.4. Relationship between redox potential and soil temperature

Mean soil redox potential showed strong inverse relationship with mean temperature 2012 [Figure 4-23]. The correlation coefficients were -0.82 for tall shade columns and -0.77 for control columns. There were no both temperature and oxidation reduction potential sensors in shade columns. Jimenez-Verceles et al. 2008 also obtained higher redox potential in colder months and lower in summer months in the presence of high water and DOC. The reasons could be due to lower solubility of oxygen in high temperature and aerobic oxidation of carbon at a higher rate in high temperature (Schmidt, 1982, Ak & Gunduz, 2013). The linear regression between redox potential and temperature was statistically significant with negative slope and R^2 of 0.67-0.59. Similar relationship was obtained in 2011.

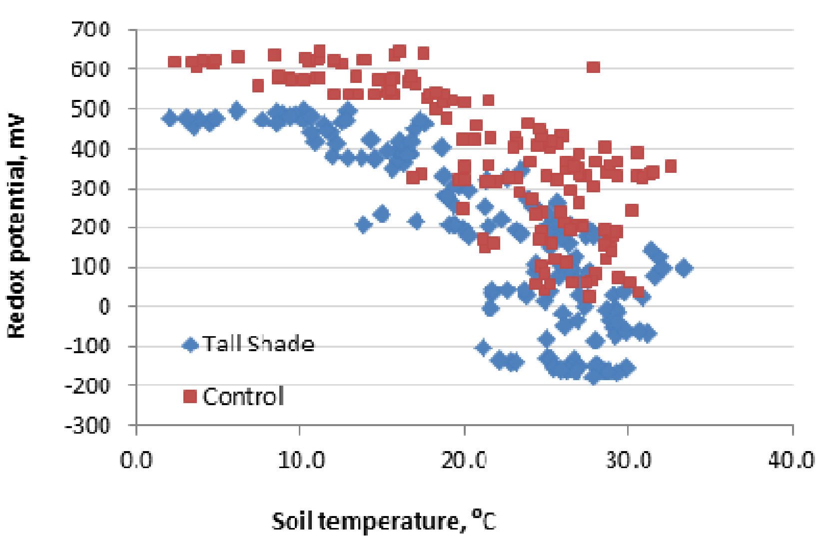


Figure 4-23 Relationship between daily soil temperature and daily redox potential in 2012

4.2.6. Nitrate

Nitrate, being highly mobile pollutant, is one of the concerns in the land application site. Nitrate was measured in leachate water in 2011, nitrate and ammonium in 2012 and in 2013-2014.

4.2.6.1. Effect of poplar trees on nitrate leaching

Nitrate leaching from columns along with influent nitrate are shown in Figures 4-24 to 4-26.

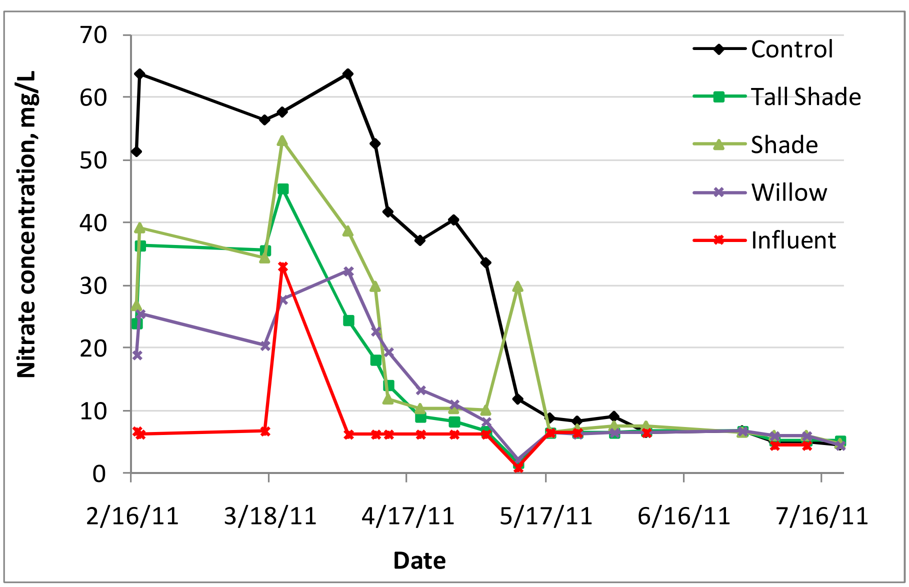


Figure 4-24 Nitrate concentration in the influent wastewater and leachate water in all treatments during 2011

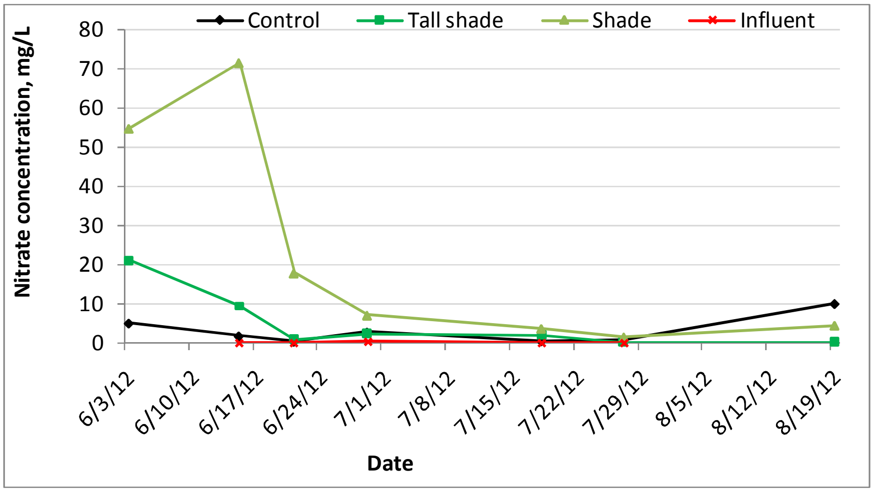


Figure 4-25 Nitrate concentration of influent and leachate samples in all treatments during 2012

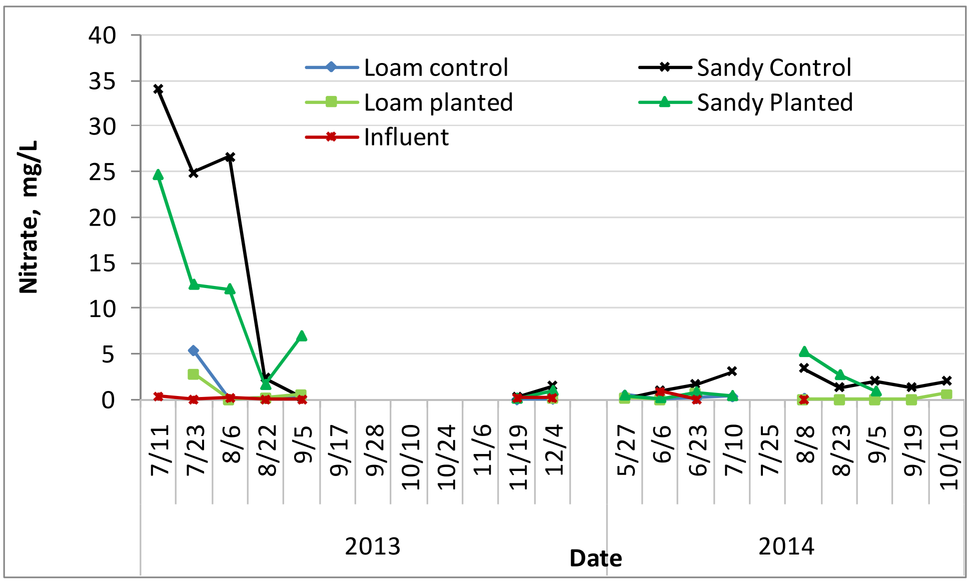


Figure 4-26 Nitrate concentration in the influent and leachate samples in all treatments in 2013-2014

Mean influent concentration of nitrate was 7.54±1.75 mg/L in 2011, 0.1±0.1 mg/L in 2012 and 0.2±0.1 mg/L in 2013. However, nitrate concentrations of leachate water in all treatments were higher than nitrate concentration of influent in all years. Mean nitrate concentration of leachate samples for control, tall shade, willow and shade in 2011 were 29.71±5.32 mg/L, 14.41±2.94 mg/L, 13.25±2.09 mg/L and 18.27±3.42 mg/L, respectively. In 2012, mean nitrate for control was 3.0±1.3 mg/L, for tall shade was 5.2±2.9 mg/L and for shade was 22.8±10.7. In 2013-2014, concentrations of nitrate for loam planted was 0.45±0.21 mg/L, for loam control was 0.60±0.44 mg/L, for sandy planted was 5±1.91 mg/L and for sandy control was 6.65±2.76 mg/L. The leaching of nitrate was much higher than 10 mg/L MCL (maximum contaminant level) during the start of the experiment in all years (US EPA) likely due to oxidizing conditions immediately after construction of soil columns. However, during the latter part of the experiment, nitrate concentration was lower than 10 mg/L in all years.

Therefore, nitrate was produced in the columns due to nitrification of ammonium and organic nitrogen under aerobic conditions (Schmidt, 1982). Nitrate that leached out of the planted columns was lower than that from control for first three months in 2011 and one and half month in 2013 likely due to plant uptake of nitrate. Statistical analysis showed that influent nitrate concentrations were significantly lower than the leachate nitrate concentrations ($p<0.05$). However, treatment groups were not different from each other ($p>0.1$) on overall basis. Plants did not show statistically significant nitrate reduction on the overall basis likely due to small plant size and low nitrate concentration in pore water during later part of the experiment. However, for the first three months in 2011 and one and half month in 2013, when the nitrate concentrations in the pore water were high, the results were significant ($p<0.05$). This result implies that poplar trees enhanced nitrate removal from soil under aerobic conditions. However, control columns had lower concentration of nitrate during first month in 2012 likely due to loss of nitrate by denitrification because of reducing conditions formed prior to other columns.

4.2.6.2. Effect of soil on nitrate leaching

Mean nitrate over 2013-3014 are shown in Figure 4-27. While planted and control columns were not different in nitrate leaching, loam columns had significantly lower nitrate leaching than sandy columns (p=0.02-0.03). The effect of soil was likely due to higher reducing conditions and soil moisture in the loam soil than in the sandy loam soil.

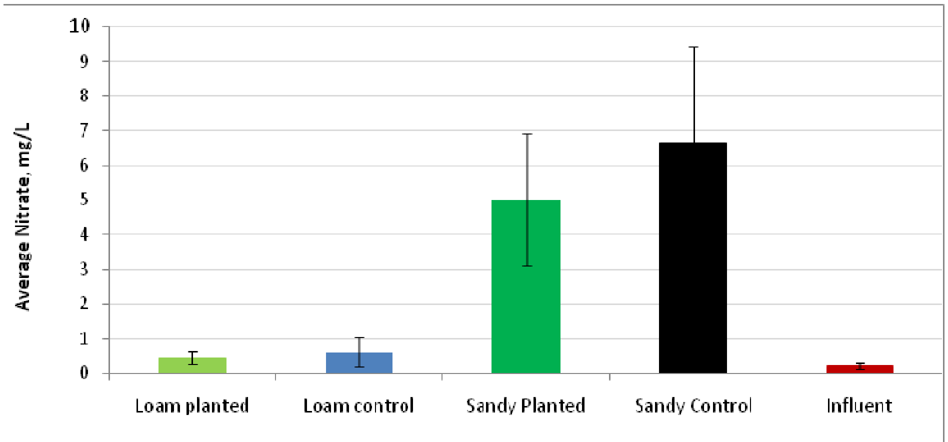


Figure 4-27 Mean nitrate concentrations in the influent and leachate water for all treatments during 2013-2014

4.2.6.3. Effect of poplar trees on ammonium leaching

Leaching and influent ammonium from all columns are shown in Figures 4-28 to 4-29.

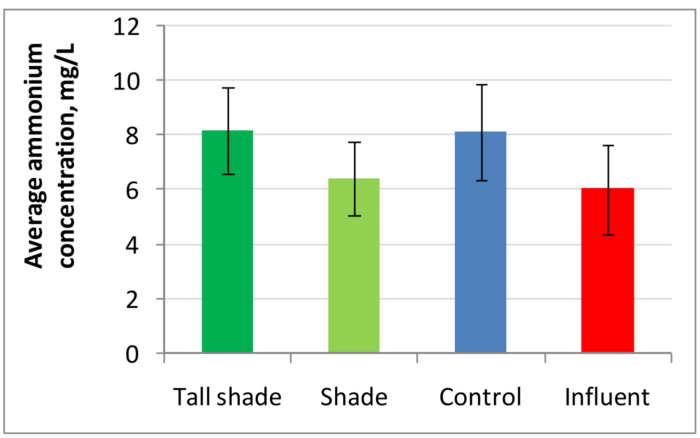


Figure 4-28 Mean ammonium concentration of influent and leachate water samples for 2012

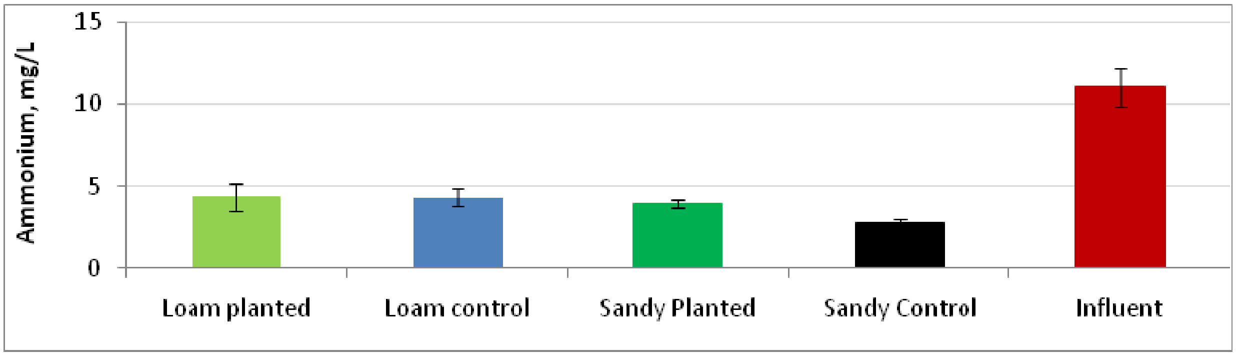


Figure 4-29 Mean ammonium concentration over experimental period in 2013-2014

The ammonium concentration of leachate was higher or similar to that of influent in 2012. Organic nitrogen could have been converted to ammonium nitrogen under reducing conditions. Higher ammonium concentration and low nitrate concentration is characteristics of reducing systems, reinforced by redox potential values and increasing ammonium concentration in leachate samples with time in 2012. The treatment groups and influent were not different among each other ($p=0.70$). Plant's role in ammonium production from organic carbon or oxidation to produce nitrate or volatilization was not observed.

In 2013-2014, ammonium concentration was high in the influent wastewater with a mean of 11.05±1.17 mg/L. However, the concentrations in the leachate were less than that in influent likely due to nitrification or volatilization or anammox or feammox (Trapp et al., 2001, Hu et al., 2011). The planted and control columns did not show distinct trend. Influent had 11.05±1.17 mg/L ammonium, loam planted had 4.36±0.81 mg/L, loam control had 4.33±0.51 mg/L, sandy planted had 3.94±0.26 mg/L and sandy control had 2.73±0.32 mg/L Therefore, there was reduction of 60.54, 60.84, 64.36 and 75.29% of influent ammonium by loam planted, loam control, sandy planted and sandy control columns, respectively. Dunn's group-wise comparison found that influent had lower ammonium than all treatments ($p<0.05$). However, there were no differences among treatments (p=0.08) indicating no plant effect on ammonium concentration of leachate.

4.2.6.4. *Nitrate, ammonia and redox potential*

Redox potential determined fate of nitrate in the columns. Comparison of the leachate concentration of nitrate in 2011 with the soil redox potential [Figure 4-30] indicated that soil redox potential was greater than 300 mV for first month. During first month, high nitrate leachate concentration was observed from columns as aerobic condition in the columns enhanced nitrate leaching. The high initial concentration of nitrate implies that alternating drying and saturation of land application site may mobilize nitrate. When redox potential decreased, nitrate concentrations in the leachate water also decreased due to denitrification which takes place in soil redox potential less than 300 mV. Denitrification used up the nitrate as an electron acceptor from the influent and from the nitrification of ammonium and organic nitrogen rapidly. By mid-May, the nitrate concentration was less than 10 mg/L and redox condition was less than 200 mV.

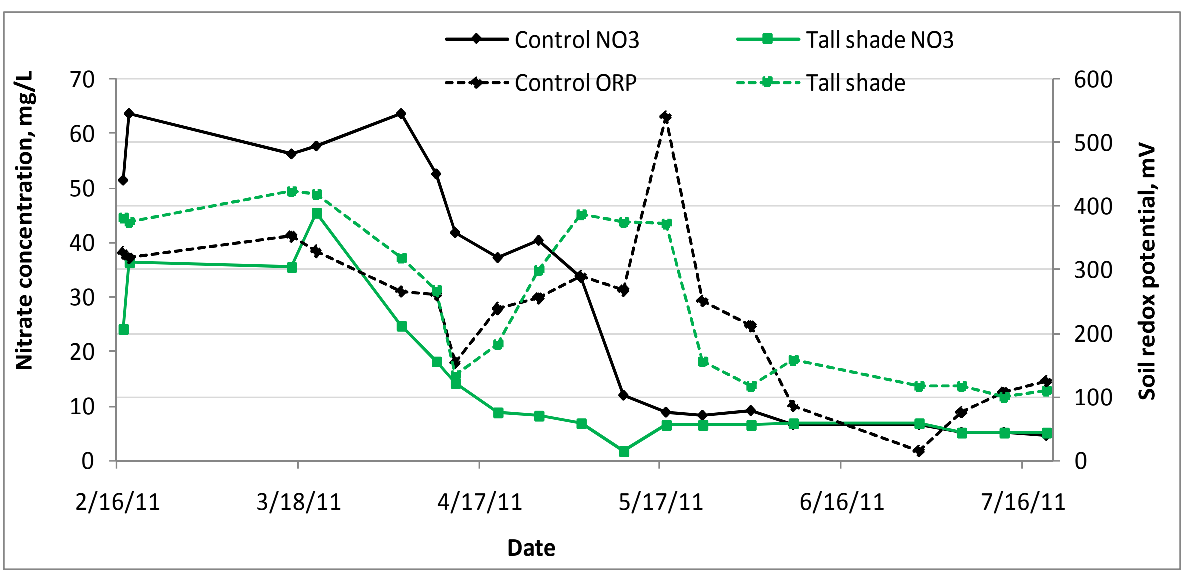


Figure 4-30 Comparison of soil redox potential and nitrate effluent with time in 2011.

Figures 4-31 to 4-32 shows the nitrogen-related processes that occurred in columns in 2012. Ammonium was relatively low and the redox potential was initially high (>200 mV) [Figure 4-32]. The aerobic conditions favored the nitrification that reduced the ammonium in the systems and increased the nitrate [Figure 4-33]. The correlation coefficient between nitrate and ammonium concentration was -0.52 for tall shade, -0.58 for shade and 0.06 for control. However, as the soil redox potential reached below 200 mV in 7/1/2012, nitrate concentrations in columns decreased to below 10 mg/L due to reducing conditions. No new nitrate was formed and the nitrate already formed was used up as electron acceptors during anaerobic respiration by microorganisms. Nitrification could have been inhibited by pH (if less than 6), oxygen, carbondioxide and temperature (Schmidt, 1982).

The concentration of nitrate and redox potential were highly correlated with correlation coefficient of 0.82 for tall shade, 0.70 for control and 0.84 for shade. Conversely, the concentration of ammonium and redox potential was negatively correlated with correlation coefficient of -0.50 for tall shade, -0.47 for shade and -0.32 for control. Therefore, redox potential strongly controlled the nitrate and ammonium concentrations in the leachate.

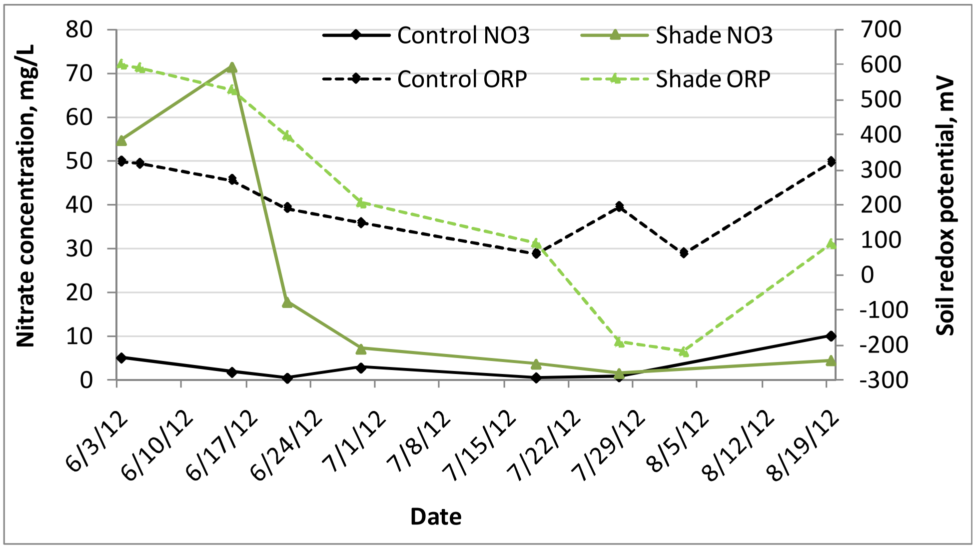


Figure 4-31 Relationship between nitrate in leachate water and soil redox potential in 2012. The correlation coefficient was 0.84 for shade and 0.70 for control.

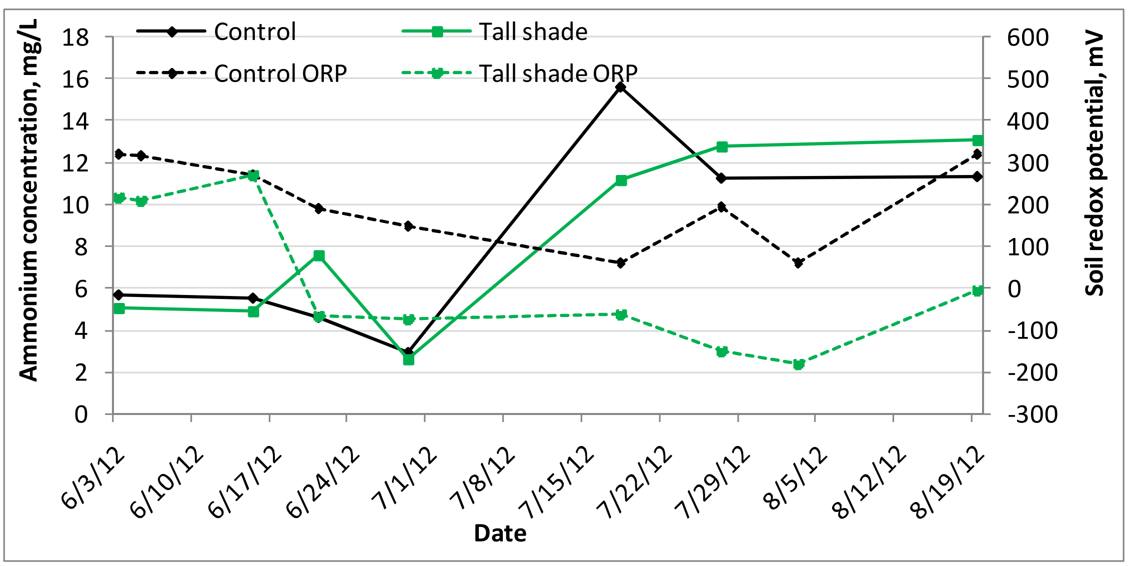


Figure 4-32 Ammonium concentration in leachate water and soil redox potential in 2012. The correlation coefficient was -0.50 for tall shade and -0.32 for control.

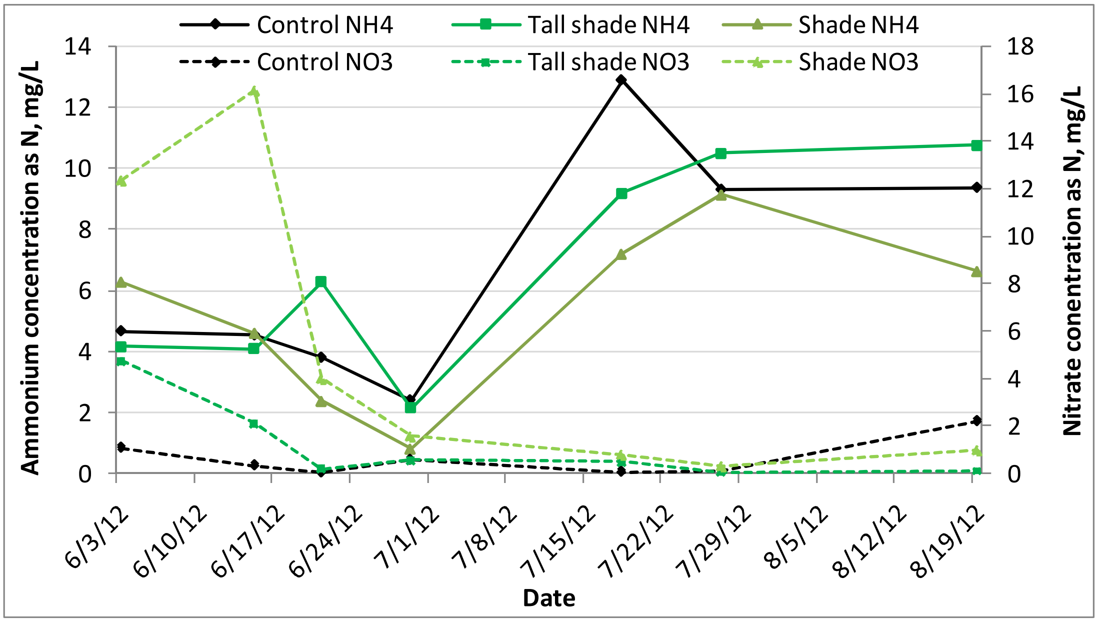


Figure 4-33 Relationship between nitrate concentration and ammonium concentration in 2012. Correlation coefficient was -0.52 for tall shade, -0.58 for shade and 0.06 for control.

4.2.6.5. Nitrogen uptake by poplar trees

Poplar tissues accumulated significantly higher concentrations of nitrogen to tissues than concentration of nitrogen in soil [Figure 4-34]. Poplar shoots accumulated concentrations of nitrogen as high as 1.58% by leaves and 0.53% by stems. The nitrogen concentration in leaves was close to typical nitrogen concentration (1.5%) in plants (ITRC, 2009).

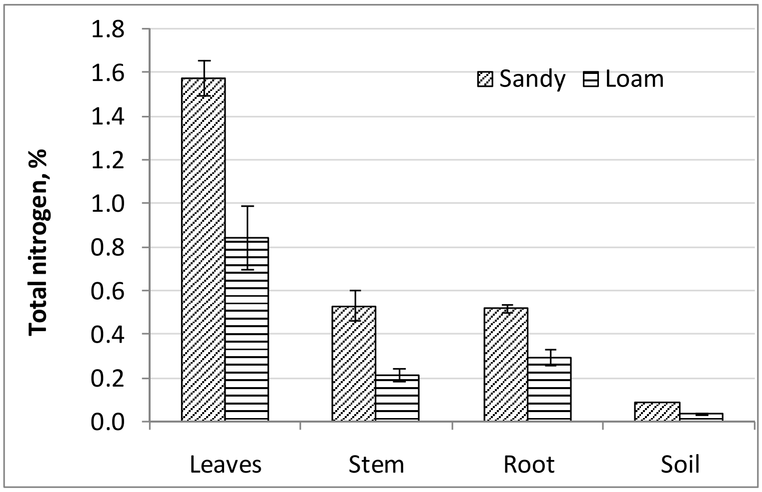


Figure 4-34 Total nitrogen in leaves, stem, root and soil at the end of the experiment 2013-2014. The accumulation by sandy loam columns was significantly higher than that by loamy columns.

Plants at sandy loam significantly accumulated higher concentration of nitrogen in its shoot tissues than plants at loam columns. The low accumulation of nitrogen by poplars in loam soil was due to below optimum nitrogen in soil..

Despite the concentration difference in soil and shoot tissues, the bioaccumulation factor, the ratio of concentration in the shoot tissues to that in soil, and translocation factor, ratio of concentration in shoot tissues to that in root, were similar [Table 4-7]. The similar accumulation factors between poplars in two soils indicated that concentration of nitrogen in shoot tissues would be higher in soil containing high nitrogen than low nitrogen. Additionally, few months old poplar trees in sandy soils may uptake total nitrogen at higher rate after few years.

Table 4-7 Bioaccumulation and translocation factors for nitrogen in 2013-2014.

	Sandy Loam soil			Loam soil		
	Leaves	Stem	Root	Leaves	Stem	Root
Bioaccumulation factor	17.6±0.90	5.92±0.79	5.77±0.23	22.9±4.34	5.86±0.90	7.96±1.16
Translocation factor	3.04±0.20	1.03±0.14		2.87±0.60	0.74±0.13	

Accumulation of nitrogen by poplar trees and leaching of nitrogen from leachate waters contributed to the nitrogen levels in the soils as shown in Table 4-8. There was no noticeable concentration difference between soil before and after experiment. However, the nitrogen in all columns followed a common trend, decrease of the total nitrogen with depth, likely due to denitrification in the more reducing lower layers of soil or plant uptake or leaching. Plants had higher root density in the lower depth of soil and could have uptaken significant nitrogen from lower depth of columns.

Table 4-8 Total nitrogen in the soil before and after 2013-2014 experiment.

Columns	Total Nitrogen, %			
	Before	After		
		30 cm	61 cm	76 cm
Sandy planted	0.10±0.02	0.090±0.000	0.073±0.003	0.067±0.003
Sandy control	0.10±0.02	0.083±0.003	0.067±0.003	0.070±0.000
Loam planted	0.03±0	0.037±0.003	0.033±0.003	0.027±0.003
Loam control	0.03±0	0.033±0.003	0.033±0.003	0.023±0.003

4.2.7. Metals

4.2.7.1. Effect of poplar trees on manganese leaching

Manganese concentrations in the leachate water and influent wastewater are shown in Figure 4-35 to 4-37.

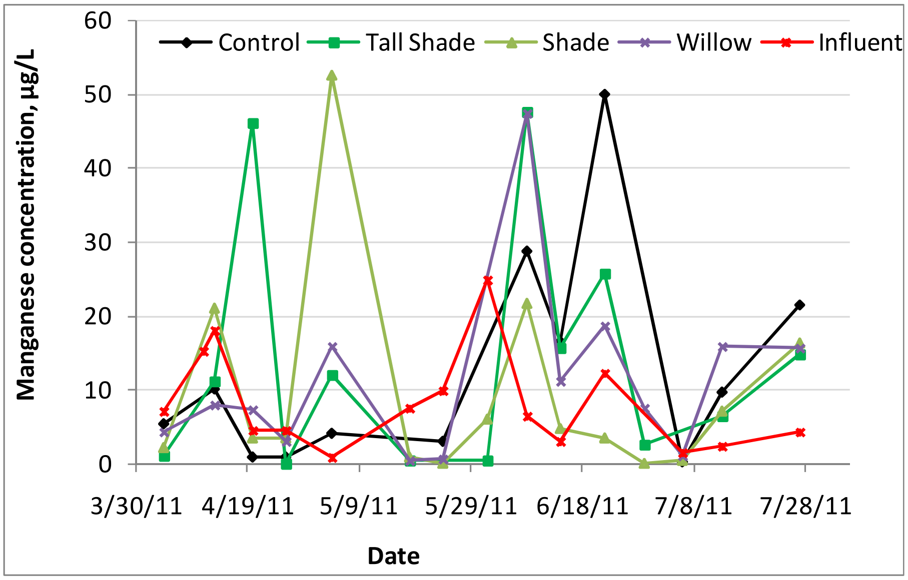


Figure 4-35 Manganese concentration in the influent wastewater and leachate water samples in 2011

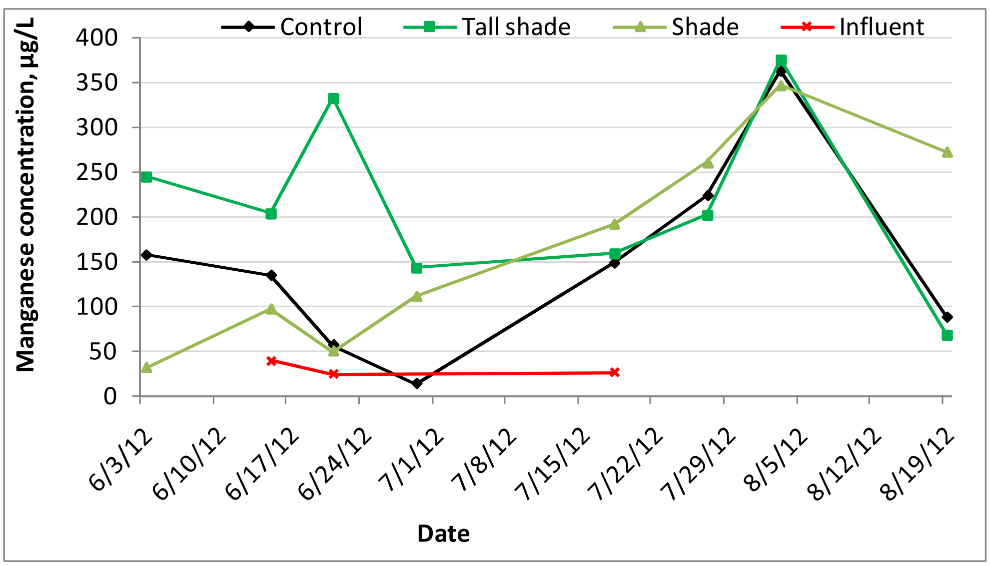


Figure 4-36 Manganese influent concentration of influent and leachate water samples in 2012

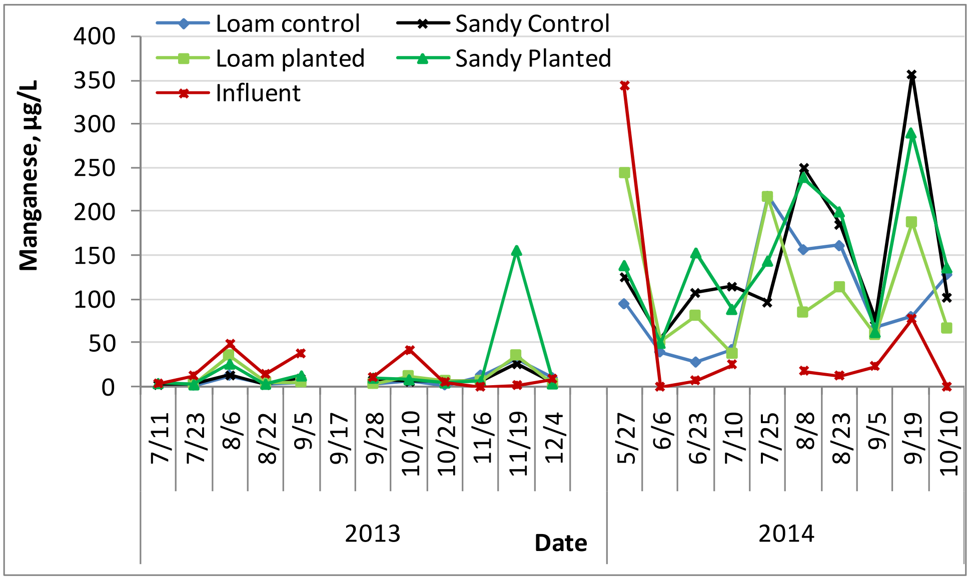


Figure 4-37 Manganese concentration for influent and leachate water samples in 2013-2014

In 2011, no net mobilization of manganese was seen as rank based ANOVA did not find any differences between treatments and influent and among treatments (p=0.88). The mean concentrations of manganese for influent, control, tall shade, shade and willow were 8.19±1.75 µg/L, 12.61±4.27 µg/L, 14.2±4.54 µg/L, 9.59±3.60 µg/L and 11.24±3.23µg/L, respectively. However, the influent was lower than the treatments (p=0.05) indicating the mobilization of manganese from the columns in 2012. Mean manganese for influent, control, tall shade and shade columns were 29.6±4.6 µg/L, 182.2±45.3 µg/L, 253.9±33.2 µg/L and 179.1±44.9 µg/L, respectively in 2012. Similarly, no net mobilization was observed in 2013 and net mobilization was observed in 2014. The MCL of 50 µg/L for manganese exceeded highly in 2012 and 2014, but not in 2011 and 2013.

In 2011, after the trees were established, the manganese concentration for control and planted columns followed similar concentration and trend eliminating any plant effect on mobilization.

Additionally, no plant effects were observed due to similar concentrations among planted and control columns in all years (p>0.05).

4.2.7.2. Effect of poplar trees on iron leaching

Total iron in the influent wastewater and leachate water are shown in Figure 4-38 to 4-40. Concentration of total iron in influent wastewater varied due to use of tap water (minimally processed groundwater), precipitation and/or incomplete dissolution of salts during preparation of synthetic wastewater.

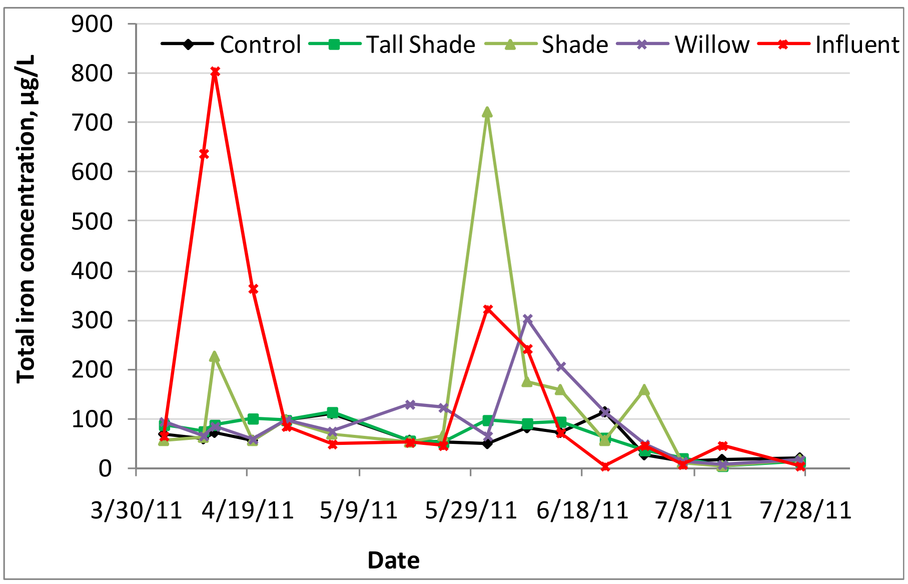


Figure 4-38 Total iron concentration of influent wastewater and leachate water samples in 2011

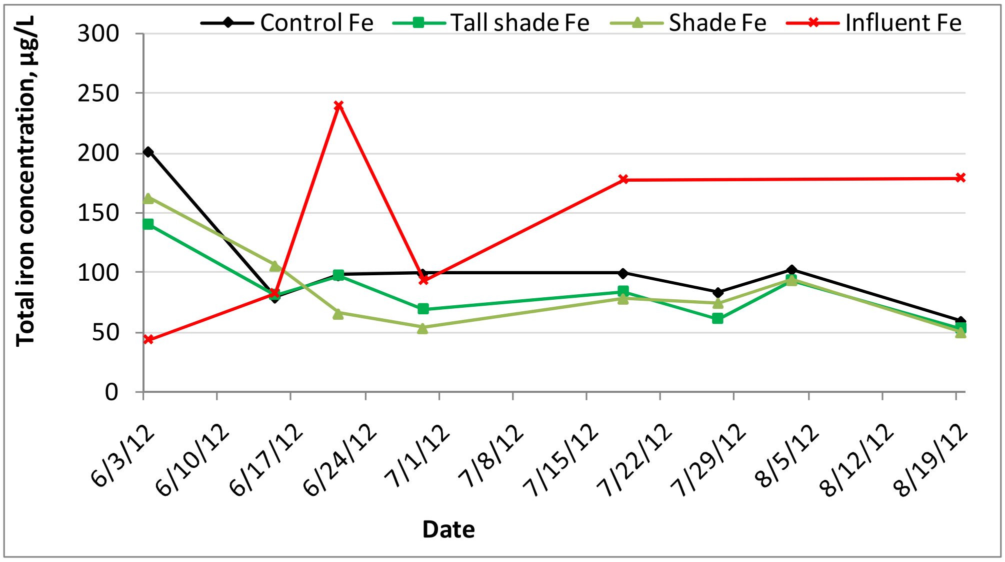


Figure 4-39 Total iron concentration of influent wastewater and leachate water samples in 2012

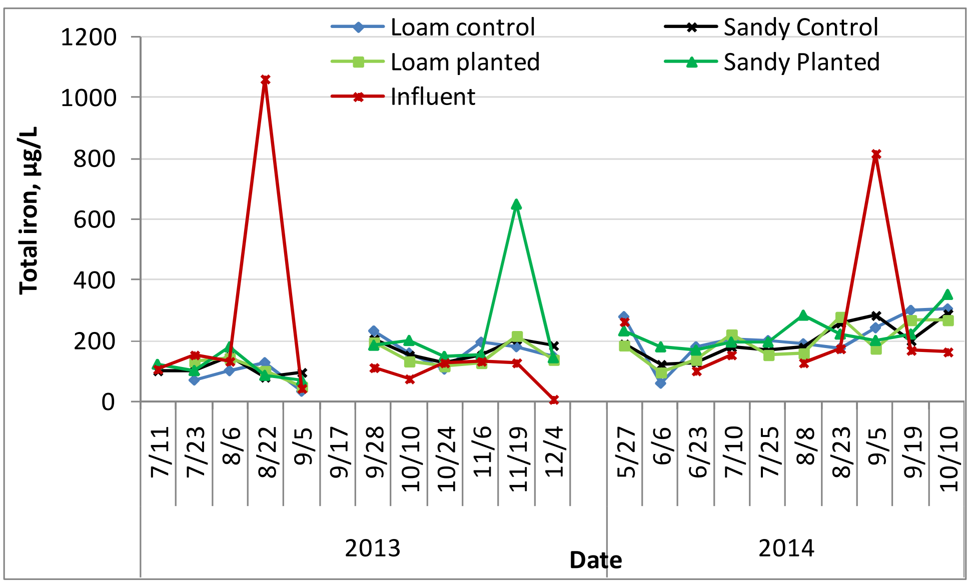


Figure 4-40 Total iron concentration of influent and leachate samples in 2013-2014

In 2011, the mean concentrations of total iron for influent, control, tall shade, shade and willow were 177.69±60.17 µg/L, 60.44±7.62 µg/L, 68.02±8.51 µg/L, 123.83±42.78 µg/L and 93.96±18.72µg/L, respectively. ANOVA on ranks did not show any difference between influent and treatments or among treatments ($p=0.81$) indicating that net mobilization did not occur in the system. In 2012, mean total iron for influent, control, tall shade and shade columns were 135.7±30.2 µg/L, 107.7± 13.7 µg/L, 89.8±8.5 µg/L and 90.8±11.8 µg/L, respectively. In 2013-2014, the mean total iron for influent, loam planted, loam control, sandy planted and sandy control were 212.65±60.62 µg/L, 165.25±13.74 µg/L, 174.81±17.08 µg/L, 204.25±26.18 µg/L and 169.81±12.74 µg/L respectively. No net iron mobilization was obtained in 2012 and 2013-2014 ($p=0.64$). Though mobilization of iron was not observed with respect to the applied influent total iron, dynamic process i.e. oxidation at the top and reduction at the bottom of the column could have occurred.

No treatments had mean iron concentration greater than 300 µg/L, secondary maximum contaminant level (SMCL) for drinking water. Therefore, total iron in leachate samples was not high enough to pose risk to human and the environment. However, occasional leachate samples had higher iron concentrations. Leachate from sandy planted columns on Nov 19, 2013 and all columns in September and October, 2014 exceeded 300 µg/L.

In all years, concentration of total iron in leachate samples from planted columns were similar to that from control columns indicating there was no reduction in metals in the leachate samples by poplar trees despite plant uptake of metals.

4.2.7.2.1 Effect of plants on metal mobilization

Poplar trees did not decrease the iron or manganese concentration in the leachate water despite plant uptake of manganese and iron. Effect of plants on metal mobilization is extremely difficult to predict as a number of factors and their combination determine it (Jacob & Otte, 2003). Root exudates, mainly low molecular organic acids, can modify pH, redox, and microbial biomass (Cervantes et al., 2011). In addition, gas exchange in the rhizosphere can precipitate metals.

Complexation by organic material secreted by roots, oxidation of organic acids secreted by roots and abundance of organic matter in the rhizosphere contribute to lower water soluble and carbonate bound fraction, lower Fe-Mn bound fraction and higher organic and sulfide bound fraction in rhizosphere soils than in non-rhizosphere soils (Wang et al., 2002). Thus, the combination of contradicting processes oxidizing capacity of plants through radial oxygen loss and the reducing capacity through production of exudates or organic matter (Jacob & Otte, 2003) may or may not influence metal concentration in leachate water.

4.2.7.3. Manganese, iron and redox potential

Relationship between manganese concentration in leachate water samples and redox potential are shown in Figures 4-40. In general, the correlation was negative. Correlation coefficient between manganese concentration and redox potential was -0.27 for tall shade, -0.11 for willow, -0.08 for shade and -0.74 for control. Thus, decrease in soil redox potential increased the manganese mobilization. However, the low value of correlation coefficient and no distinct linear relationship between redox potential and manganese concentration [Figure 4-41] indicated that other factors than redox potential controlled metal mobilization. At neutral pH, manganese is primarily reduced at 100-200 mV (DeLaune & Reddy, 2005). However, at higher pH than neutral, manganese is stable as carbonate and other salts and unlikely to get reduced (Jimenez-Carceles et al., 2008). The threshold values for mobilization of Mn was 100 to 300 mV at pH 6-7, 200 to 400 mV at pH $\leq$6-8 and -100 mV at pH 8 (Gotoh & Patrick, 1972).

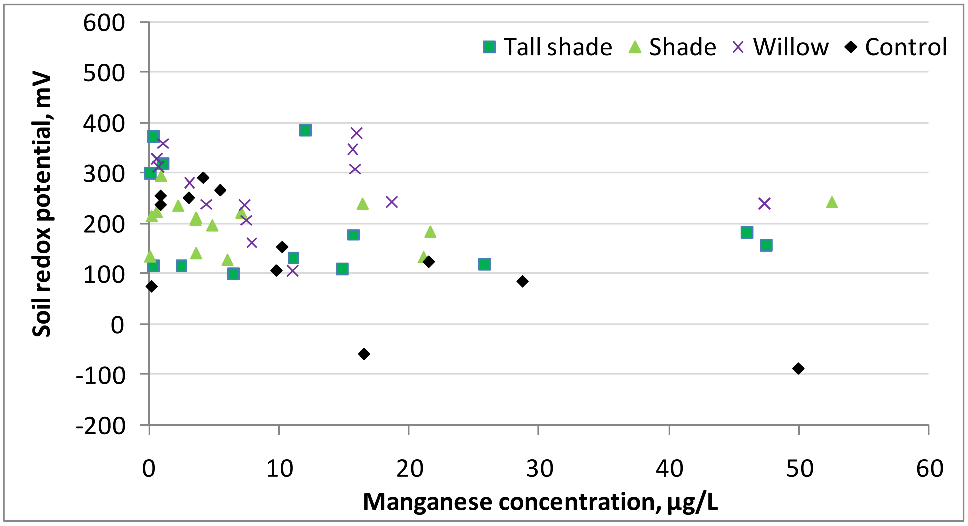


Figure 4-41 Relationship between manganese concentration of leachate samples and soil redox potential for all treatments in 2011.

In 2012, the redox potential was favorable for manganese reduction during the entire duration of water sampling [Figure 4-42]. The manganese concentration increased with decrease in redox potential as indicated by correlation coefficient of -0.19 for tall shade, -0.37 for control and -0.91 for shade. The observed manganese concentration in 2012 confirmed the manganese mobilization. Manganese concentration was up to 360 µg/L. The mobilization could be either due to increase of redox potential from heavily reducing conditions where dissolution of sulfides occurred or decrease of redox potential from oxidizing condition to slightly reducing conditions where reduction of Mn^{4+} to Mn^{2+} occurred (Jimenez-Carceles et al., 2008). The correlation coefficient values also indicated that while redox potential was a factor determining metal solubility, it was not only factor.

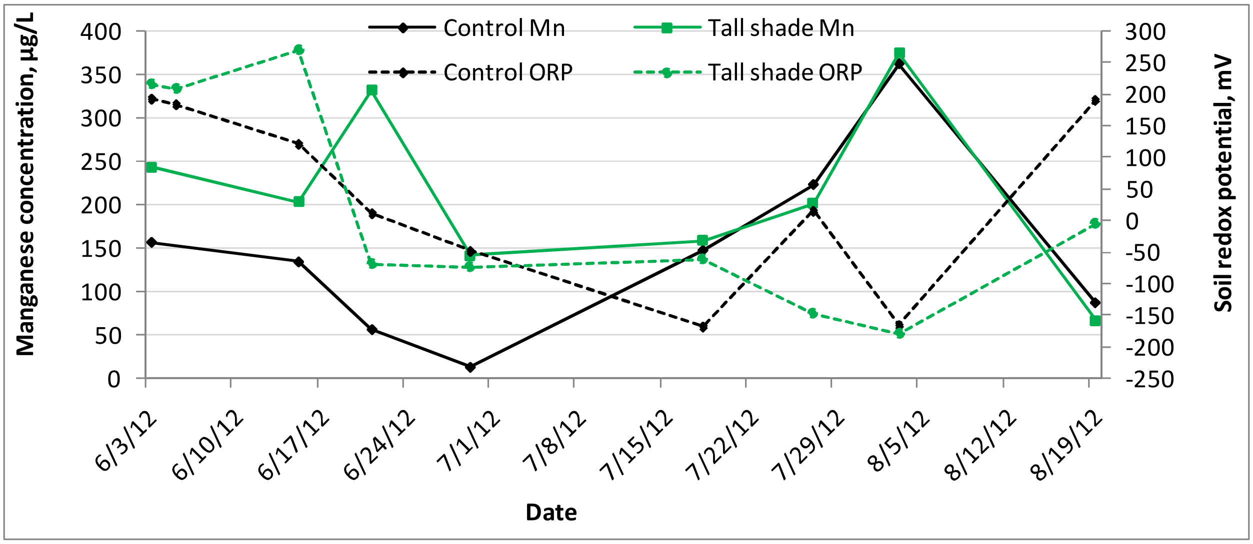


Figure 4-42 Relationship between manganese concentration in leachate water and soil redox potential in 2012. Manganese concentration and redox potential had correlation coefficient of -0.19 for tall shade, -0.37 for control and -0.91 for shade.

From Figure 4-43 to 4-45, redox potential was minimally related to leaching of iron. The correlation coefficient between iron concentration in the leachate samples and redox potential in 2011 was 0.51 for tall shade, -0.53 for shade, -0.48 for willow and 0.04 for control. Therefore, other factors such as high pH or insoluble complexes such as carbonates may have controlled the iron leaching. High pH and strong association of iron (oxy)hydroxides solids by adsorption or co-precipitation or complexation may have prevented the metal mobilization (Bayard et al., 2006, Cappuyns & Swennen, 2008). Release of iron, that occurs through mainly two mechanisms: reductive dissolution at low soil redox potential and by Fe-DOC complexes at high soil redox potential (Cappuyns & Swennen, 2008), may not have occurred. In 2012, when the redox potential was lowest (July 20-August 20), slight decrease in total iron was observed in all columns, likely due to precipitation of sulfides of iron [Figure 4-45].

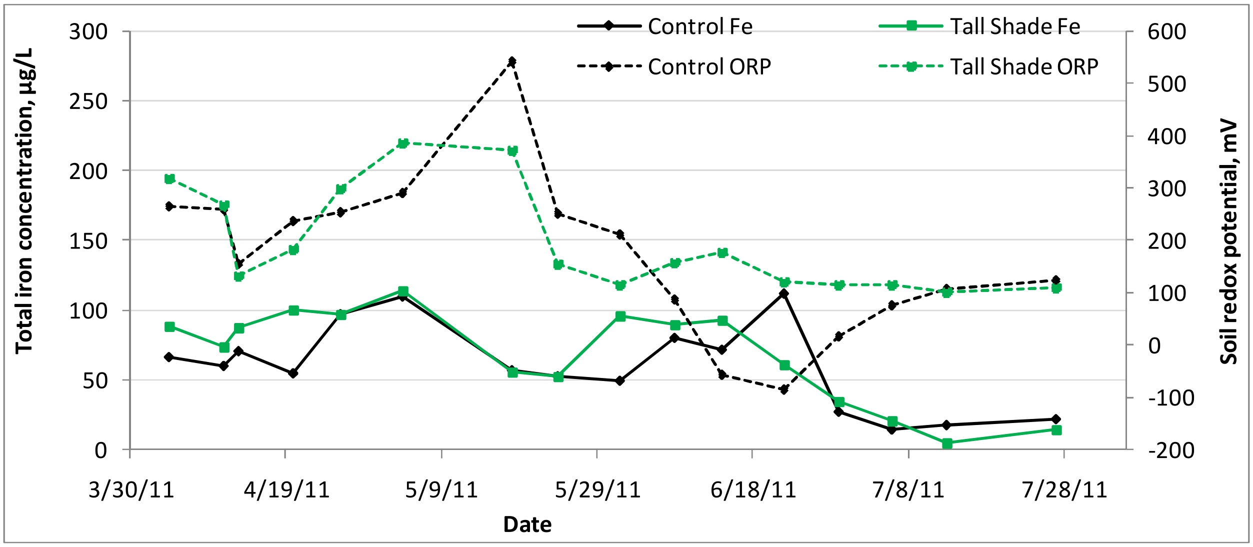


Figure 4-43 Comparison of soil redox potential and total iron concentration in leachate water in 2011.

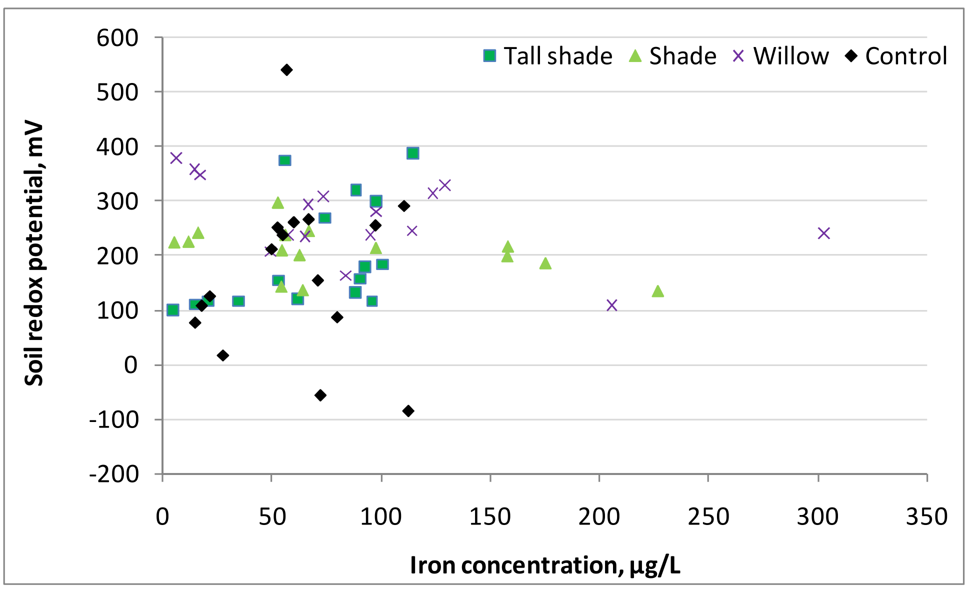


Figure 4-44 Relationship between total soluble iron concentration of leachate samples and soil redox potential for all treatments in 2011.

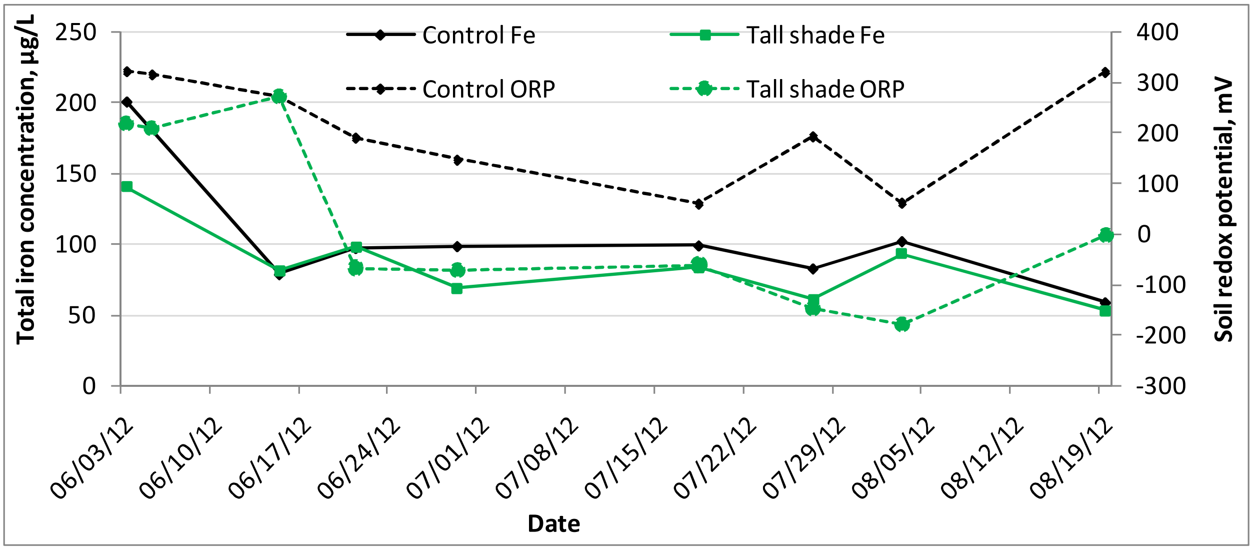


Figure 4-45 Relationship between soil redox potential and total iron in the leachate water in 2012.

4.2.7.3.1 Factors that controlled metal mobilization

Despite redox potential favoring the mobilization of iron and mangagnese during all years, only manganese net mobilization in 2012 and 2014 was observed. Iron was not mobilized despite soil redox potential falling to -250 mV during certain period. The discrepancy between metal mobilization and redox potential could be due to several reasons.

Metal mobilization is controlled by pH, redox potential, metal content, adsorption/desorption, complexation, precipitation/dissolution, salinity, organic matter, sulfer, carbonates and plants (Dassonville & Renault, 2002, Frohne et al., 2011). The critical redox potential values for mobilization of Fe was +300 mV at pH 6, +100 mV at pH 7 and -100 mV at pH 8 (Gotoh & Patrick, 1974). The threshold values for mobilization of Mn was +100 to +300 mV at pH of 6-7, +200 to +400 mV at pH ≤6-8 and -100 mV at pH 8 (Gotoh & Patrick, 1972). Higher pH than neutral obtained in the experiments may have reduced metal availability. The redox reactions (nitrate to nitrogen, Mn^{+4} to Mn^{+2}, Fe^{+3} to Fe^{+2} etc.) consume hydrogen ion, increase pH and enhance sorption of metals on organic matter. Increase in pH makes more negative surface charge and electrical potential on Fe and Mn oxyhydroxides, clay and humus and increases competition for adsorption sites from hydronium ion (Alloway, 2013). In addition, the solubility of Fe and Mn is controlled by precipitation of authigenic minerals including FeS, FeS_2, $MnCO_3$ (Otero et al., 2009). Other mechanisms that may have contributed to low mobilization of manganese and iron could be cation bridging, biosorption, rate of oxidation (Gadd, 2000, Jansen et al., 2003) and green rust formation in the presence of high chloride and sulfate (Maria-Cervantes et al., 2010, Cervantes et al., 2011). Lastly, dynamics of redox potential coupled with limited sampling of leachate water (Frohne et al., 2011) and spatial variation in redox potential (Charlatchka & Cambier, 2000) may have contributed to low metal mobilization.

4.2.7.4. Manganese, iron and arsenic uptake by poplar trees

Manganese, iron and arsenic were uptaken by plants and concentrated in its tissues [Figure 4-46]. Unlike total nitrogen accumulation, iron, manganese and arsenic were accumulated in higher concentrations by plants grown in loamy soils than in sandy soils. The higher uptake in loam soil was likely due to more available metals in loamy columns due to lower soil redox potential and higher soil moisture.

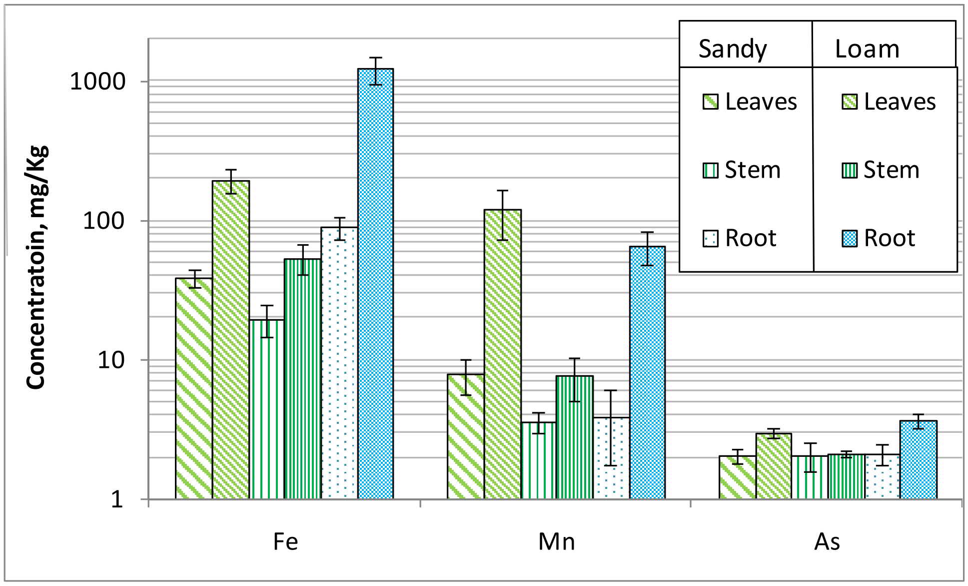


Figure 4-46 Concentrations of metals (Fe, Mn, As) in shoot tissues of plants. Leaves and stems had significantly greater concentrations of metals in plants grown in loamy soils (finer textured bars) than plants grown in sandy soils (coarse textured bars). However, root concentrations were similar.

The initial soil concentration of iron, arsenic and manganese in loam soil was significantly lower, higher and similar to those in sandy soils, respectively [Table 4-10]. Therefore, plants in the loam soil were highly efficient in taking up iron from soil as observed in bioaccumulation factors [Table 4-9]. In contrast, plants in the sandy soils were efficient in taking up arsenic.

Table 4-9 Bioaccumulation and translocation factors for metals (arsenic, iron and manganese) in 2013-2014. Error bars indicate standard error of mean.

		Bioaccumulation factors			**Translocation factors**		
		Fe	Mn	As	Fe	Mn	As
Sandy	**Leaves**	1.08±0.25	0.13±0.04	0.72±0.17	0.43±0.10	2.01±1.25	0.96±0.20
	Stem	0.55±0.17	0.06±0.01	0.73±0.22	0.22±0.07	0.92±0.53	0.98±0.28
	Root	2.52±0.63	0.06±0.04	0.75±0.19			
Loam	**Leaves**	7.04±2.41	0.86±0.38	0.10±0.01	0.16±0.05	1.84±0.88	0.81±0.12
	Stem	1.94±0.71	0.06±0.02	0.07±0.00	0.04±0.01	0.12±0.05	0.58±0.08
	Root	44.1±15.7	0.47±0.15	0.13±0.02			

The translocation factors indicated that plants in sandy columns were more efficient in translocating iron, manganese and arsenic to shoot tissues from roots than those in loam columns. Lower redox potential leads to stress in the plants as radial oxygen loss (ROL) or oxygen transported through aerenchyma to the rhizosphere can be used up during process of respiration (Pezeshki & DeLaune, 2012). Age of plants (older), more reducing conditions and more soil moisture might have stressed the plants in loam soil and resulted in the lower translocation.

Soil concentrations of manganese, iron and arsenic at various depths in the columns were assessed [Table 4-10]. The results indicated that manganese and arsenic in the loam planted columns increased with depth likely due to downward movement of metals. The plants in loam columns were growing and may have precipitated the reduced species flowing down the columns by oxidizing the soils through aeration from roots. Iron in all columns decreased considerably at the end of the experiment than at the beginning. All other concentrations remained relatively constant. Provided the low bioaccumulation factor of iron and low leaching of iron in the leachate water, the decrease of iron in the soil was confounding.

Table 4-10 Soil concentrations of metals (As, Mn, Fe) before and after experiment

	Mn, ppm				Fe, ppm				As, ppm		
	Before	After			Before	After			Before	After	
		1 ft	2 ft	2.5 ft		1 ft	2 ft	2.5 ft		1 ft	2.5 ft
Sandy planted	77.6±2.1	60.8±2.0	62.8±5.6	59.1±0.3	84.1±5.2	35.4 ±6.4	37.2 ±5.8	40.1±5.6	3.15±0.15	2.85±0.55	3.56±0.70
Sandy control	77.6±2.1	59.3±3.1	70.0±4.2	61.4±6.7	84.1±5.2	28.9 ±6.0	21.3 ±7.5	28.1±3.5	3.15±0.15	2.49±.63	2.56±0.26
Loam planted	71.3±4.70	138.7 ±26.1	204.1 ±4.7	288.8 ±43.0	27.7±7.8	9.8±2.3	14.0 ±3.7	10.2±0.9	28±2.0	28.43±0.20	30.44±1.28
Loam control	71.3±4.7	110.3 ±32.5	111.1 ±30.9	109.9 ±14.3	27.7±7.8	10.7 ±4.4	8.2±0.7	11.1±0.5	28±2.0	26.96±1.05	27.67±2.79

4.2.8. Microbial community

PLFA analysis of soil samples was performed to determine the soil microbial biomass and community across depths (0-30 cm and 61-76 cm) and among treatments.

4.2.8.1. Effect of poplar trees on microbial biomass

Phospholipid fatty acid analysis quantified as many as 38 PLFAs out of the total identified 42 fatty acids from C10:0 to C24:1w9, comparable to 37 fatty acid up to C-20 length obtained in uncontaminated sandy loam forest soil (Frostegard et al., 1993) and 38 PLFAs in sediment of harbor (Harji et al., 2010) and less than 63 PLFAs in sediments of Hiroshima Bay. The fatty acid included saturated fatty acids (C10:0 to C24:0), branched fatty acids, mono-unsaturated fatty acids and poly-unsaturated fatty acids. The total fatty acid is an indicator of total biomass in the soil as 1 mmol of PLFA is equivalent to 5.9exp13 cells or 10 g bacteria (Rajendran et al., 1992). The total fatty acids for the columns at different depths are given below [Table 4-11]. The total PLFA in columns in the soil were less than or comparable to 162.5 nmol/g obtained by Yannikos et al. 2014 in poplar rhizosphere (Yannikos et al., 2014), 65 nmol/g in arable soil (Frostegard et al., 1993) and 3.74-186.4 nmol/g soil in beech forest (Frostegard & Baath, 1996).

Table 4-11 Total phospholipid fatty acid yield per gram of soil taken from 0-30 cm and 61-76 cm depths from the columns. The values are mean±standard error of mean (n=3).

Total PLFA	Sandy planted		Sandy control		Loam control		Loam planted	
	30 cm	**61 cm**	**30 cm**	**61 cm**	**30 cm**	**61 cm**	**30 cm**	**61 cm**
µg/g soil	18.1±6.0	28.6±8.9	90.7±30.4	49.0±16.2	63.7±21.3	31.0±10.3	42.6±13.2	40.3±13.0
nmol/g soil	58.0±19.2	91.7±28.6	290.7±97.4	157.1±61.9	203.4±68.4	99.2±32.9	136.4±42.3	129.1±41.7

The total biomass at 30 cm was higher than that at 61 cm in sandy control, loam control and loam planted columns likely due to higher carbon accumulation and oxygen. Lower microbial biomass at deeper depth and less decrease in total biomass with depth for sandy loam than loam has been previously reported (Vestal & White, 1989). Poplar trees in the loam columns enhanced the microbial biomass at 61 cm, but not at 30 cm. Effect of poplar trees in sandy loam soil were disregarded due to small plants (15 to 60 cm) and shallower roots. Therefore, effect of poplar trees on microbial growth was minimal in column soils. The hypothesis that poplar trees increases microbial biomass in the soil was not true at 30 cm and true at 61 cm in columns.

4.2.8.2. Effect of poplar trees on microbial diversity

Diverse communities of microorganisms, both aerobic and anaerobic, both prokaryotes and eukaryotes and both gram positive and gram negative bacteria were present in soils. Groups of specific fatty acids based on classification of functional groups (Findlay & Dobbs, 1993, Ibekwe & Kennedy, 1999, Steer & Harris, 2000, Buyer, 2003, Lee et al., 2004, Hinojosa et al., 2005, Kaur et al., 2005, Harji et al., 2010, Ruess & Chamberlain, 2010) along with their % abundance are shown in Table 4-12. Some of the PLFAs may fall under multiple functional groups which have been considered in only one functional group.

Table 4-12 Abundance (% relative abundance) of PLFAs grouped under different functional groups. Values provided are mean±standard error of mean (n=3).

	Loam control		Loam planted		Sandy control		Sandy planted	
	30 cm	**61 cm**	**30 cm**	**61 cm**	**30 cm**	**61 cm**	**30 cm**	**61 cm**
Microeukaryotes								
C18:3w3	12.4±7.2	8.6±4.9	13.6±7.9	10.6±6.1	10.4±6.0	10.7±6.2	10.7±6.2	9.1±5.2
C18:3w6	0.0	0.0	0.0	0.1±0.1	0.1±0.1	0.0	0.1±0.1	0.0
C20:0	0.1±0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4±0.2
C20:3w3	11.5±6.6	8.1±4.7	15.1±8.7	10.5±6.1	9.7±5.6	10.3±6.0	2.1±1.2	6.5±3.7
C20:3w6	0.6±0.3	0.6±0.4	0.8±0.5	0.5±0.3	0.5±0.3	0.5±0.3	0.7±0.4	0.6±0.3
C20:4w6	0.2±0.1	0.4±0.2	0.4±0.2	0.2±0.1	0.3±0.1	0.2±0.1	0.4±0.2	0.4±0.2
C20:5w3	0.1±0.0	0.0	0.1±0.0	0.1±0.0	0.0	0.0	0.0	0.0
C22:0	0.6±0.4	0.7±0.4	0.9±0.5	0.6±0.4	0.6±0.3	0.7±0.4	0.8±0.5	0.5±0.3
C22:2w6	9.6±5.6	7.0±4.0	12.8±7.4	9.0±5.2	8.1±4.7	8.9±5.1	3.2±1.8	5.4±3.1
C22:6w3	2.2±1.3	0.2±0.1	0.6±0.4	0.4±0.2	0.2±0.1	0.5±0.3	0.3±0.2	0.2±0.1
C23:0	8.1±4.7	5.9±3.4	10.5±6.0	7.4±4.3	6.5±3.8	7.0±4.0	6.4±3.7	4.4±2.6
C24:0	0.6±0.4	4.7±2.7	1.0±0.6	2.7±1.6	0.7±0.4	0.7±0.4	0.6±0.3	0.3±0.1
Total	**45.9±12.2**	**36.3±9.1**	**55.8±15.2**	**42.2±11.1**	**37.1±10.2**	**18.7±10.8**	**25.4±7.6**	**27.7±7.6**
Aerobic prokaryotes and eukaryotes								
C14:1w5	0.0	0.1±0.1	0.0	0.0	0.1±0.0	0.0	0.2±0.1	0.1±0.0
C16:1w7	3.0±1.7	1.2±0.7	0.2±0.1	1.9±1.1	4.6±2.7	4.1±2.4	5.1±2.9	6.9±4.0
C17:1w7	0.0	0.0	0.0	0.2±0.1	0.2±0.1	0.2±0.1	0.0	0.0
C18:1w9 C+T	2.7±1.5	1.3±0.8	0.3±0.2	1.6±0.9	7.6±4.4	5.1±2.9	6.8±3.9	6.8±3.9
C18:2w6 C+ T	8.3±4.8	7.0±4.0	9.8±5.7	7.5±4.3	7.0±4.0	7.0±4.1	6.8±3.9	5.6±3.2
C20:1w9	0.2±0.1	0.7±0.4	0.3±0.2	3.6±2.1	0.5±0.3	0.1±0.1	0.2±0.1	0.1±0.0
C20:2w6	12.1±7.0	18.1±10.4	16.1±9.3	13.5±7.8	10.5±6.1	12.7±7.3	9.5±5.5	7.0±4.0
C22:1w9	0.4±0.2	1.4±0.8	0.6±0.3	0.4±0.2	0.4±0.2	0.4±0.2	0.4±0.2	0.3±0.2
C24:1w9	5.1±2.9	4.2±2.4	6.3±3.6	4.5±2.6	3.9±2.3	4.5±2.6	3.9±2.2	3.4±2.0
Total	**31.7±9.3**	**20.0±11.5**	**33.6±11.5**	**16.7±9.6**	**34.9±9.2**	**16.5±9.6**	**32.9±8.6**	**13.6±7.9**
Gram positive prokaryotes and other anaerobic bacteria								
C14:0	0.0	0.0	0.0	0.2±0.1	0.3±0.2	0.2±0.1	0.3±0.2	0.5±0.3
C15:0	0.0	0.7±0.4	0.1±0.0	0.6±0.3	0.7±0.4	0.5±0.3	1.1±0.6	1.1±0.6
a-15:0	0.9±0.5	0.3±0.2	0.2±0.1	0.7±0.4	3.0±1.7	1.7±1.0	3.8±2.2	3.1±1.8
i-15:0	1.0±0.6	0.0	0.1±0.0	0.5±0.3	1.4±0.8	1.1±0.6	1.9±1.1	2.2±1.3
i-16:0	0.3±0.2	0.7±0.4	0.5±0.3	0.5±0.3	1.4±0.8	1.2±0.7	2.0±1.2	2.2±1.3
i-17:0	0.5±0.3	0.0±0.0	0.0	0.2±0.1	1.3±0.8	0.5±0.3	1.4±0.8	1.2±0.7
Total	**2.7±0.8**	**1.7±0.6**	**0.8±0.3**	**2.7±0.7**	**8.0±2.2**	**5.2±1.4**	**10.4±2.9**	**10.3±2.7**

Table 4-12 (cont'd)

	Loam control		Loam planted		Sandy control		Sandy planted	
	30 cm	**61 cm**	**30 cm**	**61 cm**	**30 cm**	**61 cm**	**30 cm**	**61 cm**
Sulfate reducing bacteria and other anaerobic prokaryotes								
2-OH C12:0	0.9±0.5	0.0	0.0	0.6±0.3	0.0	0.0	1.1±0.6	2.3±1.3
C16:0	4.2±2.4	2.3±1.3	0.3±0.2	2.4±1.4	4.2±2.4	3.3±1.9	5.0±2.9	6.1±3.5
3-0H C12:0	2.5±1.5	2.1±1.2	2.0±1.2	2.0±1.2	2.8±1.6	2.7±1.5	3.7±2.1	3.9±2.2
C17:0	0.5±0.3	0.1±0.1	0.0	0.3±0.1	0.4±0.3	0.2±0.1	0.6±0.3	0.3±0.2
17:0 cy	1.2±0.7	0.3±0.2	0.1±0.0	1.0±0.6	1.2±0.7	0.9±0.5	1.5±0.8	2.2±1.2
C18:0	6.0±3.5	5.0±2.9	5.4±3.1	4.6±2.7	4.4±2.5	4.6±2.6	5.1±3.0	4.6±2.7
C19:0	2.8±1.6	17.4±10.1	1.2±0.7	9.5±5.5	5.3±3.0	8.7±5.0	12.7±7.3	10.9±6.3
C19:0 cy	1.1±0.6	0.0	0.2±0.1	0.7±0.4	0.4±0.2	0.2±0.1	0.3±0.2	0.1±.1
2-OH C16:0	0.3±0.2	0.6±0.4	0.5±0.3	0.3±0.2	0.5±0.3	0.5±0.3	0.4±0.2	0.9±0.5
Total	**19.6±4.9**	**28.0±10.6**	**9.6±3.4**	**21.4±6.4**	**19.2±5.0**	**21.0±6.2**	**30.4±8.8**	**31.3±8.2**

Microeukaryotes originate from plant roots (example: 18:1w9, 18:3w3, 20:5w3), algae (18:3w3, 18:1w9), diatoms (20:5w3) or animal origin such as protozoa (20:3w6, 20:4w6) (Vestal & White, 1989). Microeukaryotes were high in abundance in all plots (18.7 to 55.85%). Except for sandy planted, all columns had higher microeukaryotes at 30 cm than at 61 cm. Loam planted columns had 9.9% and 5.9% higher relative abundance of microeukaryotes than loam control.

The general fungi marker 18:1w9 or 18:2w6 (Hinojosa et al., 2005) and 16:1 (Kaur et al., 2005) were also found in high abundances. The sum of fungi and aerobic prokaryotes accounted for 13.6 to 34.9% of total microorganisms in abundance. Presence of large abundances of fungi indicated that aerobic activity was occurring in soils. Aerobic activity explained the high carbon removal rate observed in the columns. The total aerobic biomass was less at 61 cm than at 30 cm for all columns. The lower biomass at 61 cm was expected due to less oxygen diffusion and higher moisture than at 30 cm. Both planted and control plots had similar abundances of aerobic microbial biomass indicating no effect of plants on aerobic microbial biomass. The effects of plants if any may have been limited by the heterogeneity of soils and limited or no plant growth.

In contrast, poplar trees enhanced fungal but bacterial species than alfalfa in PLFA analysis of soil profile (Yannikos et al., 2014).

As shown in the Table 4-12, biomarkers of sulfate reducing and anaerobic bacteria were present in the column soils. These organisms were present in approximately similar relative concentrations to fungi and aerobic prokaryotes. These organisms contributed to very high biomass proportions in the columns, ranging from 10.6% to 41.6%. The anaerobic bacteria, both gram positive and gram negative, increased significantly in proportion with depth in all columns except sandy planted. Presence of higher biomass of anaerobic microorganisms including sulfate reducing bacteria at deeper soil depth was also supported by lower redox potential values at deeper depths. The relative increase was pronounced for loamy soils consistent with higher soil moisture, higher redox potential and finer soil particles. Loamy control had higher relative proportion of sulfate reducing bacteria and anaerobic bacteria than loamy planted (9.6 for planted vs 19.6 for control at 30 cm and 21.4 for planted vs 28% for control at 61 cm). Lower contribution of anaerobic bacteria in planted columns than in control columns indicate more anaerobic activity occurring in control columns and likely more metal mobilization.

The clustering of columns based on the relative presence of fatty acids using the UPGMA method and Euclidean distance of standardized data resulted in dendrogram shown in Figure 4-47. The agglomerative coefficient was 0.71 and the correlation with Cophenetic distance using was 0.78. Mantel test showed that the clustering was statistically significant (p=0.0009).

At 30 cm, all columns were different based on the pattern of the PLFAs. The aerobic prokaryotes and fungi in all three columns (Loam control, loam planted, sandy planted) were high. Therefore, the top of the columns, represented by groups at the top of the dendrogram, were very different from the bottom. The bottom clusters were separated based on abundances of anaerobic bacteria. Loamy control columns at 61 cm were different than loamy planted and sandy control at 61 cm. Thus, separation of loamy planted columns from loamy control columns in clustering also supported the role of poplar trees.

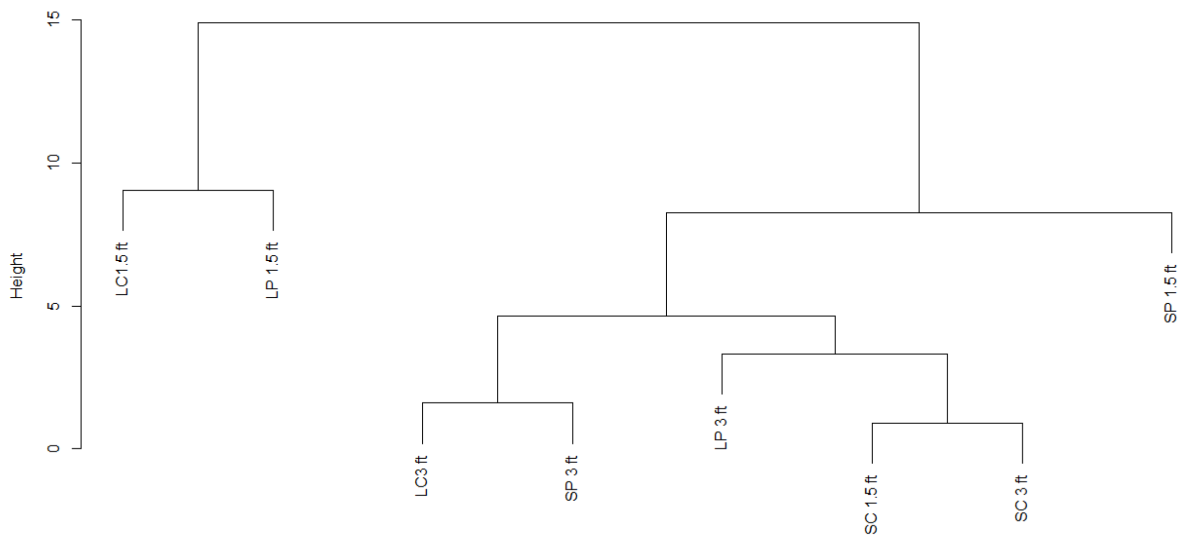


Figure 4-47 Clustering of columns using UPGMA algorithm based on Euclidean distances for standardized PLFA data. SP, SC, LP and LC represent sandy planted, sandy control, loamy planted and loamy control columns.

Therefore, both total microorganisms and aerobic microorganisms decreased with depth. However, anaerobic microorganisms increased with depth. Poplar trees influenced relative presence of microbial populations in the soil. Microeukaryotes, fungi and aerobic prokaryotes and anaerobic bacteria in planted columns were higher, similar, and lower than those in control columns for loam soil, respectively. The presence of different types of microorganisms in soils supported the consumption of nitrate, sulfate and oxygen by microbial community simultaneously to remove organic carbon in wastewater. Similar diversity and DOC treatment was observed by (Essandoh et al., 2013) in the column soils.

4.2.9. Strengths and limitations of the study

The columns provided accurate measurements of water balance that otherwise could not have been achieved in actual field. Columns provided control over variables such as soil, wastewater composition and application rate and facilitated measurement of continuous redox potential and sampling of soil and water. The results of the experiment should well represent the geochemical, microbial and hydrologic processes occurring in the actual site except for temperature effects.

Contrarily, the greatest limitation of this study was poor poplar growth in 2013 or 2014. Colder than average and prolonged winter in 2013-2014 killed the plants in the sandy columns. The growth of the trees in loam columns also did not occur well. Poor nutrients in the loam soil could have also contributed to poor plant growth in loam columns. The temperature data in the columns showed that the soil was frozen completely in the winter at all levels in the columns. The frozen temperature limited the biological activity in the columns unlike in an actual site where soil is not frozen below certain depth and biological activity continues to occur.

Because moisture balance was not possible in 2011 or 2012, the evapotranspiration assessed in 2013-2014 was for young poplars and/or for poplars without optimal growth. While the data when poplars were not growing well was disregarded, it also limited the quantity of data to support or refute hypothesis. Additionally, temperature profile was not the true representative of the actual field due to freezing of the column soil even at its bottom. Use of columns also affected the rooting pattern and likely some moisture flow and retention. The roots were found highly dense at the bottom of the columns. Water content was usually higher and roots were confined within a closed environment compared to field conditions, possibly magnifying the effect of roots in a column experiment (Maria-Cervantes et al., 2010). The collection rate of leachate water was used to monitor the preferential flow. Preferential flow through channels or along the edges of the columns occurred at times. The abundance of roots at the top 30 cm of the columns and bottom of the columns may have been due to preferential flows.

Other limitations include the one-time analysis of plant parts for metals and of soil for microbial analysis. Any dynamics of metal uptake and change in soil microbial composition was not captured by the study.

4.2.10. Conclusions

Poplar trees significantly evapotranspired higher moisture than control. Sandy loam and loam columns with poplar trees removed 1.55±0.23 and 1.42±0.11 times greater moisture than by control columns, respectively. The second year value of the sandy planted columns was not used due to death of plants in spring of 2014. Evapotranspiration coefficient of poplar trees in loam columns decreased from 1.50 in first year to 1.36 in the second year due lack of nutrients and poor growth. The lack of nutrients indicated that nutrients in the wastewater were not enough for poplar trees.

The reduction of soil moisture in columns was mainly seen at top (30 or 46 cm) and bottom (76 or 122 cm) depths, consistent with observed dense roots in top 30 cm and bottom of the columns. However, rooting pattern along depths needs further consideration.

Sandy soils achieved greater than 90% efficiency of COD treatment during all years. Loamy soil had lower treatment efficiency than sandy soils and ranged from 69% to 85%. Therefore, selection of site is crucial to achieving higher treatment efficiency of carbon. While increasing sand content is advantageous, it should be done within the limit so that nutrients and moisture in the soil can support the grasses and/or trees. In the site with fine textured soil, planting poplar trees could aid the carbon treatment. Even with poplar plantation, application rate need to be lower in the finer textured soils. This experiment had the same rate of application for sandy loam and loamy soils. Many times in 2013, loamy soils were flooded. Therefore, in 2014, the application rate was halved. While frequency of flooding was not the same as in 2013, it was considerably low in 2014.

Platinum sensors were fabricated in the laboratory, tested and used to study the redox dynamics. The redox potential accurately predicted processes that control nitrates and ammonium. However, mobilization of metals was not accurately predicted by redox potential despite the high correlation. The research results demonstrated that metal mobilization may not occur at land treatment sites despite low redox potential. Regarding the plant effect on redox potential increase, inconsistent results were observed. Soil redox potential was higher in planted columns in 2011, but reverse was true in 2012. In 2013-2014, planted and control columns were not different on the redox conditions.

Diverse groups of microorganisms were present in the soils. The microbial diversity included microeukaryotes, aerobic prokaryotes and eukaryotes, fungi, anaerobic prokarytoes, sulfate reducing bacteria, gram positive prokaryotes. Total biomass and aerobic microorganisms were mostly higher at surface soils than at deeper soils (except one instance). There were significant relative abundances of aerobic bacteria and anaerobic bacteria. Anaerobic bacteria were higher in the deeper soils as expected. Minimal positive influence on biomass was seen as the result of the poplar trees. Loamy planted columns had lower anaerobic microorganisms. Clustering with UPGMA method for PLFA data grouped columns very well, especially at 30 cm depth.

Metals (manganese and iron) usually were not mobilized to very high concentrations. Plants did not show significant effects on either reduction of leachate concentrations or soil concentrations of metals. Though, no significant effects were observed, plants accumulated large concentrations of metals (arsenic, manganese and iron) on to its shoot tissues. This may translate into significant reduction of metals when the poplar biomass is high.

For nitrate, there were strong indications that poplar trees enhanced the removal. First, there was high nitrate uptake with similar bioaccumulation factor despite differences in soil concentrations. Second, poplar trees reduced leaching of nitrate when the columns had high nitrate concentration in leachate water due to aerobic conditions in 2011 and 2013.

4.3. Field experiment

Following were the objectives of the field experiment under application of food processing wastewater.

1. Evaluate treatment of chemical oxygen demand (COD) in wastewater by poplar trees
2. Quantify evapotranspiration and moisture reduction of soils by poplar trees
3. Evaluate the effect of poplar trees on soil microbial biomass and community
4. Assess the decrease of metals and nitrates mobilization including plant uptake of nitrate and metals.

The results of plot P2 represented planted and of plot P1 represented poplar under high loading. The results of the field experiment are categorized to four sections, each for an objective.

4.3.1. Carbon treatment

The primary objective of the land application of food processing wastewater is to treat high carbon in the wastewater. Biochemical oxygen demand of the influent and chemical oxygen demand of the leachate water from May to November, 2013 are shown in Figure 4-48. The BOD of the leachate water would be higher than the COD, however, no measurement was made. One report suggested that COD was equal to 74% of BOD in food processing wastewater (Esvelt, 1970).

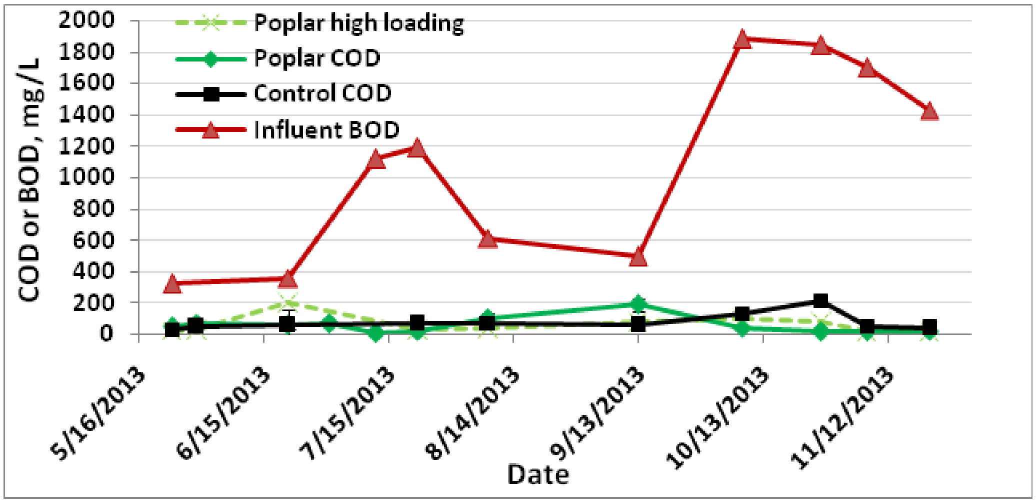


Figure 4-48 COD of leachate water collected at 0.9 m and 1.8 m below ground surface

As expected, the biochemical oxygen demand (BOD) of the influent varied considerably from 322 mg/L to 1880 mg/L during May to November, 2013. Even at the same factory, characteristics of the influent varies with the food being processed, processing operation and storage time of the wastewater in the reservoir (Bruner & Burgard, 2003, MDEQ, 2007). The facility processes fruits and vegetables such as apples, tart cherries, blueberries, plums, celery, asparagus primarily for freezing, making juices and concentrates. Significant treatment of carbon was achieved (p-value of 0.001 for control and 0.0006 for poplar planted). The average COD concentration of leachate was 57.4±14.5 mg/L, 62.1±18.3 mg/L and 78.5±17.6 mg/L for planted (P2), planted with high loading (P1) and control plots, respectively. The average influent BOD was 1095.6±193.9 mg/L BOD. Single factor ANOVA test indicated concentration of leachate COD in the planted plot P2 did not differ from that in control plots ($p=0.47$). Higher loading did not increase the concentration of leachate in the poplar planted plots ($p=0.97$).

4.3.2. Evapotranspiration and soil moisture

Water quantity parameters such as soil moisture and evapotranspiration were assessed and are discussed below along with precipitation and drainage.

4.3.2.1. Precipitation

As the raingage reading for the snow is not reliable and the factory does not apply its wastewater using the pivot in the studied field in the winter, only months from May to November were considered. Monthly rainfall and wastewater applied in each plot is shown in Figure 4-49. The total rainfall at the field from May to November in 2013 was 551 mm. October had the greatest amounts of rainfall in 2013 totaling 176 mm. The wastewater application from May to November in 2013 was 458 mm. However, the changed settings (opening of terminal large sprinkler starting field P1) of the rotating pivot that sprayed wastewater led to difference in the amount of wastewater applied to field P1 and other fields. Spray rate measurements across and along pivot using buckets during the application of wastewater at all fields showed that plot P1 received 1.33 times higher wastewater than other plots (P2, C2, C1) on average [Table 4-13]. Therefore, application rate to field P1 and other plots were adjusted accordingly. After adjustment, the wastewater application rate was 523 mm for P1 and 393 mm for other plots.

Table 4-13 Volume of wastewater collected in buckets at different locations in the field

Pivot pass	Locations	P1	C1	P2	C2
1	1	220	195	155	128
2	2	228	188	160	157
3	2	295	245	200	265
	Sum	743	564 (averaged for C1, P2, C2)		

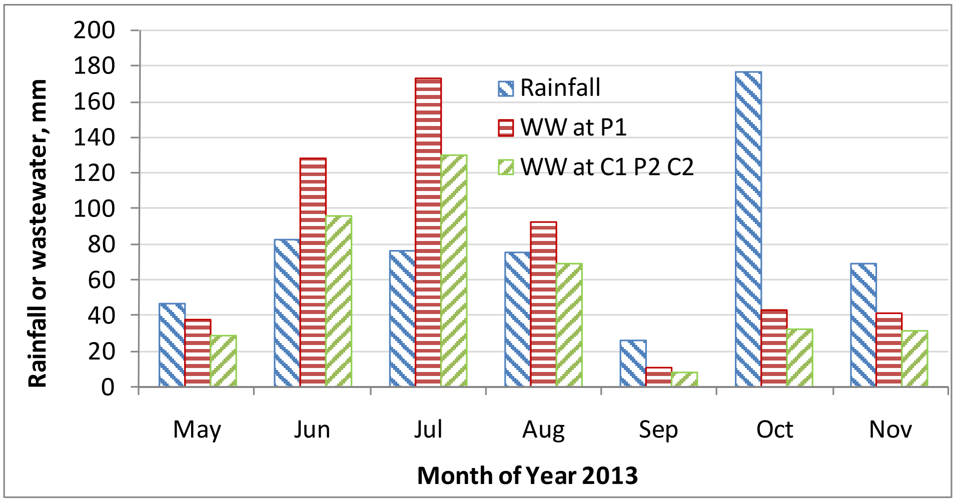


Figure 4-49 Monthly rainfall or wastewater at all plots. Plot P1 obtained greater amount of wastewater (red) than plots C1, P2 and C2 (green) due to difference in setting of pivot.

Plot P1 received total of 1074 mm hydraulic loading that included 523 mm of wastewater and 551 mm of rainfall in the studied period. Whereas, plots C1, P2 and C2 received 945 mm hydraulic loading that included 393 mm wastewater and 551 mm rainfall in the same period.

4.3.2.2. Drainage

4.3.2.2.1. Effect of trees on drainage

Monthly drainage and hydraulic loading are shown in Figure 4-50. The cumulative drainage during May to September in different plots is shown in Figure 4-51. Despite low hydraulic loading, drainage was higher in May than June likely due to spring thaw. In June and September, all plots had low drainage. Poplar planted plot had lower drainage than control plots in July and August indicating positive influence of poplar trees in summer months.

4.3.2.2.2. Effect of loading on drainage

The planted plots P1 and P2 from May to September drained 287 mm and 262 mm respectively. Thus, on overall basis, higher hydraulic loading resulted in higher drainage. In July and August, when plot P2 had reduced drainage, plot P1 had similar drainage to control plots indicating that hydraulic loading is crucial to minimize drainage despite poplar plantation. Alternatively, for same drainage, higher hydraulic loading can be applied in poplar planted plots than in control plots.

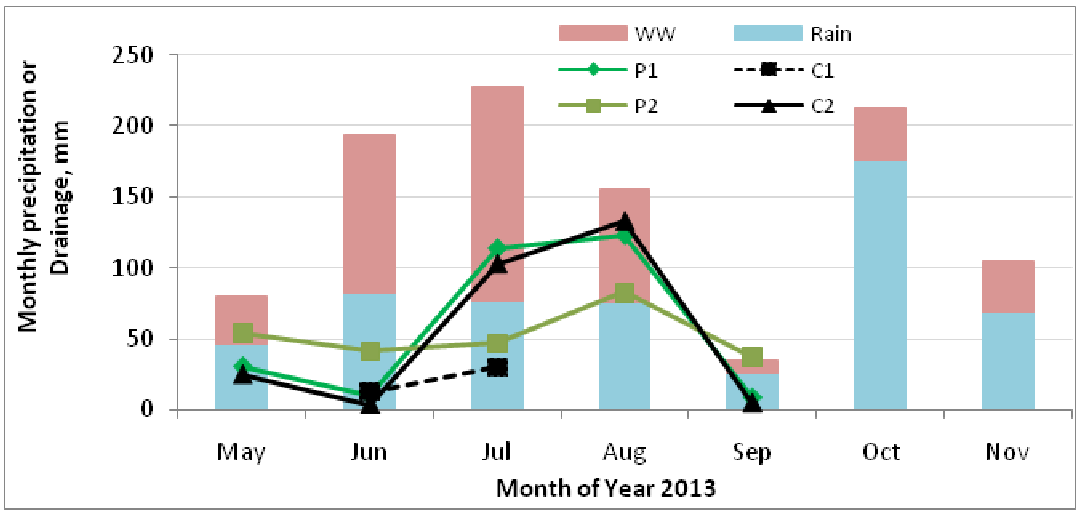


Figure 4-50 Monthly drainage from all plots. Total precipitation (rainfall and wastewater) on a monthly basis are also shown.

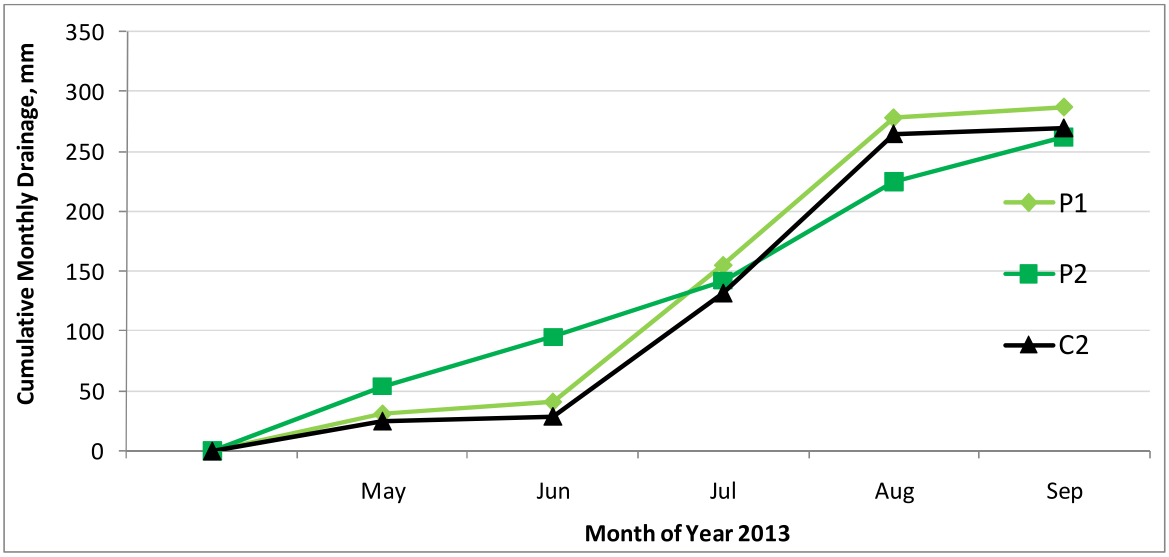


Figure 4-51 Cumulative monthly drainage of plots from May to September, 2013.

4.3.2.3. Evapotranspiration

Evapotranspiration of the field plots were calculated from drainage data, raingage data and monthly wastewater application data provided by the factory using equation given below.

$$\boldsymbol{Evapotranpiration\ (ET) = Rainfall + Wastewater - Drainage} \qquad \textbf{Equation 16}$$

Change in storage was considered negligible due to less variation in soil moisture and monthly time period considered. As expected, evapotranspiration was high in June and July [Figure 4-52]. Planted plots had higher ET during July and August as compared to control plots. Comparison of evapotranspiration between poplar-planted and control plots, is shown in the following Figure 4-52.

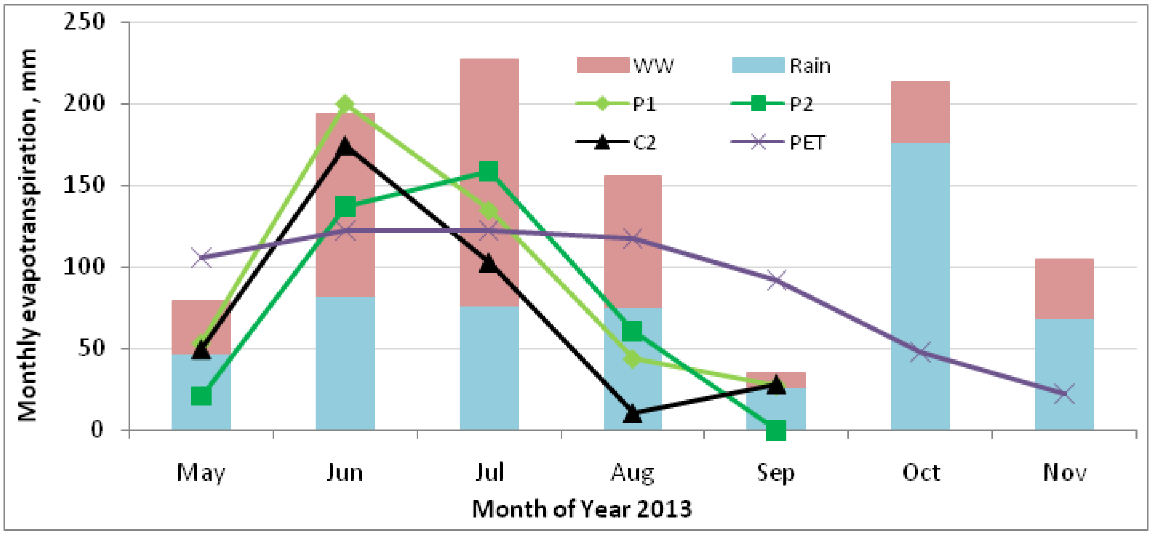


Figure 4-52 Monthly evapotranspiration in different plots. Reference evapotranspitaion and precipitation are also shown. WW stands for wastewater.

The average ratio of evapotranspiration by planted plots to that by control plots was 1.5±0.4 and 1.7±0.8 respectively for P1 and P2 [Figure 4-53]. There was no statistical difference for evapotranspiration between planted and control plots for both P1 (p value of 0.26) and P2 (p value of 0.43). However, during summer months of July and August, the crop coefficient was significantly higher (p=0.04 for P2, 0.01 for P1). It is very likely that poplar trees accelerated the moisture use from soil to contribute to its growth and evapotranspiration. On the other hand, the growth is slowed down from September for poplar trees and less water use is expected. Ambient temperature also does not support high moisture use by plants after September. Therefore, the no-effect of poplar trees after September was expected for the young trees (mostly 2 year trees).

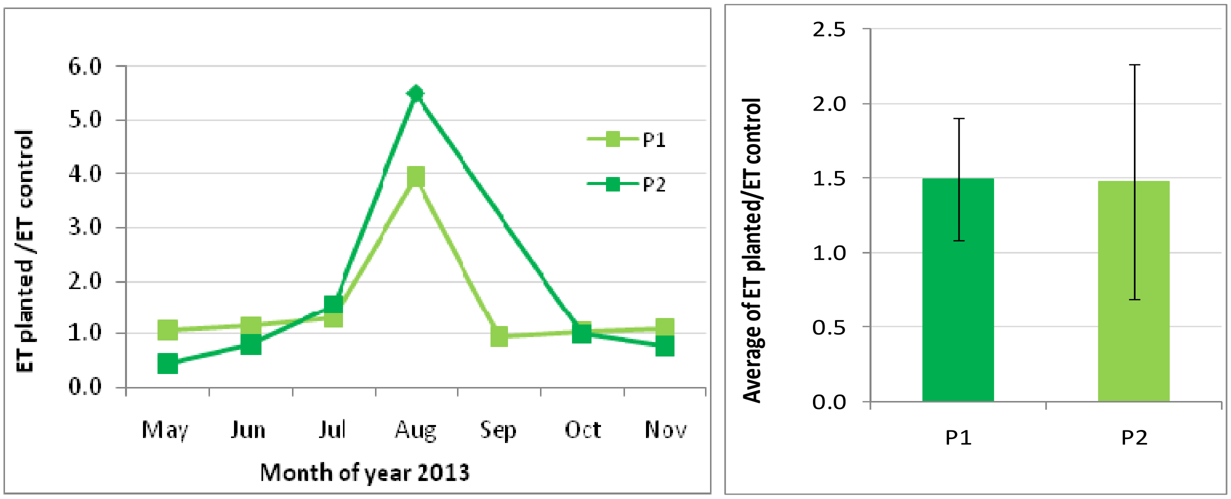


Figure 4-53 Ratio of evapotranspiration of planted plots to that of control plot (C2) for each month (on the left). Mean ratio of evapotranspiration of poplar to that of control plots on the right. Error bars indicate standard error of mean.

4.3.2.4. Soil moisture

Soil moisture was monitored at three locations at each plot along several depths. Since plot P1 got 1.33 times higher wastewater than other plots, plot P1 is compared with plot P2 to study effect of hydraulic loading on moisture content under poplar plantation. The average of control plots C1 and C2 was then compared to plot P2 to study the effect of poplar trees on soil moisture.

4.3.2.4.1. Variation of soil moisture along depth

As seen in Figure 4-54 to 4-57, soil moisture at all depths generally changed with precipitation indicating that moisture sensors responded well with the change in the soil moisture. Soil moisture at 46 cm was highest except in plot P1 where moisture was highest at 122 cm. This could be due to high soil moisture in plot P1 than at other plots at all times. The moisture at 46 cm and 122 cm level were higher in C1 and C2 than in P2. When the soil moisture was increasing (May-August), moisture at 46 cm was higher than at other depths. However, when the soil moisture was decreasing (August to October), the moisture at 46 cm was lower than that at other depths. The recharge of soil moisture due to precipitation took place at shallower depth before influencing the deeper depths. Some of the moisture at shallower depth never reached deeper depth due to evapotranspiration loss. Similarly, once the addition of wastewater (precipitation) was stopped, the moisture loss was faster at shallower depth and the soil moisture quickly decreased to lower value than in deeper depth.

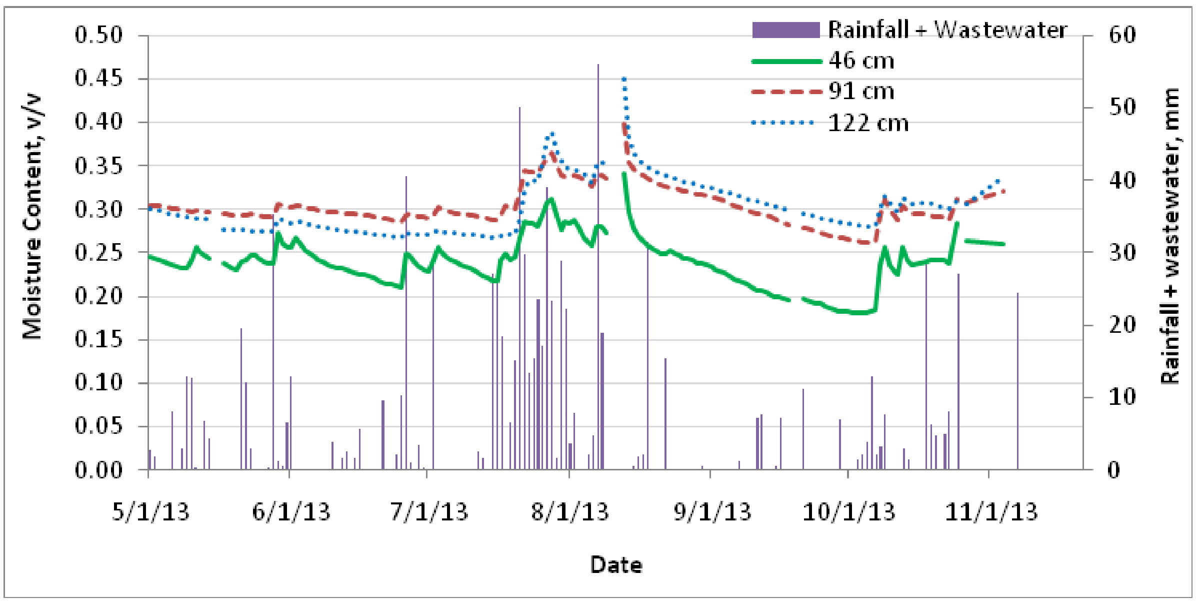


Figure 4-54 Variation of moisture along depth at plot P1 in the presence of poplar trees. Wastewater and rainfall as recorded by the raingage at the site is also shown.

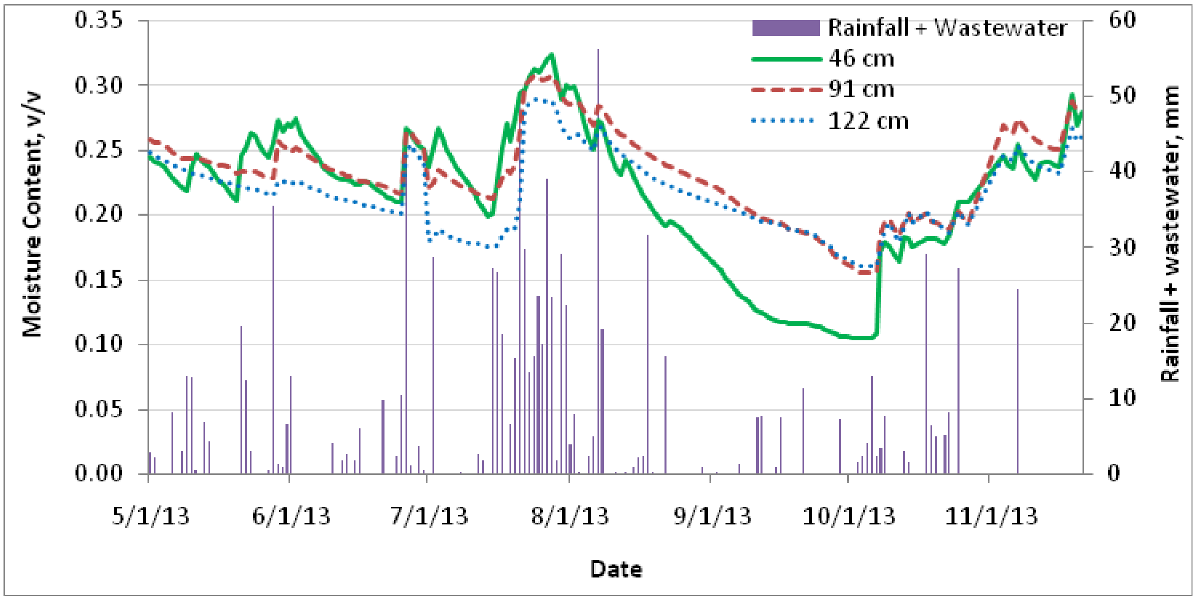


Figure 4-55 Variation of soil moisture along depth for plot P2 in the presence of poplar trees. Wastewater and rainfall as recorded by the raingage at the site is also shown.

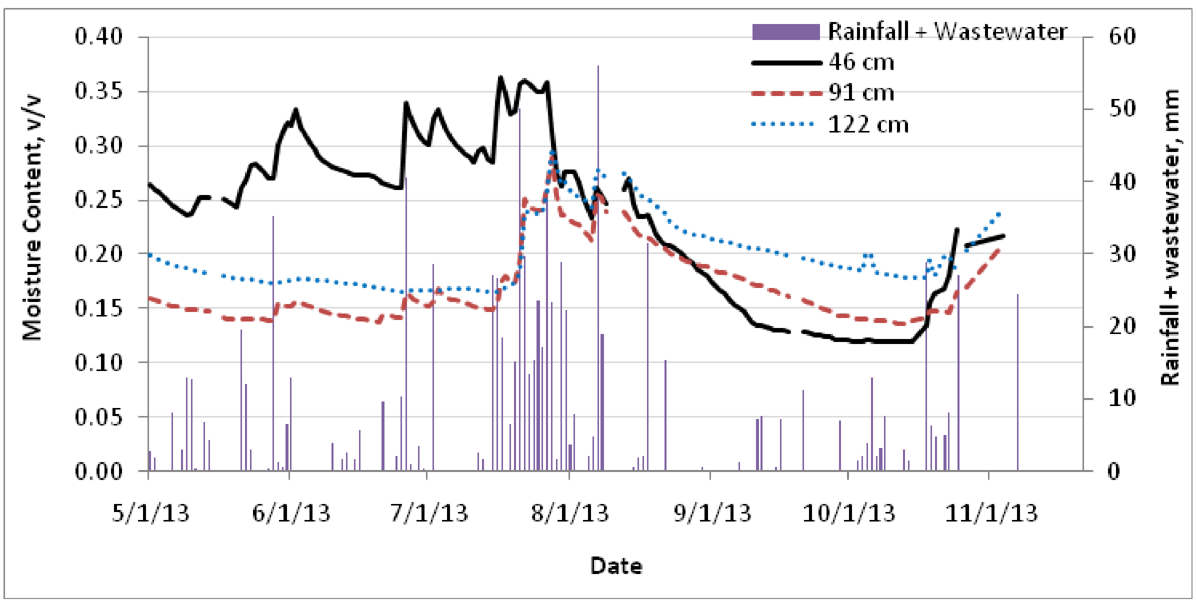


Figure 4-56 Variation of soil moisture along depth for plot C1 in the absence of poplar trees. Wastewater and rainfall as recorded by the raingage at the site is also shown.

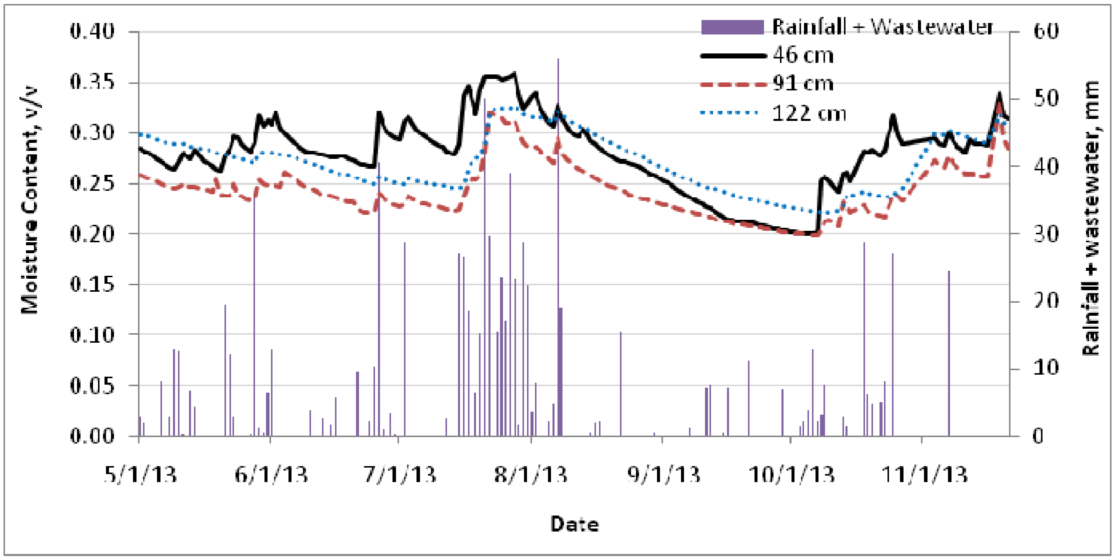


Figure 4-57 Variation of soil moisture along depth for plot C2 in the absence of poplar trees. Wastewater and rainfall as recorded by the raingage at the site is also shown.

When average moisture at each level was considered, the moisture at 46 cm below ground was 0.214±0.004 for planted and 0.259±0.004 for control, at 91 cm as 0.232±0.002 for planted and 0.211±0.002 for control and at 122 cm was 0.216±0.002 for planted and 0.239±0.002 for control. Soil moisture in planted plot was lower than that at control plots at 46 cm level (p value of <0.0005) and 122 cm level (p value of <0.0005). However, surprisingly, control plots had lower soil moisture at 91 cm level than planted (p value of 0.0005) plot. The most contrasting trend between planted and control plots was a reduction in soil moisture from 91 cm level to 122 cm level in planted plots, but increase in moisture in control plots [Figure 4-58]. The higher soil moisture in planted plots at mid-level depth (91 cm) compared to control was consistent with our observation in large column study. Rooting pattern of poplars will describe the phenomenon.

The roots of poplars at or below 122 cm depth could have contributed to the decrease in the soil moisture beneath 122 cm. In our large column experiment, we observed dense roots even at the bottom of the columns 1.2 m deep. Though poplar roots can extend more than 2 m (Tufekcioglu et al., 1998, ITRC, 2009), >90% of poplar roots during phytoremediation do not extend beyond 1.5 m and 70-80% roots are shallower than 0.6 m (ITRC, 2009, Douglas et al., 2010). However, the proportion of roots less than 2 mm diameter increased with depth (Douglas et al., 2010). And, irrigation influenced the poplar rooting pattern in a study at East Lansing. Irrigated poplar trees had more fine roots at 0-30 cm depth, but non-irrigated poplar trees had most fine roots at 30-100 cm (Dickmann et al., 1996). Therefore, number, length and density of poplar roots at the field might have been affected by moisture dynamics. In other words, in dry periods, poplar roots might have grown longer in pursuit of moisture and the intermediate depth might have had less roots. In such a case, removal of higher moisture at surface and below 100 cm depth can be expected.

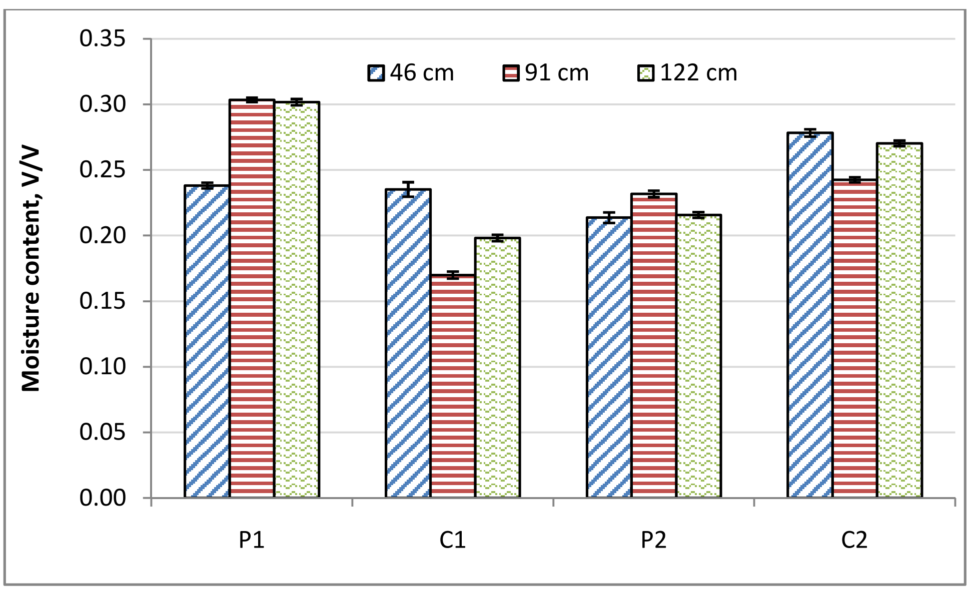


Figure 4-58 Mean soil moisture at different depths for each plot. Error bars represent standard error of mean.

The correlation between planted and control plots for overall average soil moisture was 0.66, for 46 cm level soil moisture was 0.84, for 91 cm level soil moisture was 0.52 and for 122 cm level soil moisture was -0.11. The correlation coefficient indicated that for the first two depths, the soil moisture increased in controls and planted plots at the same time; however, the converse was true at 122 cm level. One explanation for this could be due to poplar roots penetrating below 91 cm depth unlike grasses whose roots are confined to shallower than 91 cm.

4.3.2.4.2. Effect of trees on soil moisture

Figure 4-59 shows the soil moisture in planted plot P2 and control plots. Except at the beginning of the season (May) and when there was overwhelming amount of precipitation (late July), planted plots had lower soil moisture than control plots. Beginning of the season was characterized by initiation of re-emergence of leaves after the winter. Therefore, significant moisture reduction was not expected at the beginning of the season. On the other hand, when the moisture or precipitation was overwhelming, the evapotranspiration rate may not have been significant to show decrease in soil moisture.

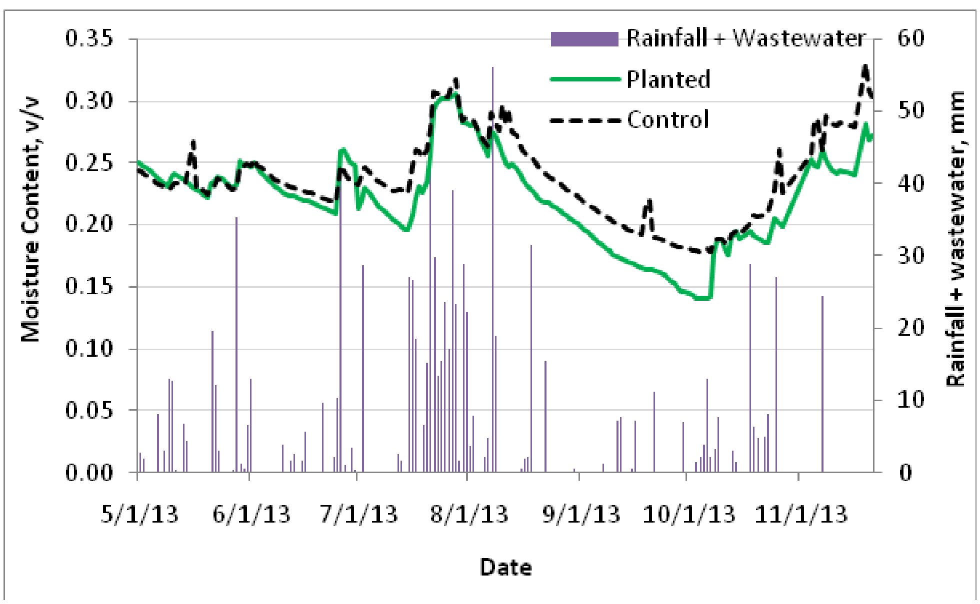


Figure 4-59 Mean soil moisture at all depths and locations for planted plot (P2) and control plots (C1 and C2 average)

The soil moisture at planted and control plots were 0.2220±0.003 and 0.236±0.001 respectively. The soil moisture at the control plots was significantly higher than that at planted plots (p <0.0005) indicating that poplar trees reduced soil moisture compared to control plots.

4.3.2.4.3. Effect of loading rate on soil moisture under presence of trees

Planted plot P1 received 1.33 times higher wastewater than planted plot P2. Therefore, higher soil moisture is expected at plot P1 than at plot P2. Under the presence of trees, the soil moisture at plots P1 and P2 are shown in Figure 4-60. Average soil moisture at plot P1 (0.281±0.002) was higher than (p value of <0.0005) average soil moisture at plot P2 (0.220±0.003). P1 and P2 had average soil moisture of 0.250 ± 0.002 and 0.223±0.002 at 46 cm, 0.299±0.001 and 0.236±0.001 at 91 cm and 0.293±0.002 and 0.206±0.001 at 122 cm, respectively. Therefore, the soil moisture at plot P2 was lower than that at plot P1 at all depths (p<0.0005). These results indicated that in the presence of poplar trees, higher loading contribute to higher soil moisture.

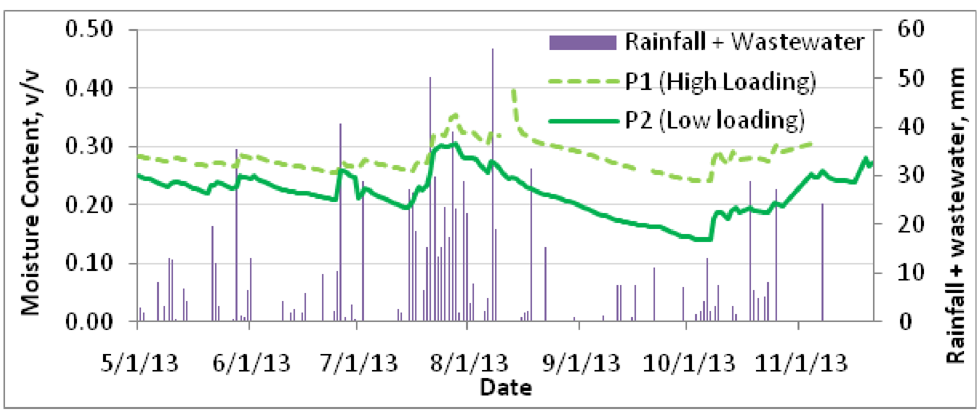


Figure 4-60 Average soil moisture at plot P1 and P2

The correlation coefficient for soil moisture at P1 and P2 was 0.82 for overall average, 0.67 for 46c m, 0.67 for 91 cm and 0.60 for 122 cm. The correlation coefficients signify that the moisture data was highly correlated between plot P1 and P2 at all depths. The trend in increase or decrease in soil moisture was similar in both plots, except for magnitudes of soil moisture. This observation highlights that the plant mechanisms appear to work in the same way for moisture regardless of the hydraulic loading at least for the two hydraulic rates.

4.3.2.4.4. Variation of soil moisture with COD

Plot of COD of leachate water from each plot and the soil moisture on the day of sampling of leachate water is shown in Figure 4-61. There was no significant linear relationship between soil moisture and COD for all plots. However, soil moisture was negatively correlated to COD with a correlation coefficients of -0.60, -0.32 and -0.42 for P1, C, and P2, respectively. The negative correlation indicated that at high soil moisture, lower COD in the leachate water was obtained. Aerobic degradation rate of carbon is inhibited by high soil moisture, however, the drainage may have occurred quickly during high soil moisture limiting the time of degradation of carbon. Moreover, the week correlation coefficient demonstrated that soil moisture was only partially related to COD of leachate.

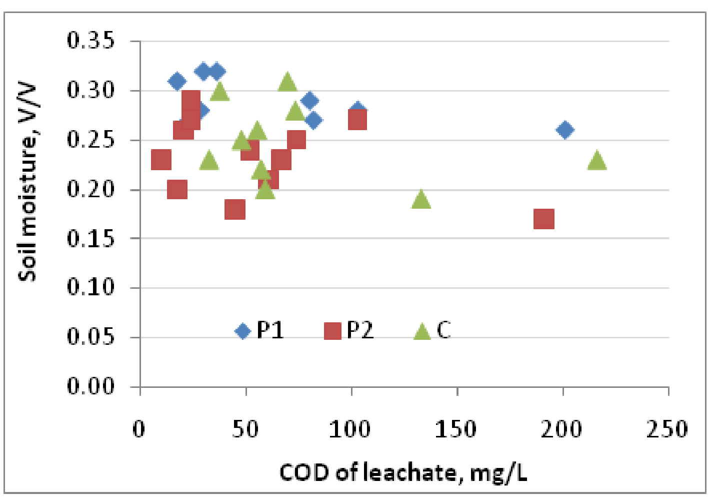


Figure 4-61 Relationship between COD of leachate samples and soil moisture on the day of sampling

4.3.2.4.5. Variation of soil moisture with ET

Plot of monthly evapotranspiration with monthly mean of soil moisture is shown in Figure 4-62. Evapotranspiration was low when the soil moisture was low likely due to dearth of moisture. When the evapotranspiration was highest, soil moisture was again low likely due to high removal of moisture. Soil moisture was highest at moderate evapotranspiration.

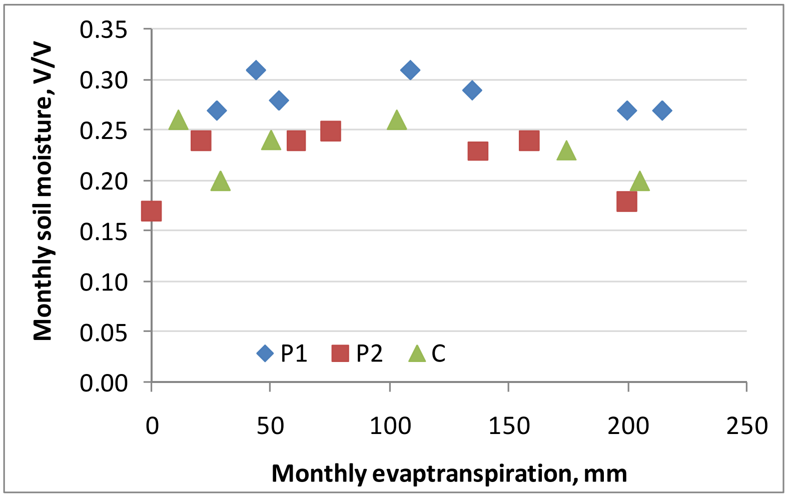


Figure 4-62 Relationship between soil moisture and evapotranspiration

4.3.3. Microbial biomass and community

PLFA analysis represents the quantitative and qualitative status of the soil microbes on 10/16/2014 when soil was sampled from field.

4.3.3.1. Effect of poplar trees on microbial biomass

Phospholipid fatty acid analysis quantified as many as 39 PLFAs out of the total identified 42 fatty acids from C10:0 to C24:1w9, comparable to 37 fatty acid up to C-20 length obtained in uncontaminated sandy loam forest soil (Frostegard et al., 1993), 38 PLFAs in sediment of harbor (Harji et al., 2010) and less than 63 PLFAs in sediments of Hiroshima Bay. The fatty acids included saturated fatty acids (C10 to C12), branched fatty acids, mono-unsaturated fatty acids and poly-unsaturated fatty acids. The total fatty acid is an indicator of total biomass in the soil as

1 nmol of PLFA is equivalent to 5.9×10^7 cells or 0.01 mg bacteria (Rajendran et al., 1992). The total fatty acid for the field plots are given in Table 4-14.

Table 4-14 Total phospholipid fatty acid yield per gram of dry soil taken from 0-30 cm depth from the field plots. The errors are propagated values from sum of standard error of mean (n=3).

PLFA in soil	P1	C1	P2	C2
μg/g	61.06±3.64	42.62±5.40	126.17±21.00	67.01±4.19
nmol/g	195.70±11.68	136.59±17.32	404.38±67.41	214.78±13.41

The total PLFA of 195.70 nmol/g soil for P1, 136.59 nmol/g for C1, 404.38 nmol/g for P2 and 214.78 nmol/g for C2 were higher than or comparable to 162.5 nmol/g obtained by (Yannikos et al., 2014) from poplar rhizosphere, 65 nmol/g from arable soil (Frostegard et al., 1993) and 3.74-186.4 nmol/g soil from beech forest (Frostegard & Baath, 1996). The observed high biomass was expected in top roots-rich soil with regular addition of carbon-rich wastewater. The microbial biomass was significantly higher in planted plots than in control plots ($p=0.003$- 0.005). Our hypothesis that poplar trees enhance total microbial biomass in the rhizosphere was supported by the microbial data. In contrast, higher organic loading in plot P1 did not yield higher biomass in P1 than in P2.

4.3.3.2. Effect of poplar trees on microbial diversity

Diverse communities of microorganisms, both aerobic and anaerobic, both prokaryotes and eukaryotes and both gram positive and gram negative bacteria were present in soils. Groups of specific fatty acids based on classification of functional groups (Vestal & White, 1989, Findlay & Dobbs, 1993, Ibekwe & Kennedy, 1999, Steer & Harris, 2000, Buyer, 2003, Lee et al., 2004, Hinojosa et al., 2005, Kaur et al., 2005, Harji et al., 2010, Ruess & Chamberlain, 2010) along with their % abundance are shown in Table 4-15.

Table 4-15 Abundance (% relative abundance) of PLFAs grouped under different functional groups. Values provided are mean±error propagated from standard error of mean (n=3) for individual fatty acid.

	P1	C1	P2	C2
Microeukaryotes				
C18:3w3 α +ϒ	12.81±0.36	10.77±2.12	13.53±0.06	12.78±0.70
C18:3w6	0.12±0.07	0.17±0.09	0.18±0.09	0.23±0.05
C20:0	0.05±0.03	0.14±0.10	0.02±0.01	0.06±0.03
C20:3w3	12.75±0.98	8.53±3.80	13.77±0.54	13.32±0.65
C20:3w6	0.74±0.02	0.50±0.25	0.79±0.02	0.74±0.06
C20:4w6	0.36±0.02	0.48±0.09	0.38±0.03	0.32±0.03
C20:5w3	0.08±0.00	0.10±0.01	0.09±0.01	0.07±0.01
C22:0	0.80±0.03	0.57±0.28	0.89±0.02	0.84±0.05
C22:2w6	10.86±0.91	7.18±3.21	11.70±0.60	10.50±1.16
C22:6w3	0.06±0.01	0.05±0.03	0.26±0.19	0.27±0.10
C23:0	8.90±0.76	5.77±2.66	9.73±0.61	9.56±0.42
C24:0	1.20±0.19	0.74±0.37	1.17±0.09	1.09±0.19
Total	**48.73±1.59**	**35±6.05**	**52.51±1.04**	**49.78±1.42**
Aerobic prokaryotes and eukaryotes				
C14:1w5	0.12±0.02	0.29±0.16	0.09±0.03	0.11±0.03
C15:1w5	0.01±0.01			0.02±0.02
C16:1w7	2.30±0.77	6.75±3.71	1.25±0.31	1.56±0.44
C17:1w7	0.14±0.04	0.39±0.22	0.02±0.02	0.46±0.39
C18:1w9 C+T	5.36±1.82	13.85±7.79	2.73±0.59	3.65±1.33
C18:2w6 C+ T	8.73±0.24	6.62±2.16	9.59±0.25	8.97±0.42
C20:1w9	0.46±0.09	1.12±0.69	0.32±0.08	1.62±1.19
C20:2w6	13.58±0.90	9.21±3.81	14.67±0.42	13.19±1.58
C22:1w9	0.48±0.03	0.49±0.09	0.58±0.04	1.44±0.91
C24:1w9	5.34±0.49	3.55±1.53	5.92±0.41	5.66±0.27
Total	**36.52±2.24**	**42.27±9.82**	**35.17±0.83**	**36.68±2.67**
Gram positive prokaryotes and other anaerobic bacteria				
C14:0	0.11±0.04	0.28±0.15	0.06±0.01	0.07±0.03
C15:0	0.24±0.07	0.54±0.26	0.14±0.04	0.34±0.19
a-C15:0	0.78±0.24	1.89±1.02	0.45±0.10	0.56±0.14
i-C15:0	0.50±0.17	1.35±0.80	0.28±0.08	0.40±0.09
i-C16:0	0.75±0.10	1.26±0.41	0.64±0.07	0.89±0.21
i-C17:0	0.80±0.29	1.14±0.78	0.33±0.09	0.57±0.22
Total	**3.18±0.43**	**6.46±1.60**	**1.9±0.18**	**2.83±0.40**

Table 4-15 (cont'd)

	P1	C1	P2	C2
Sulfate-reducing bacteria and other anaerobic prokaryotes				
C16:0	1.90±0.61	4.43±2.41	0.96±0.25	1.38±0.41
C17:0	0.25±0.07	0.66±0.40	0.14±0.05	0.15±0.05
C17:0 cy	0.45±0.15	1.10±0.60	0.26±0.09	0.29±0.07
C18:0	5.04±0.11	4.21±0.97	5.32±0.17	4.98±0.37
C19:0 cy	0.09±0.03	0.31±0.18	0.06±.02	0.01±0.01
2-OH C10:0	0.02±0.01		0.01±0.00	0.02±0.02
2-OH C12:0	0.18±0.10	0.58±0.23	0.04±0.01	0.09±0.05
3-OH C12:0	2.22±0.18	2.46±0.24	2.19±0.15	2.00±0.24
2-OH C16:0	0.43±0.01	0.38±0.05	0.45±0.02	0.42±0.05
Total	**10.58±5.59**	**14.13±2.72**	**9.43±0.35**	**9.34±0.61**

Microeukaryotes were high in abundance in all plots (48.7% in P1, 35.6% in C1, 52.5% in P2 and 49.8% in C2). Microeukaryotes can come from plant roots (example: 18:1w9, 18:3w3, 20:5w3) as there were abundant roots of poplar trees and/or grasses in the plots, from algae (18:3w3, 18:1w9), from diatoms (20:5w3), or from animal origin such as protozoa (20:3w6, 20:4w6) (Vestal & White, 1989). The lower relative abundance of microeukaryotes in the C1 plot could be due to lower plant roots. Poplar planted plot P2 had 17.5% and 2.73% higher relative abundance of microeukaryotes than C1 and C2, respectively.

As shown in the Table 4-15, the sum of biomarkers of fungi and aerobic prokaryotes accounted for 36.5% in P1, 42.3% in C1, 35.2% in P2 and 36.7% in C2. Fungi and aerobic prokaryotes aerobically degrade carbon at a rate faster than their anaerobic counterparts (Rittmann & McCarty, 2001) and convert organic nitrogen and ammonia to nitrate (Ponnamperuma, 1972). Presence of large proportions of fungi and aerobic prokaryotes indicate that aerobic activities were occurring in 0-30 cm soil. Though enhancement of fungal species in poplar rhizosphere was been reported before (Yannikos et al., 2014), no effect of poplar was observed in this experiment.

Biomarkers of sulfate reducing bacteria, gram positive prokaryotes and other anaerobic bacteria were found in the field soil. These organisms contributed 13.8% in P1, 20.6% in C1, 11.3% in P2, 12.2% in C2, much lower than in column soils. Presence of anaerobic microorganisms indicated that anaerobic activities including denitrification occurred in all plots. Lower contribution of anaerobic bacteria in planted plot P2 than in control plots (11.3% vs. 16%) indicated more anaerobic activity occurred in control plots.

The clustering of field plots based on the relative presence of fatty acids using the UPGMA method using Euclidean distance of standardized data resulted in dendrogram shown in Figure 4-63. The agglomerative coefficient was 0.54 and the correlation with Cophenetic distance using was 0.96. Plot C1 was different from rest of the plots. C1 had high anaerobic biomass and low microeukaryotes. P1 and C2, though got different loading, had similar proportions of microeukaryotes and aerobic biomass. Anaerobic biomass was greater by 1.6% in C1 than in P1 indicating the separation in the dendrogram was again due to anaerobic microorganisms. P2 differed with C2 in anaerobic biomass. Thus, the field plots were mainly separated based on anaerobic biomass and planted plot P2 was different than both control plots C1 and C2.

Thus, PLFA analysis of soil for microbial biomass and community indicated that poplar trees enhanced total microbial biomass and microeukaryotes and decreased anaerobic microorganisms in rhizosphere. Influence in aerobic microorganisms due to poplar trees was not observed. Consumption of nitrate, sulfate and oxygen by diverse microbial community simultaneously to remove organic carbon in wastewater occurred at field site. Similar diversity and DOC treatment was observed by Essandoh et al. 2013 in the column soils.

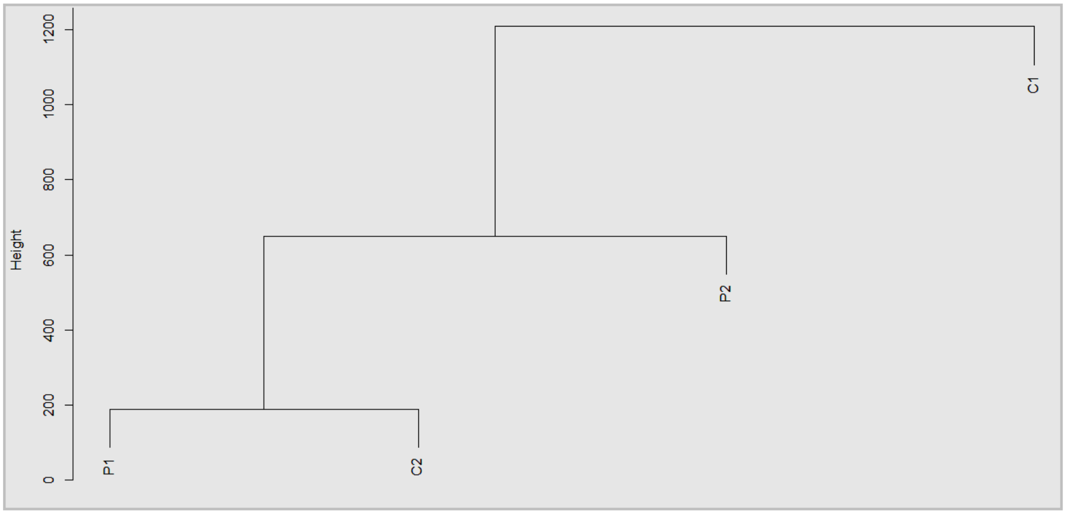


Figure 4-63 Clustering of field plots using UPGMA algorithm based on Euclidean distances for field PLFA standardized data

4.3.4. Metals and nitrate treatment

4.3.4.1. pH of leachate water

As shown in Figure 4-64, pH of the influent samples ranged from 4.4 to 6.4 with an average±standard error of 5.54±0.18. The slightly acidic influent samples were treated by buffering capacity of soil by virtue of its cation exchange capacity and high exchangeable calcium in the soils. The leachate samples had mostly neutral pH of 6.21 to 8.01 for planted P2 samples (7.42±0.13) and 6.10 to 8.23 (7.48±0.11) for control samples. Though pH for planted plot was slightly lower than that in no-tree control plot, t-test showed no significantly difference (p=0.44). One-way ANOVA showed no difference in pH for all four plots (p value of 0.25).

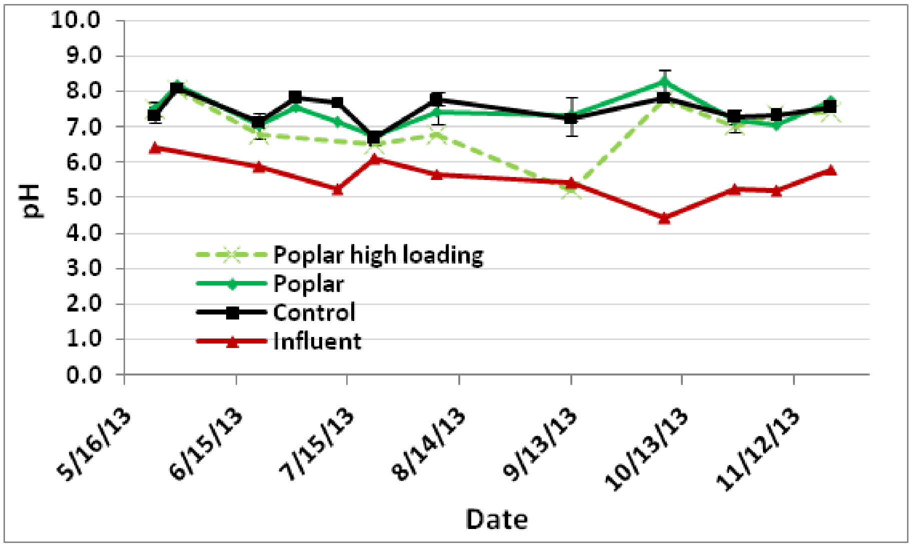


Figure 4-64 pH of leachate water collected at 91 cm and 200 cm below ground surface. The error bars are standard error of mean (n up to 8 based on availability of sample).

4.3.4.2. Nitrate leaching

The trend for concentration of nitrate in leachate water from both planted and control plots with time was similar ($p=0.46$). However, there was distinct concentration difference in July and August when the nitrate leaching from planted plots was much lower than that from control plots [Figure 4-65]. Combined with the lower drainage and higher evaporation from planted plots during July and August, the poplar plantation reduced the nitrate leaching both in mass basis and concentration basis.

The average nitrate concentration of leachate samples was 53.8±15.8 mg/L, 66.4±28.2 mg/L and 3.3±1.1 mg/L respectively for planted, control and influent samples. High leachate nitrate concentrations than the influent nitrate concentration indicated that there was production of nitrate in the soil from conversion of organic nitrogen and/or ammonium to nitrate by nitrification.

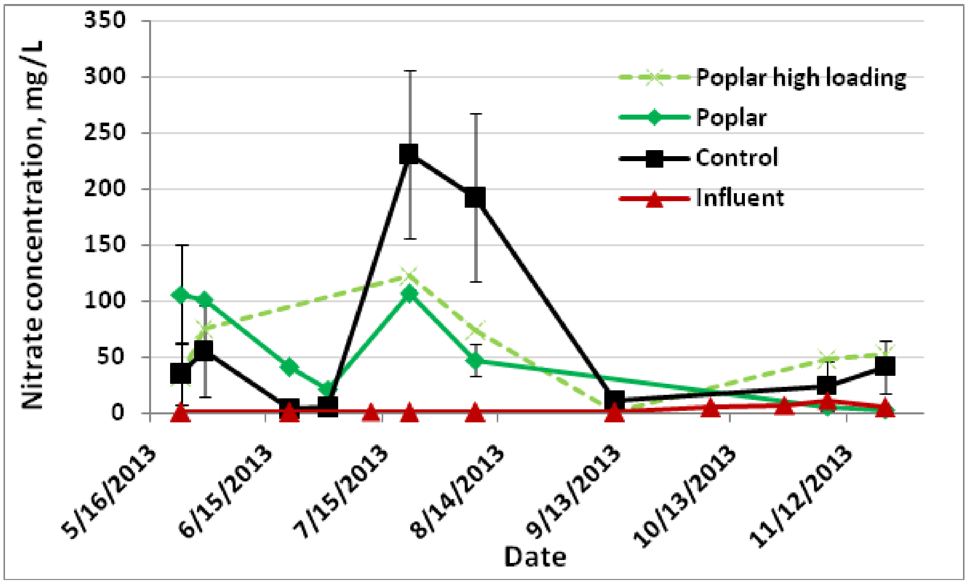


Figure 4-65 Nitrate concentration in the leachate water with time. Error bars represent the standard error of mean.

4.3.4.3. Ammonium leaching

Ammonium in the leachate water was undetected for most samples. The lack of water samples combined with no ammonium detection in leachate water samples led to sparse points in Figure 4-66. The highest ammonium concentration in the control leachate sample was 27.15 mg/L and in the planted leachate sample was 1.6 mg/L. Low ammonium was indicative of the aerobic conditions consistent with microbial and nitrate data.

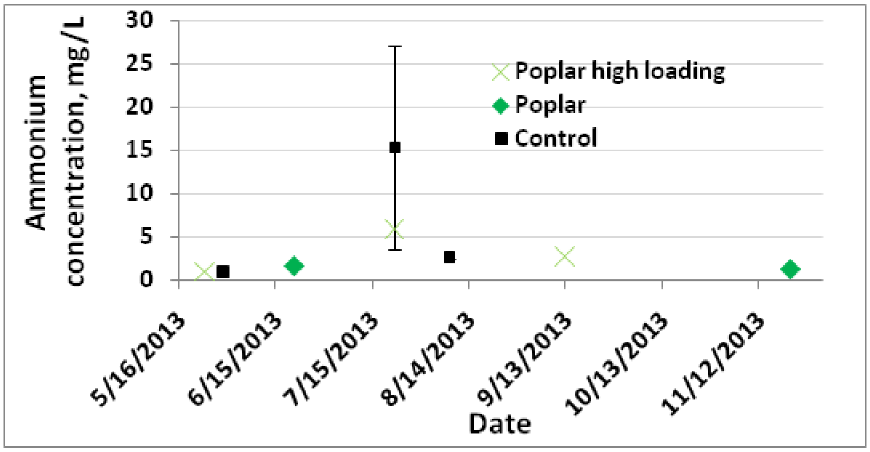


Figure 4-66 Concentration of ammonium in leachate water

Though the concentration of ammonium was higher than nitrate in the most food processing wastewater, it was not the case in the factory wastewater; ammonium was minimal. The average ammonium concentration of leachate samples was 1.44±0.15 mg/L, 6.28±4.57 mg/L and 0.14±0.01 mg/L respectively for planted, control and influent samples. As with nitrate, ammonium was produced in the soil. Except on 7/22/2013, the ammonium concentration of leachate samples was always lower than 5 mg/L. Both ammonium and nitrate production implied that ammonification and nitrification of organic nitrogen occurred. Regular wastewater application during week of 7/22/2013 may have contributed to more reducing conditions and accumulation of ammonium.

4.3.4.4. Total nitrogen

The high uptake of the nitrogen by the poplar trees [Figure 4-67] indicated that poplar trees potentially can uptake large amount of nitrogen in its biomass. As the application rates are dependent on the nitrogen uptake of the plants on the field, potentially higher application rate is possible at poplar planted field. Though poplar trees accumulated higher nitrogen in its tissues when loaded with higher loading (P1) than with lower loading (P2), the uptake rates were not statistically different. Poplar with higher loading had higher concentration in leaves (p=0.23) and roots (p=0.09) and lower concentration in stem (p=0.45) compared to poplar with lower loading.

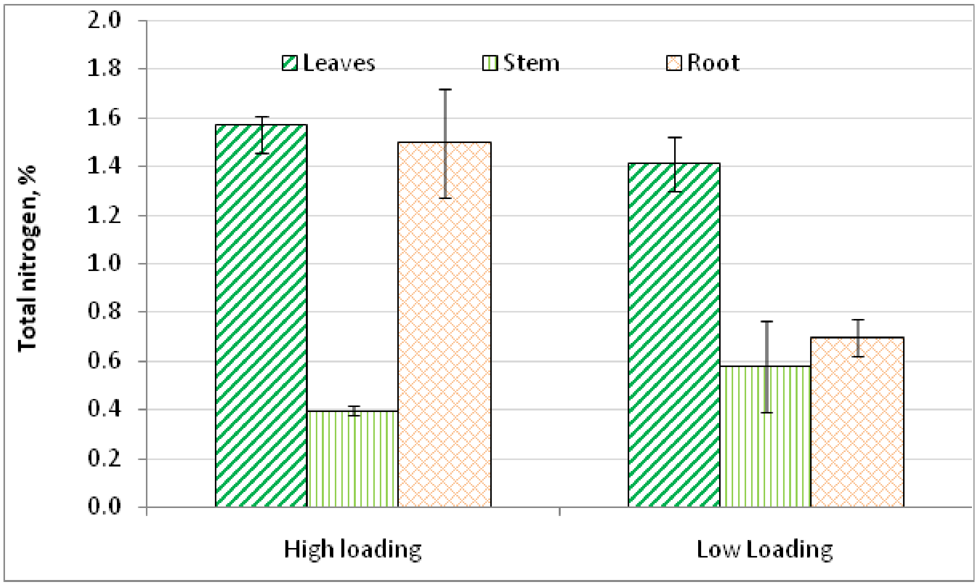


Figure 4-67 Total nitrogen in the plant parts at field site.

If poplar production of 20-25 Mg/ha/yr (Ceulemans & Deraedt, 1999) is assumed, nitrogen removal can be 300-375 kg/ha/yr using average of 1.5% N content. The uptake rate is higher than previously reported 133-159 kg/ha/yr for 4th year poplar and similar to 350 kg N/ha/yr (Deckmyn et al., 2004). The uptake rate is higher than loading of 318 kg/ha/yr total nitrogen in growing season (April to September) in Minnesota (Zvomuya et al., 2006).

Concentration of the total nitrogen in the soil between planted plots and control plots [Table 4-16] at the end of the experiment were not different. It is likely that the nitrogen in the soil was too high to see any decrease in the concentration.

Table 4-16 Total inorganic nitrogen at the beginning of the experiment and total nitrogen at the end of the experiment in the soil.

Parameter (unit)	Before experiment	After experiment			
		P1	P2	C1	C2
TKN (%)	0.11	0.13±0.04	0.14±0.04	0.16±0.02	0.13±0.05
Nitrate N (ppm)	25.4				

4.3.4.5. Metal leaching

Iron and manganese concentrations were monitored in the leachate samples when the samples were available [Figure 4-68]. Total iron concentration at the beginning of the summer was low. Plants growth and lack of wastewater application may have contributed to the aerobic conditions when iron was mostly in insoluble (oxy)hydroxides. The concentration of total iron increased with wastewater application from May-July and remained constant for the remainder of the season. The average total iron concentration in the leachate water collected from the control field and planted field were respectively 117.09 ±15.3 µg/L and 116.13±17.13 µg/L. Total iron in the leachate samples was less than 300 µg/L, SMCL in the drinking water. As such, the concentration in leachate did not pose any concern to human and ecosystem health.

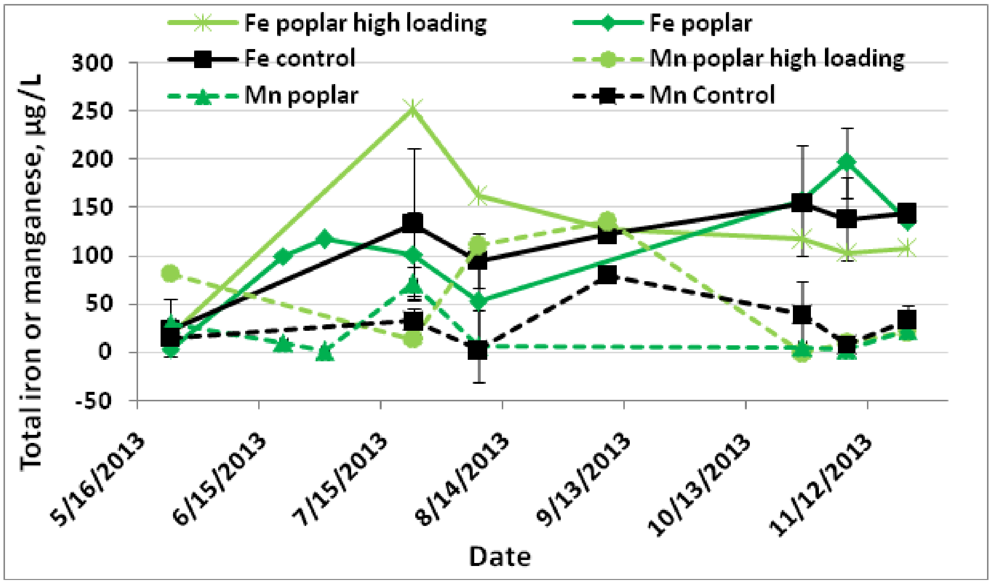


Figure 4-68 Concentration of total iron and manganese in leachate water along with time

Manganese concentration in the leachate water was up to 136 µg/L in planted plot and 81 µg/L in control plot. Contrary to the total iron concentration that increased from May to July, the manganese concentration increased from July to September. However, from September, the manganese concentration in leachate water decreased. The average manganese concentration in the leachate water from the control field and planted field were respectively 37.59±14.39 µg/L and 30.54±9.82 µg/L. Therefore, though metals leached to the ground water, the leaching was not enough to pose any concern. Poplar plants did not show effect on the metal mobilization at the low concentration observed in the leachate water.

Moreover, iron, manganese and arsenic were analyzed in plant parts (triplicates of roots, stems and trees from each plot) and soils (triplicate samples from 0-30 cm from each plot). Soil concentrations of metals did not show any conclusive evidence if poplar trees reduced the concentration of metals in the soil [Table 4-17]. Due to short duration of the study and limited biomass produced, significant plant uptake to change the soil concentration was not expected.

Iron in the top soil decreased in all plots, whereas manganese increased. On the other hand, arsenic increased in planted plot P1 and decreased in others. However, the power of the test being very low did not enable to come to conclusion on the soil concentrations of metals.

Table 4-17 Metals in the soil before and after experiment (n=3)

Parameter (unit)	Before	After			
		P1	P2	C1	C2
Mn (mg/kg)	29.5±1.7	50.6±8.9	44.7±8.5	56.1±2.4	32.2±5.9
Fe (mg/kg)	210.2±1.7	129.3±25.4	157.7±7.2	138.6±8.4	144.5±3.9
Arsenic (mg/kg)	3.7	4.1±0.6	2.8±0.4	2.9±0.3	3.2±0.5

Neither large concentration of metals was lost from the leachate, nor was large biomass produced to significantly affect the fate of the metals during the short duration of the study. However, there was significant accumulation of iron and manganese in poplars from both plots (P1 and P2). Unlike our small columns study, leaves accumulated the highest concentration of iron and manganese, followed by root and stem. However, arsenic was slightly higher in roots than in leaves. Leaves, stems and roots had 2.14±0.38 mg/kg, 1.55±0.22 mg/kg and 2.74±0.44 mg/kg arsenic respectively [Figure 4-69]. Except for arsenic in stems, all plant parts from plot with high loading (P1) accumulated higher concentrations of metals than from plots with low loading (P2), though insignificant statistically (p=0.15-0.56). The higher uptake could be due to more available metals under higher organic and hydraulic loading. Poplar as a strategy first plants uptake more soluble form of metals (Mihucz et al., 2012).The longer growth period of poplar trees under high loading may uptake significantly higher metals. The poplars in the field accumulated similar concentration of metals in leaves and lower concentration of metals in roots and stem than the small column study. Iron concentrations were similar to previously reported (Mihucz et al., 2012). Shoot concentrations of arsenic (2.2-1.3 mg/kg) were lower than the leaf concentrations observed in poplars grown at a contaminated field site near Tuscany, Italy (e.g., 9 to 12 mg/kg after 12 months) (Ciurli et al., 2014). Poplar tree applied with landfill leachate in uncontaminated soil had concentration of 194.6±5.4 mg/kg, 58.4±2.0 mg/kg and 73.66±5.7 mg/kg manganese in leaves, stems and shoots, respectively (Zalesny & Bauer, 2007). In field experiment, corresponding uptake of manganese for leaves, stems and leaves by poplar were 259.6±51.0 mg/kg, 19.6±5.3 mg/kg and 35.4±6.9 mg/kg, respectively.

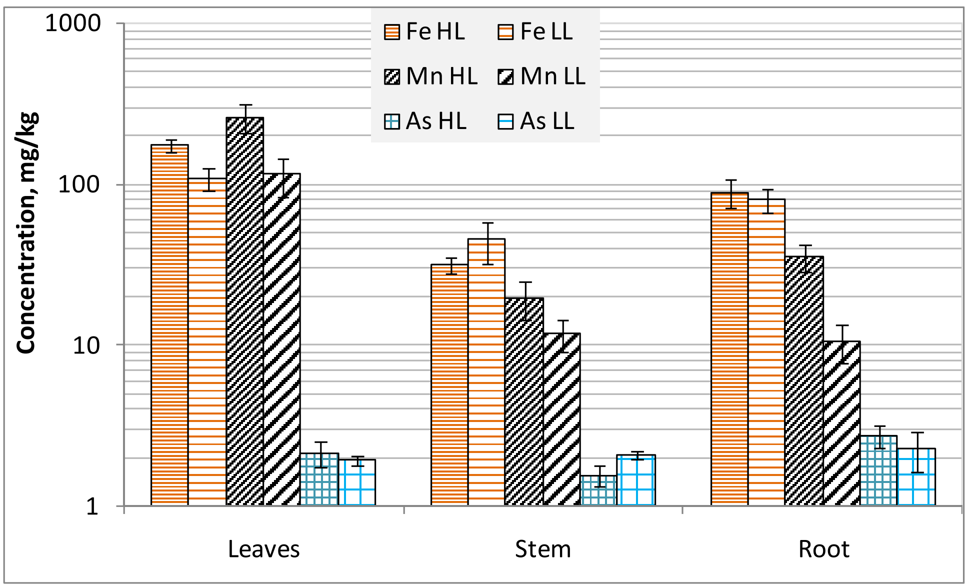


Figure 4-69 Concentration of iron, manganese and arsenic in poplar tree parts. HL indicate high loading rate (P1) and LL indicate low loading rate (P2). There was no difference between HL and LL for any plant part for any metal.

The bioaccumulation factors, ratio of plant concentrations to that of soil concentrations and the translocation factors, the ratio of stem or leaf concentration to that of root concentrations are provided in the table 4-18.

Table 4-18 Bioaccumulation factors and translocation factors for iron, manganese and arsenic in the field experiment

	Fe HL	**Mn HL**	**As HL**	**Fe LL**	**Mn LL**	**As LL**
Bio- accumulation factors						
Root	0.69±0.19	0.70±0.18	0.67±0.15	0.51±0.09	0.24±0.08	0.81±0.25
Stem	0.24±0.06	0.39±0.12	0.38±0.08	0.29±0.08	0.26±0.08	0.74±0.12
Leaves	1.34±0.29	5.13±1.35	0.52±0.12	0.69±0.12	2.56±0.85	0.69±0.11
Translocation factors						
Stem	0.35±0.08	0.55±0.18	0.57±0.12	0.56±0.19	1.11±0.39	0.91±0.26
Leaves	1.95±0.44	7.33±2.03	0.78±0.19	1.36±0.32	10.83±4.09	0.85±0.25

HL indicate higher loading (P1) and LL indicate lower loading (P2)

Arsenic bioaccumulation factors were higher for lower loading rate than for higher loading rate for all plant parts, whereas, manganese bioaccumulation was higher for higher loading than for lower loading. This could be supported by the manganese reducing condition of the field. Overall, manganese was the easiest to transport to leaves and stems from the roots.

4.3.4.5.1. *Effect of moisture on metal leaching*

Manganese concentrations of the leachate samples and mean soil moisture on the day of sampling when plotted against each other [Figure 4-70] did not show definitive relationship between moisture content and metal leaching. Correlation coefficient were 0.71, 0.15 and -0.16 between moisture content and manganese leaching for plot P1, control plots and plot P2, respectively. Similar result was obtained for iron leaching when plotted against moisture content. No-distinct relationship and poor correlation indicate that metal leaching in the field was not only related to moisture content of the soil.

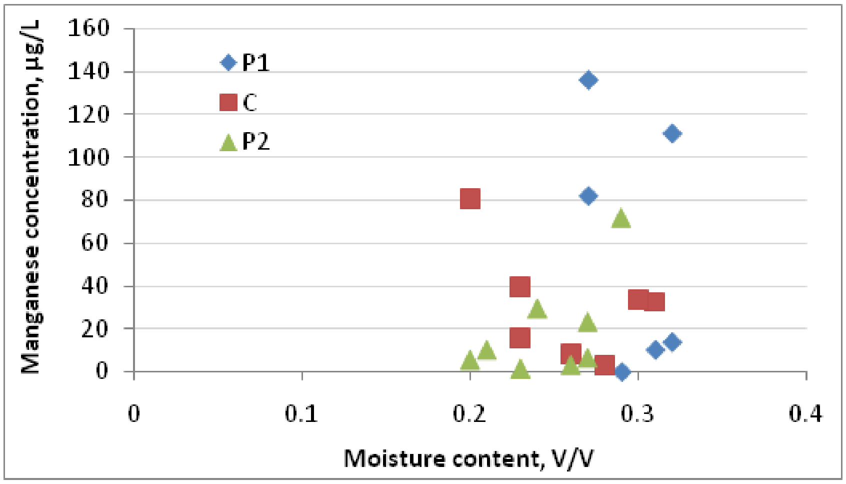


Figure 4-70 Relationship between manganese leaching and soil moisture content

To sum up, the conditions in the field was mostly aerobic due to i) significant aerobic microbial populations, ii) low ammonium, iii) high nitrate and iv) low metals mobilization. Though anaerobic activities were likely in some parts of the field as indicated by presence of anaerobic bacteria, metal mobilization did not occur significantly. The metals in the water were below 0.25 mg/l during the experiment. Poplar shoots successfully accumulated high concentration of iron (31-200 µg/kg), manganese (6.41-350.5 µg/kg) and arsenic (1.3-2.6 µg/kg). In this experiment, the accumulation was not significant enough to decrease the leachate concentration or mass flow of metals.

4.3.5. Strengths and limitations of the study

The study represented the real condition and scenario of the land application of food processing wastewater such as wastewater composition, wastewater application rate, soil and management practices. The poplar trees growth took place under application of actual carbon-rich wastewater. The instrumentation provided the actual variation of soil moisture, temperature and drainage.

However, the application rate was lower than reported elsewhere and was not in the control of researcher. Breakage of wastewater applying pivot prevented wastewater application during several occasions. The lack of enough wastewater application did not yield enough number and quantity of leachate samples. As with field conditions, the nuisances such as deer eating poplar trees, rat eating bark of trees and cables were also representative of any land application site. The sampling of plants and soil did not capture the dynamics of plant uptake of nitrogen and metals, microbial population change and change in metal and nitrate concentration of soils. Another limitation of the field study was death of plants due to rodents. While replanting was done to replace the dead plants, size of plant was still compromised. Survival of most poplars near the instruments and sampling locations likely provided better results.

4.3.6. Conclusions

The treatment efficiency of the carbon-rich wastewater was high as the application of 332-1880 mg/L BOD resulted in 19-216 mg/L COD. Presence of abundant aerobic microorganisms degraded the organic carbon present in the food processing wastewaters. Poplar trees did not decrease the leaching of carbon to the groundwater.

Evapotranspiration of the poplar planted plots were higher than that at grasses only plots during months of July to September. On average, the crop coefficients of the poplar plots were 1.5±0.4 and 1.7±0.8. Thus, poplar trees removed moisture at the rate of 1.5 to 1.7 times faster than that by control plots. The higher evapotranspiration significantly decreased soil moisture in poplar planted plots compared to control plots at 46 cm and 122 cm depth. At 91 cm, control plots had higher moisture than planted plots. The rooting pattern may have influenced by the irrigation frequency of the wastewater. The evapotranspiration during the summer months was high and reduced the drainage and mass flow of contaminants to the ground water. However, other than from July to September, poplar trees did not enhance evapotranspiration or mass removal of nitrate and metals.

PLFA analysis of soil samples from poplar rhizosphere and control plots showed that the diversity of microorganisms were similar in both plots. Though both planted and control soils had high microorganisms, total microbial biomass was higher in poplar planted plots than in control plots. The type of microorganisms included abundant microeukaryotes, aerobic prokaryotes and eukaryotes, gram positive prokaryotes, other anaerobic bacteria and sulfate reducing bacteria.

Metal mobilization in the field was not of concern likely due to low loading rate. The loading rate of 55 cm/year applied in the field site was significantly less than the highest reported application rate (1.5 cm/day) in Michigan even though the reported rate is not known to have metal mobilization. Poplar trees did take high concentrations of metals (up to hundreds of mg/kg) into its root and shoot tissues. By virtue of its high biomass potential, poplar trees may reduce the metal mobilization to the ground water when they grow to maturity, however, in this study, we did not see the effect of metal accumulation by poplars on the soil or water concentrations.

More concerning than metals, nitrate was mobilized at higher rate than safe for ecosystems and human health. Poplar trees showed signs of decreasing the ground water pollution by nitrate in the summer. As summer months get high application of wastewater on to the field, this trait of poplars could be beneficial. Again, older and matured trees will have higher evapotranspiration and higher total uptake of nitrate. Therefore, these kinds of study need to continue for years until poplar trees peak their growth.

4.4. Plant-associated processes

This section summarizes briefly and discusses each hypothesis including the metal and nitrate mobilization, often contrasting between column experiments and field experiment.

4.4.1. COD treatment

The removal efficiency of 82.12±1.73%, ≥92.0 ± 1.4%, and 90±1% were obtained in sandy loam soils in small-scale columns, large-scale columns and field studies, respectively. In loam soils of large-scale column experiments, ≥72.3±4.8 treatment efficiency was obtained. These treatment efficiency are higher than previously reported 58-3%-61.4% (Ak & Gunduz, 2013) and comparable to 91.7% (Law et al., 1970). Higher treatment by sandy soil than by loam soil was obtained before also (Law et al., 1970). Microorganisms use carbon as electron donor for energy and in the process use oxygen, nitrate, oxidized metals, sulfate and carbon dioxide as electron acceptors in the order (Froelich et al., 1979, Rittmann & McCarty, 2001). Microbial communities present in the experimental soils confirmed the removal of carbon mostly by aerobic respiration and to some extent by anaerobic respiration.

Poplar trees enhanced mass removal of carbon in small-scale columns and in the loam soils in the large-scale columns. Plants affect the carbon in the soil by photosynthesis, production of exudates and radial oxygen loss (Pezeshki & DeLaune, 2002, Jacob & Otte, 2003). Hybrid poplar exudates, that contain 10-120 mg/L dissolved organic carbon and 1-10 mg/L acetic acid (Schnoor et al., 1995), can change the soil redox potential in conjunction with root oxygen, reactions in the soils and the microbial growth and biomass. While root exudates increase the oxygen demand in the soil (Otero et al., 2009), radial oxygen loss can supply oxygen. Net effect of these plant-products in the loam soil was greater reduction of carbon from the wastewater.

4.4.2. Evapotranspiration and soil moisture

Poplar planted small-scale columns had evapotranspiration coefficient of 3.25±0.31 compared to controls, large-scale columns had 1.55±0.23 in sandy planted and 1.42±0.11 on loam planted and field experiment had 1.5±0.4 and 1.7±0.8 for two planted plots. These values are comparable to 1.06 to 4.25 in Italy for 1-2 year poplars when used as filter strips (Guidi et al., 2008). While column results would be more accurate due to actual water measurement and balance, field studies is more realistic due to actual field conditions. Column evapotranspiration values may have been overestimated due to confined columns. Column studies showed that plants accelerated loss of soil water due to high evapotranspiration at all months in the growing period (April 15 to November 15) except when the plants come out of spring dormancy. However, at field, only months from July to September had higher evapotranspiration than control.

Poplar trees decreased soil moisture on overall basis in all experiments. However, unexpectedly, in both column and field experiments, the soil moisture was lower in control than in planted columns/plots at middle depth (91 cm or 61 cm). Though poplar roots grow at least 180 cm deep in soil (Tufekcioglu et al., 1998), the root number, density and root area ratio decreases with depth. Poplar had 50%, 38% and 12% of roots less than 30, 30-60 and greater than 60 cm deep respectively (Coleman et al., 2000). Poplar root density decreased from 23 roots/100 cm^2 at 0-50 cm, to 8 roots/100 cm^2 at 50-100 cm, to 3 roots/100 cm^2 at 100-125 cm and to 1 root/100 cm^2 at 125-165 cm in a study at Iowa (Tufekcioglu et al., 1998). However, the proportion of roots less than 2 mm diameter increased with depth (Douglas et al., 2010). Moreover, irrigated poplar trees had more fine roots at 0-30 cm depth, but non-irrigated poplar trees had most fine roots at 30-100 cm (Dickmann et al., 1996). The field site could be representative of non-irrigated poplar trees due to intermittent application of wastewater and prolonged period without wastewater application at times. Number, length and density of poplar roots at the soil may have been affected by moisture dynamics or wastewater application frequency. In such a case, removal of higher moisture at surface and below 100 cm depth can be expected.

4.4.3. Soil oxygenation

In large-scale soil column experiment that used measurement of soil redox potential as proxy for soil oxygenation, poplar trees showed inconsistent results. In 2011, poplar and willow increased the redox potential compared to no-tree controls. Similar results obtained before in the presence of *Phragmites* and *Spartina* have been attributed to transport of oxygen from aerial parts to rhizosphere and radial oxygen loss and evapotranspiration (Otero et al., 2009, Maria-Cervantes et al., 2010, Wu et al., 2014). In addition to oxygen, plants provide habitat for microorganism and more rapid drying of soil (Cervantes et al., 2011). Cracks or macropores by roots may facilitate diffusion of oxygen to soils. All these plant-associated mechanisms can enhance the soil redox potential. However, in 2012, control columns had higher redox potential than poplar planted columns. In 2013, sandy control had higher redox potential than sandy planted, whereas, loam control and loam planted were close to each other. This variability in results can be attributed to the contrasting mechanisms that plant exhibit in soil treatment systems. First, plants produce exudates that increase oxygen demand in the soil and negatively affect the redox potential (Jacob & Otte, 2003). Second, plants enhance microbial biomass that may consume oxygen in the soil quickly (Ponnamperuma, 1972). Third, plants supply oxygen to the rhizosphere through roots (Brix & Schierup, 1990, Wu et al., 2014). The net effect can either enhance or decrease redox potential of the soil.

4.4.4. Rhizostimulation

Diverse microorganisms were present in the soils of large-scale columns and field. The experiments (columns and field) observed higher total biomass in poplar planted soils than control soils consistent with higher populations of total heterotrophs, denitrifiers, pseudomonads obtained in rhizosphere of hybrid poplar trees before (Jordahl et al., 1997). The increase in microbial biomass can be attributed to production of root exudates that contain as much as 10-20% of total photosynthesis in the forms of sugars, alcohols and acids (Schnoor et al., 1995).

Overall, the abundance of microorganisms decreased with depth in large-scale columns, consistent with a study by Ak and Gunduz (2013). Additionally, more aerobic microorganisms were present in the top (0-30 cm) than deep soil (30-60 cm), while opposite was true for anaerobic microorganism. High fungal biomass present in both field and columns can convert organic nitrogen to ammonium (Ponnamperuma, 1972). In both columns and field, aerobic as well as anaerobic activities were occurring as supported by presence of anaerobic as well as aerobic biomarkers, redox potential and concentration of redox sensitive species. However, more anaerobic activity occurred at columns due to higher relative abundance of anaerobic fatty acid markers than at field.

4.4.5. Plant uptake

Summary of concentrations of nitrogen, iron, manganese and arsenic in all experiments are given in Table 4-19.

Table 4-19 Summary of plant uptake of nitrogen and metals in all experiments.

Experiments	N, %			Mn, mg/kg			Fe, mg/kg			As, mg/kg		
	Root	Stem	Leaves	Root	Stem	Leaves	Root	Stem	Leaves	Root	Stem	Leaves
Small-scale columns	0.67	0.38	1.03	272	118	254	5379	335	241	40	5	5
Large-scale columns sandy loam	0.52	0.53	1.58	4	4	8	89	19	38	2	2	2
Field experiment	1.50	0.40	1.57	20	35	51	31	90	129	2	3	4

The accumulation of metals was expected to be high in small-scale or large scale columns. The data showed that metal accumulation was high in plants from small-scale columns than others in all plants parts likely due to more available metals resulting from application as high as 30 mm/day wastewater. Field experiment had higher metal accumulation than large-scale column experiment except iron and arsenic by roots. The noticeable trend was the high translocation factor in field experiment than in column experiments. Higher translocation factor at field implies higher segregation of metals by poplar aerial parts and removal from ground in actual conditions. Typical tissue concentration of iron and manganese are 100 mg/kg and 50 mg/kg in plants (ITRC, 2009). Poplar trees extracted higher, lower and similar concentrations iron and manganese than normal plant concentration in small-scale, large-scale and field experiments, respectively. Using poplar production of 20-25 Mg/ha/yr (Ceulemans & Deraedt, 1999) and average of 51 mg Mn/kg, 129 mg Fe/kg and 4 mg As/kg shoot concentration from the field experiment, manganese, iron and arsenic removal can be 1.02-1.23 kg/ha/yr, 2.58-3.23 kg/ha/yr and 0.08-0.1 kg/ha/yr, respectively. Therefore, there is potential for fully grown poplars to be a significant sink of manganese, iron and arsenic at land treatment sites.

Nitrogen uptake by roots was greatest in field experiment. Concentration of leaves and stem was similar for all experiments. The nitrogen concentration is close to typical nitrogen concentration in plants, i.e. 1.5% (ITRC, 2009). If poplar production of 20-25 Mg/ha/yr (Ceulemans & Deraedt, 1999) and average of 1.5% N content is used, nitrogen removal can be 300-375 kg/ha/yr, higher than previously reported 133-159 kg/ha/yr for 4th year poplar and similar to 350 kg N/ha/yr (Deckmyn et al., 2004). The uptake rate is higher than loading of 318 kg/ha/yr total nitrogen in growing season (April to September) in Minnesota (Zvomuya et al., 2006). Therefore, poplar trees have potential to treat nitrogen in the food processing wastewater by plant uptake. In the process, poplar trees may decrease the nitrate concentration of leachate water as obtained in field experiment during July-August and in column experiments during start of the season.

4.4.6. Nitrate mobilization

Nitrate in the influent wastewater was less than 1 mg/L in both column and field studies. Ammonium was high in the column studies (>6 mg/L) and low (<1 mg/L) in the field study. Total nitrogen may have been high due to presence of organic nitrogen in the field, but the data was not available. The nitrate concentrations of effluent wastewater from all experiments were significantly higher than the influent nitrate concentrations. Mean nitrate leaching observed was 1,038 mg/L in small-scale columns, 30 mg/L in large-scale columns and 66 mg/L in field study. Mean leachate ammonium concentration in small-scale columns, large-scale columns and field study were 3 mg/L, 8 mg/L and 6 mg/L, respectively. The mean influent ammonium in the wastewater was 6 mg/L in small-scale columns, 11 mg/L in large-scale columns and 0.14 mg/L in field. Therefore, nitrate was formed in all experiments and ammonium was removed from all columns experiments. The decrease of ammonium and formation of nitrate was due to nitrification (Schmidt, 1982).

The concentrations of nitrate in the leachate water from columns were high at the beginning of the experiment that rapidly decreased to lower than 5 mg/L as the experiment progressed. The decrease could due to denitrification or anammox or feammox. The monitoring of redox condition in large-scale soil columns showed that the conditions in the columns were anaerobic, favorable for denitrification and significant denitrification was expected. Anammox (anaerobic ammonium oxidation), a process in which ammonium is oxidized coupled with nitrite reduction to produce nitrogen gas, contributed 4-37% of total nitrogen loss in agricultural soils (Hu et al., 2011). However, as the oxidation of nitrite to nitrate is rapid, soils do not usually have nitrite more than in trace amount (Schmidt, 1982) and occurrence of significant anammox in columns was not expected. Whereas, Feammox (anaerobic ammonium oxidation) is a process in which oxidation of ammonium is coupled to iron reduction to produce nitrogen gas (Trapp et al., 2001). Occurrence of feammox in the columns could not be ruled out due to fluctuating redox conditions.

In field, ammonium in addition to nitrate was also formed likely from ammonification of organic nitrogen by group of microorganisms, mostly fungi, in aerobic soils (Ponnamperuma, 1972). The microbial analysis of soil had >35% relative abundance of aerobic prokaryotes and eukaryotes indicating ammonification may have occurred.

In small-scale columns, poplar trees reduced the mass leaching of the nitrate consistent with results obtained by Mankin et al. 2010. In large-scale columns, poplar trees reduced the nitrate leaching both in mass and concentration basis during the period when nitrate leaching was high due to predominant nitrification and insignificant denitrification. In field, during summer (July to September), nitrate leaching was significantly reduced in poplar planted plots on both concentration and mass basis. The reduction in nitrate was likely due to plant uptake of nitrogen.

Alternatively, poplars may have enhanced formation of the conditions conducive to nitrate loss, mainly denitrification. Ammonium concentration was not affected by poplar trees.

4.4.7. Metal mobilization

Mean iron and manganese concentrations in large-scale columns were less than 123.83±42.78 µg/L and 14.2±4.54 µg/L in 2011, less than 107.7± 13.7 µg/L and 253.9±33.2 µg/L in 2012 and less than 204.25±26.18 µg/L and 82.56±19.36 µg/L in 2013-2014, respectively. At field, the concentration of metals in leachate water was even lower. The mobilization obtained was much lower than 100 to 2,800 µg/L manganese and 50 to 44,600 µg/L iron under the application of 20-300 mg/L glucose carbon (Ugwuegbu et al., 2001) and 180 µg/L manganese and 7,500 µg/L iron from municipal waste landfill soil (Di Palma & Mecozzi, 2010).

Soil redox potential in large-scale columns varied from -100 mV to +450 mV (mostly between +100 to +300 mV), moderately reduced, in 2011, from -200 to +450 mV, reduced to moderately reduced, in 2012 and from -250 to +50 mV, highly reduced to reduced, in 2013-2014. Based on the redox data, the expected high mobilization of Mn and Fe due to dissolution of sulfides or reduction of manganese and iron oxy(hydroxides)(Moore et al., 1988, Jimenez-Carceles et al., 2008) did not occur.

First, poplar trees can influence metal concentration through producing exudates and oxygenating rhizoshpere (Jacob & Otte, 2003). Exudates can modify pH, redox and precipitation/dissolution and complexation by organic matter (Wang et al., 2002, Cervantes et al., 2011). However, redox influence was inconsistent in large-scale columns. As the concentration in leachate water from poplar planted columns were not different than that from no plant controls, rhizostimulation likely was also not influential. Therefore, plant related processes did not control metal mobilization.

In large-scale column experiments, mean pH during 2011 was >7.78±0.05, during 2012 was >8.12±0.10 and during 2013-2014 was >7.64±0.04. In field experiment, pH of planted and control samples were 7.42±0.13 and 7.48±0.11, respectively. In small-scale columns, pH of planted and control were 7.85±0.50 and 7.41±0.41, respectively. Higher pH of leachate water than neutral in all experiments reduced the critical redox potential for mobilization of both Mn and Fe. The critical redox potential values for mobilization of Fe was +300 mV at pH 6, +100 mV at pH 7 and -100 mV at pH 8 (Gotoh & Patrick, 1974). Soluble iron was least at pH 8 (Di Palma & Mecozzi, 2010). In another experiment, the threshold values for mobilization of Mn was +100 to +300 mV at pH of 6-7, +200 to +400 mV at pH ≤6-8 and -100 mV at pH 8 (Gotoh & Patrick, 1972). In addition, the redox reactions (nitrate to nitrogen, Mn^{+4} to Mn^{+2}, Fe^{+3} to Fe^{+2} etc.) consume hydrogen ion, increase pH and enhance sorption of metals on organic matter (Alloway, 2013). Therefore, it is evident from above discussions and pH values that high pH increased the stability of the manganese and iron.

Other biogeochemical processes that may have reduced the mobilization of iron and manganese could be precipitation of authigenic minerals including FeS, FeS_2, $MnCO_3$ (Otero et al., 2009), (co)precipitation of Ca, Fe and Mn as carbonates in sub-oxic or anoxic condition (Otero et al., 2009, Cervantes et al., 2011), cation bridging, biosorption and rate of oxidation under high carbon (Gadd, 2000, Jansen et al., 2003) and green rust formation (Cervantes et al., 2011) (Maria-Cervantes et al., 2010). Finally, redox dynamics or insufficient time for facultative anaerobes or anaerobes to acclimatize, (Frohne et al., 2011), sampling frequency and spatial variation in soil redox potential (Charlatchka & Cambier, 2000) may have contributed to low metal concentration in leachate samples. Thus, the microbial reduction and plant uptake were minor processes in controlling the mobilization of manganese and iron. Instead, geochemistry, mainly pH and soil redox potential controlled the mobilization of iron and manganese in the column as well as field experiments.

CHAPTER 5: CONCLUSIONS AND FUTURE RESEARCH

Phytoremediation technologies depend on the performance of plants in the mix of complex processes including soil and microorganisms. Success of such technology is largely determined by plant growth. Poplar trees should be able to withstand continuous soil moisture and high-organic carbon containing wastewater in order to have any phyto-treatment. Small-scale column studies evaluated this aspect and found that the addition of 1-2 times the highest known rate in Michigan did not prohibit poplar growth. However, it was discovered at the end of the experiment that roots mass, length and number was decreased compared to water-applied plants. As multiple-nutrients and metals were uptaken significantly higher by the wastewater applied plants than by the water applied plants, trees had healthy and functional roots, but small rhizosphere. This implies that the root density and rhizosphere need study before any design to treat food processing wastewater.

Poplar trees were able to withstand continuous saturation of soils while maintaining high evapotranspiration rates. Poplar trees evapotranspired 3.25 times more water than soil-only control columns in small-scale columns, 1.55 than sandy loam soil-only large-scale columns, 1.42 than loam soil-only large-scale columns and 1.5 and 1.7 (by poplar+grasses) than grasses only field plots.

To our knowledge, this is the first study where the soil moisture along depths due to influence of poplar trees in land treatment site has been studied. Soil moisture monitoring in large columns and field plots indicated that moisture level was reduced by poplar trees at the top and bottom levels of measurement, but not at the middle level. This was an unexpected result obtained at multiple locations (both field and columns). Irrigation pattern may have influenced the rooting pattern of the poplar trees and need further study.

The main objective of the treatment, reduction of carbon from the wastewater, was achieved from all systems. In small-scale columns, that had just 30 cm soil depth, 82% of influent COD was removed. In large-scale columns, greater than 90% and 69-85% COD removal were achieved from sandy loam and loamy soil columns, respectively. Though poplar trees did not reduce COD on a concentration basis, trees reduced COD significantly on a mass basis in small-scale columns. However, in large-scale columns, poplar trees enhanced COD treatment only in fine textured soils. The results indicated that selection of the site is crucial for better treatment of carbon in the food processing wastewater. While selecting the coarse textured soils tends to increase treatment, there is threshold where plants (grasses or trees) do not grow appreciably. Therefore, study to optimize the soil mixture is required. Conversely, on the fine textured soils, poplar plantation increases COD treatment.

In-situ redox potential was continuously monitored as a proxy for soil oxygenation in large-scale columns. The effect of poplar trees on redox potential was inconsistent. Soil redox potential was higher in planted columns in 2011, but lower in 2012 compared to control columns. In 2013-2014, planted and control columns were not different on the redox conditions. Contrasting plant processes including production of root exudates, oxygenation of soil and microbial enhancement contributed to inconsistent results. However, the plants were not growing healthily for most duration or were very young and small. Therefore, it would be worthy to study the effects of poplar trees on soil redox potential when the plants mature and the rooting density is higher.

Microbial biomass and community study using PLFA analysis for field and large column soils obtained very high biomass in the soil. In both cases, microbial diversity included microeukaryotes, aerobic prokaryotes and eukaryotes, fungi, anaerobic prokarytoes, sulfate reducing bacteria and gram positive prokaryotes. However, the presence of relative proportions of each type of microorganisms varied between field and column experiments. Total microbial biomass was higher in poplar planted plots than in control plots in the field indicating the contribution of poplar trees to microbial enhancement. In column studies, loamy planted columns had lower anaerobic microorganisms than loamy control. Anaerobic microorganisms were in lower abundances in field plots than in columns. Total biomass and aerobic microorganisms were higher at surface soils than at deeper soils. Generally, anaerobic bacteria were higher in the deeper soils as expected. PLFA analysis does not get the resolution to the species level. Therefore, identifying individual strains of carbon degraders, metal reducers and studying their presence, relative abundance and activities can better determine the role of poplar plantation in microbial enhancement.

Reduction in metal and nitrate mobilization, the primary aim of the research was evaluated for all studies. Poplar trees in the small-scale columns reduced the leaching of iron and manganese in the leachate water on the mass basis mostly due to uptake of the metals to shoot tissues. In large-scale soil columns, high mobilization of metals (manganese and iron) was not observed. Manganese was moderately mobilized in 2012 and 2014 and iron was not mobilized in all years. Plants did not show significant effects on either reduction of leachate concentrations of metals or of soil concentrations of metals. Geochemical processes dominated the fate of metals. High pH of the leachate water indicated that metals were strongly adsorbed or precipitated rather than solubilized even at low redox potential. Plant associated processes may have worked together with geochemical processes, however, no significant effects of plants only was observed in reducing metal mobilization. However, poplar trees accumulated large concentrations of metals (arsenic, manganese and iron) on to its shoot tissues. High biomass production may show effects on metal reduction and need further study.

Nitrate leaching was significantly reduced on mass basis by poplar trees in small-scale columns due to significant nitrogen uptake. In large-scale columns, there were signs that poplar trees enhanced the nitrate removal. First, there was high nitrogen uptake with similar bioaccumulation factors despite difference in soil concentrations. Second, plant reduced nitrate concentration of leachate water when the columns were predominantly aerobic in 2011 and 2013 and high nitrate mobilization occurred. In field, nitrate decrease by poplar trees were observed during July and August. As large quantity of wastewater is land applied in the summer months, this trait of poplars could be beneficial. Though plant uptake and significant accumulation of nitrogen and metals were observed, the accumulated mass was not significant enough to show any effect on either soil or leachate concentration. The projected mass accumulation using concentration and total biomass after years was promising. Therefore, these kinds of study need to continue for years until poplar trees peak their growth.

5.1. Contribution to science

This is, to our knowledge, first study that studied potential of poplar plantation to reduce metal mobilization from any wastewater applied land. Moreover, this study applied unique approach to study soil redox potential and moisture at different levels in the soil profile using the combination of different scale experiments under poplar plantation and food processing wastewater application.

Research demonstrated that moisture loss pattern along depths is more complex than increasing or decreasing trend from surface to depth. Roots of grasses and trees affected the moisture and contributed to lower or higher soil moisture at depths depending on where the reference soil moisture depth was. More specifically, moisture at intermediate depth was not decreased by poplar trees compared to bottom and top levels monitored in this research.

The research confirmed that though wastewater applied poplar trees at land application site had higher uptake of metals (manganese, iron and arsenic) both in concentration and mass in their shoot tissues than water applied poplar trees, metal mobilization was not reduced compared to control. Metal mobilization was controlled by geochemical processes than by plant-related processes. Though redox potential was low enough to mobilize metals, high pH, high adsorption and precipitation of metals may have contributed to low concentration of iron and manganese in leachate water. For metal mobilization, geochemical processes, plant uptake, evapotranspiration, microbial stimulation and oxygenation of soils were important in the decreasing order. Plant uptake of nitrogen was a significant process to reduce the nitrate concentration in the leachate water during summer months or peak nitrate mobilization. For nitrate mobilization, plant uptake, evapotranspiration, microbial stimulation and soil oxygenation were important in the decreasing order. Though several prior studies have indicated oxygenation of soils by plants, poplar trees did not oxygenate soils in the magnitude enough to affect the soil redox potential.

Therefore, this research provided an insight into fate of metals and nitrate in the presence of poplar trees under the application of food processing wastewater. Scientific community will find the results of this study to be applicable to land treatment of wastewater that have high carbon loading.

5.2. Plant-soil-microbial activities in winter

Average growing season for study area in MI is 160 days and depend on air temperature, day length, soil temperature and first and last frost date (USDA, 2014). During non-growing season, colder temperature limits the activity of aerobic microorganisms and the degradation of organic carbon (MDEQ, 2007). However, microbial activities in soil continue to occur in winter. Rhizospheric respiration, due to live toots and associated microorganisms, as well as heterotrophic respiration, due to microbial oxidation of soil organic matter, continued during winter (Kähkönen et al., 2001, Scott-Denton et al., 2006, Tucker et al., 2014). The contribution was higher from heterotrophic respiration during onset of winter and from rhizospheric during the end of winter (Scott-Denton et al., 2006). Plants released high levels of sucrose during late-spring even when dormant and increased the rhizospheric respiration by providing carbon source to the microbial community. Though no or limited root growth is expected during winter as the critical temperature for significant root growth is 6°C (Alvarez-Uria & Koerner, 2007), fine root grew even in the dormant season from the coppice of poplars using nitrogen and carbon reserves (Dickmann et al., 1996). In sub-alpine ecosystems, microbial respiration increased by 2-3 folds from January to May in the winter and was greatest in the summer (Tucker et al., 2014). The winter respiration in soil was 4-25% of that in summer in which rhizosphere contributed 33-39% of wintertime respiration (Tucker et al., 2014). Even when under 91 cm of snow and unlikely photosynthetic processes occurring, soil microbial respiration was observed (Tucker et al., 2014). Thus, small but significant activities in the rhizosphere in the winter can be expected. As such, poplars may be beneficial even in the winter. However, enhanced removal is expected when the rhizosphere activities and plant growth are at optimum, in the summer time. Additionally, high temperature during summer can help to overcome the energy barrier to adsorption or desorption and metal movement in general (Alloway, 2013).

5.3. Merits and demerits of poplar plantation at land treatment sites

Phytoremediation is low-cost and effective method for contaminants treatment (Ciurli et al., 2014). Economically, the poplar plantation technology at food processing land application site may be viable as poplar tree grew well at field site. The return on investment of hybrid poplar is as high as an estimated $10,000 per acre per year at the end of 10 year growing cycle with production cost of $450 per acre per year (St John, 2001).

However, poplar plantation at the land application sites has challenges. First, the pivot which is mostly used in irrigation has to be replaced by sprinklers that rise from sub-surface in order for poplar to not stand on the way of pivot rotation. Second, metals at the higher concentration may be toxic to plants (Ciurli et al., 2014). While no toxic effect of wastewater application on vegetative growth was observed in small-scale columns, roots were less developed than in controls. Third, the planting density, clones, harvesting interval and stump removal need to be figured out. Planting density depends on many factors such as market, rotation length, cost of plantation, cost of maintenance and harvesting cycle. In Idaho, harvesting cycle based on plant spacing were 1-3 years for 0.6×1.2 m, 5 years for 1.5×1.5 m, 8 years for 2.4×2.4 m or 2.4×3 m, 10 years for 3×3 m, 12 years for 3.6×3.6 m or 4.2×3.6 m (St John, 2001). Harvesting cycle need to leave enough poplar trees on ground at any time to continue treatment. Harvesting can be done by thinning or partial cuts in the field. Stump removal is one of the problems associated with harvest. Short harvest cycle of 4-5 years will allow multiple harvests, up to three, from same stump before replanting (St John, 2001). However, longer harvest cycle will produce unreliable, susceptible and weaker plants. Lastly, because the technology is still at infancy, potential contaminants in the biomass and the management of contaminants in the biomass are other challenges.

APPENDIX

Table A-1 Textural analysis of new soils used in six columns in 2012.

Column with new soil	Sand	Silt	Clay	Texture
Planted, 32	64.7	16.6	18.7	Sandy loam
Planted, 14	66.7	18.6	14.7	Sandy loam
Planted, 23	66.7	18.6	14.7	Sandy loam
Average	66.7	17.9	16.0	Sandy loam
Control, 12	58.7	20.6	20.7	Sandy clay loam
Control, 24	68.7	12.6	18.7	Sandy loam
Control, 31	60.7	21.6	17.7	Sandy loam
Average	62.7	18.3	19.0	Sandy loam

Table A-2 Soil characteristics at the end of the experiment in 2011. Values reported are mean and standard error of mean (n=3).

Description	Old soil from 2011
pH	8.03±0.03
P (ppm)	24.33±0.88, O
K (ppm)	106.00±9.29, O
Mg (ppm)	130.33±20.50, AO
Exchangeable K (%)	2.13±0.26
Exchangeable Mg (%)	8.47±1.12
Exchangeable Ca (%)	89.37±0.85
Ca (ppm)	2273.33±96.28
Zn (ppm)	5.90
Mn (ppm)	92.77±7.52
Cu (ppm)	5.87±0.09
Fe (ppm)	112.23±17.98
OM (%)	2.93±0.23
TKN (%)	0.10
CEC (meq/100 g)	13.10
Nitrate N (ppm)	3.07±0.58
Lead (ppm)	16.00±1.53
Arsenic (ppm)	3.53±0.74

AO: Above Optimum, O: Optimum

Table A-3 Characteristics of soil used in 2012 experiment. Values provided are mean and standard error of mean, n=3.

Description	Old soil from 2011	New soil in 2012
pH	8.03±0.03	8.20±0.06
P (ppm)	24.33±0.88, O	108.67±11.33, AO
K (ppm)	106.00±9.29, O	425.33±19.43, AO
Mg (ppm)	130.33±20.50, AO	236.00±13.65, AO
Exchangeable K (%)	2.13±0.26	8.80±0.50
Exchangeable Mg (%)	8.47±1.12	15.55±0.35
Exchangeable Ca (%)	89.37±0.85	75.60±0.80
Ca (ppm)	2273.33±96.28	1776.33±31.18
Zn (ppm)	5.90	6.70
Mn (ppm)	92.77±7.52	98.43±5.98
Cu (ppm)	5.87±0.09	4.43±0.12
Fe (ppm)	112.23±17.98	120.90±15.21
OM (%)	2.93±0.23	4.00±0.50
TKN (%)	0.10	0.14
CEC (meq/100 g)	13.10	12.00
Nitrate N (ppm)	3.07±0.58	3.40±1.13
Lead (ppm)	16.00±1.53	12.33±0.33
Arsenic (ppm)	3.53±0.74	2.10±0.06

AO: Above optimum, O: Optimum

Table A-4 Soil characteristics before and after the experiment in 2012.

Description	Before experiment (n=3 each)		After experiment (n=2)
	Old soil from 2011	New soil in 2012	
pH	8.03±0.03	8.20±0.06	8.1±0.1
P (ppm)	24.33±0.88, O	108.67±11.33, AO	22.5±1.5 AO
K (ppm)	106.00±9.29, O	425.33±19.43, AO	256±7 AO
Mg (ppm)	130.33±20.50, AO	236.00±13.65, AO	152±1 AO
Exchangeable K (%)	2.13±0.26	8.80±0.50	4.6±0.1
Exchangeable Mg (%)	8.47±1.12	15.55±0.35	8.9±0
Exchangeable Ca (%)	89.37±0.85	75.60±0.80	86.5±0.1
Ca (ppm)	2273.33±96.28	1776.33±31.18	2464.5±10.5
Zn (ppm)	5.90	6.70	6.85±0.25
Mn (ppm)	92.77±7.52	98.43±5.98	77.55±2.05
Cu (ppm)	5.87±0.09	4.43±0.12	6.2±0.1
Fe (ppm)	112.23±17.98	120.90±15.21	84.1±5.2
OM (%)	2.93±0.23	4.00±0.50	3.1±0.1
TKN (%)	0.10	0.14	0.10±0.02
Ammonium-N (ppm)			2.05±0.05
CEC (meq/100 g)	13.10	12.00	14.25±0.05
Nitrate N (ppm)	3.07±0.58	3.40±1.13	10.3±0.7
Lead (ppm)	16.00±1.53	12.33±0.33	15.5±0.5
Arsenic (ppm)	3.53±0.74	2.10±0.06	3.15±0.15

AO: Above Optimum, O: Optimum

Table A-5 Characteristics of soil used in 2013-2014 experiment. Values provided are mean and standard error of mean (n=2).

Description		Sandy Loam	Loam
pH		8.1±0.1	7.95±0.05
P (ppm)		22.5±1.5 AO	3.5±1.5 BO
K (ppm)		256±7 AO	44.5±2.5 BO
Mg (ppm)		152±1 AO	263.5±9.5 AO
% Exchangeable bases	K	4.6±0.1	0.6±0
	Mg	8.9±0	11.95±0.25
	Ca	86.5±0.1	87.45±0.25
Ca (ppm)		2464.5±10.5	3216±42
Zn (ppm)		6.85±0.25	1.25±0.15
Mn (ppm)		77.55±2.05	71.3±4.7
Cu (ppm)		6.2±0.1	7.4±0.7
Fe (ppm)		84.1±5.2	27.7±7.8
OM (%)		3.1±0.1	2.2±0.1
TKN (%)		0.10±0.02	0.03±0
Ammonium-N (ppm)		2.05±0.05	1.8±0.1
CEC (meq/100 g)		14.25±0.05	18.4±0.3
Nitrate N (ppm)		10.3±0.7	6.1±1.1
Lead (ppm)		15.5±0.5	22.5±1.5
Arsenic (ppm)		3.15±0.15	28±2

AO: Above Optimum, BO: Below optimum

Table A-6 Characteristics of soil before and after experiment in 2013-2014.

		Before	After		Before	After	
Description		Sandy	Sandy planted	Sandy control	Loam	Loam planted	Loam control
pH		8.1±0.1	8.5±0.0	8.5±0.0	7.95±0.0	8.4±0.1	8.4±0.0
P (ppm)		22.5±1.5 AO	15.7±0.3	16.3±0.3	3.5±1.5 BO	3.3±0.3	3.3±0.3
K (ppm)		256±7 AO	494±11	437±20	44.5±2.5 BO	358±46	363±39
Mg (ppm)		152±1 AO	207±6	218±18	263.5±9.5 AO	224±11	215±3
Ca (ppm)		2465±11	2373±43	2311±41	3216±42	3202±28	3164±5
% Exchangeable bases	K	4.6±0.1			0.6±0		
	Mg	8.9±0			11.95±0.25		
	Ca	86.5±0.1			87.45±0.25		
Zn (ppm)		6.85±0.25			1.25±0.15		
Mn (ppm)		77.55±2.05			71.3±4.7		
Cu (ppm)		6.2±0.1			7.4±0.7		
Fe (ppm)		84.1±5.2			27.7±7.8		
OM (%)		3.1±0.1			2.2±0.1		
TKN (%)		0.10±0.02			0.03±0		
Ammonium-N (ppm)		2.05±0.05			1.8±0.1		
CEC (meq/100 g)		14.25±0.05			18.4±0.3		
Nitrate N (ppm)		10.3±0.7			6.1±1.1		
Lead (ppm)		15.5±0.5			22.5±1.5		
Arsenic (ppm)		3.15±0.15			28±2		

AO: Above Optimum, BO: Below Optimum

Table A-7 Characteristics of the field soil. Number of sample is 1 for composite soil and 2 for top soil (mean±standard error).

Parameter (unit)		Composite sample 0-1.2 m	Top soil
pH		6.8	6.6±0.0
Sand (%)		72.6	62.4±1.5
Silt (%)		12.0	22.0±2.0
Clay (%)		15.4	15.6±0.5
Textural class		Sandy loam	Sandy loam
P (ppm)		83 AO	167.5±14.5 AO
K (ppm)		98 O	154.5±22.5 AO
Mg (ppm)		119 AO	127.5±17.5 AO
% Exchangeable bases	K	7.9	9.1±0.1
	Mg	31.2	24.45±0.15
	Ca	60.9	66.45±0.35
Ca (ppm)		388	576.5±72.5
Zn (ppm)		3.2	6.2±1.6
Mn (ppm)		20.5	29.45±1.65
Cu (ppm)		1.5	7.15±2.75
Fe (ppm)		90.9	208.5±1.7
OM (%)		0.9	2.1±0.5
TKN (%)		0.05	0.11
CEC (meq/100 g)		3.2	4.35±0.55
Nitrate N (ppm)		1.3	25.4
Lead (ppm)		5.5	14
Arsenic (ppm)		2.2	3.7

AO: Above optimum, O: Optimum

Table A-8 Nutrients and metals in the soil before and after experiment for field soil.

Parameter (unit)		Composite 0-4 ft	Top soil	Top soil	P1	P2	C1	C2
pH		6.8	6.6	6.6	7.2	7.2	7.2	7.1
P (ppm)		83 AO	153 AO	182 AO	143	193	176	156
K (ppm)		98 O	177 AO	132 AO	224	244	260	196
Mg (ppm)		119 AO	145 AO	110 AO	239	236	269	210
Ca (ppm)		388	649	504	143	193	176	156
% Exchangeable bases	K	7.9	9.2	9				
	Mg	31.2	24.6	24.3				
	Ca	60.9	66.1	66.8				
Zn (ppm)		3.2	7.8	4.6				
Mn (ppm)		20.5	31.1	27.8	50.6±8.9	44.7±8.5	56.1±2.4	32.2±5.9
Cu (ppm)		1.5	9.9	4.4				
Fe (ppm)		90.9	210.2	206.8	129.3±25.4	157.7±7.2	138.6±8.4	144.5±3.9
OM (%)		0.9	2.6	1.6				
TKN (%)		0.05	0.11		0.13±0.04	0.14±0.04	0.16±0.02	0.13±0.05
CEC (meq/100 g)		3.2 4.1	4.9	3.8 5.9				
Nitrate N (ppm)		1.3	25.4					
Lead (ppm)		5.5		14				
Arsenic (ppm)		2.2		3.7	4.07±0.60	2.82±0.40	2.94±0.33	3.20±0.53

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