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Wastewater Treatment Using Poplar Plants: Processes

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Abstract: Phytoremediation is used to treat wastewater, wherein plants, microorganisms, and soil work together to remediate pollutants. We evaluated the plant processes that can affect metal mobilization during phytoremediation. The experimental columns were filled with silica sand and soil mixture spiked with redox-sensitive metal(loid)s—arsenic, manganese, and iron, and fitted with an ORP probe and oxygen sensors. Three columns were planted with poplars and three others were no-plant controls. Carbon-rich, synthetic food-processing wastewater was applied at 15.4 mm/day to the columns. Leachate water was analyzed every other week for water quality. Both soil and plant tissue samples were analyzed for metal concentrations, and soils were analyzed for microbial populations. Both treatments reduced 65–70% carbon. ORP ranged from −321 mV to 916 mV and affected metal mobilization. Oxic conditions in planted treatments yielded high ORP, oxygen concentration, and nitrates. Microbial communities were enhanced in both treatments, but the planted columns had more microbial abundance and evenness. Plants successfully accumulated metals in roots from soil with an accumulation factor of up to 40 for some metals and translocated to shoots from roots with a translocation factor of 10.62. The crop coefficient was 1.88, indicating accelerated loss of water in planted columns compared to control columns. The results demonstrated the benefits of plants in creating more oxic conditions, removing more wastewater from the rhizosphere, accumulating and translocating metals in the biomass, and enhancing rhizodegradation of pollutants by microbial population enhancement. Knowledge of the soil–plant–microbial processes is useful in designing engineered phytoremediation systems.

Keywords: phytoremediation; rhizosphere processes; heavy metal contamination; groundwater; land treatment of wastewaters; phytoprocesses

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1. Introduction

One of the popular methods of food-processing wastewater (FPWW) treatment is land application, which relies heavily on microbial metabolism to oxidize organic waste [1,2]. During land application, especially under continuous saturation and anoxic conditions, metals in native soil become reduced and mobile and can contaminate groundwater. Thus, heavy metals are often found in excess of the permissible levels in groundwater [3]. Heavy metals are defined generally as metals with high densities (relative to water) and high atomic weights (greater than that of iron) that are toxic at even minute concentrations (ppb). In contrast, metalloids are elements with properties that are intermediates between metals and non-metals. One of the most common contaminants in groundwater systems is trace elements, more specifically, metallic elements such as arsenic, cadmium, lead, chromium, copper, nickel, and mercury [4].

Due to natural, agricultural, industrial, domestic, and other sources, heavy metal pollution has been a problem that has been plaguing the environment and negatively impacting human health for some time. The increase in urbanization and industrialization has led to the rise in heavy metal pollution [4]. In North Carolina, 50% of well waters have

Mn levels above the state's maximum contaminant level (MCL) [5]. Even more concerning is the levels of arsenic in North Carolina's groundwater [6].

Once a groundwater source is contaminated, the water can be unsafe for human consumption. This is because these metals enter the body through ingestion through food and water [7]. Increasing human exposure through food and water due to the mobility and bioavailability of heavy metals is a global concern [8]. Exposure to heavy metal or metalloid contaminants such as Pb, Cd, Zn, Cu, Hg, and As threaten human health. Of all trace elements, Pb and Cu are some of the most hazardous environmental pollutants. Duffus (2002) noted that even at minor concentrations and low levels of exposure, some metalloids, like arsenic, are able to induce toxicity [9].

But it is not only humans that are at risk. Excess concentrations of heavy metals in soils can lead to a loss of microbial activity, which leads to reduced soil fertility, resulting in yield loss. That is why it is vital to protect groundwater from contamination from these toxins. If contaminated by these metals, the soil and water are very difficult and expensive to remediate because they are immutable by biochemical reactions, unlike organic contaminants that may be broken down into smaller molecules.

For decades, researchers have studied soil–microbe–metal interactions to deduce the best method for solving this environmental crisis. The metals of interest in this study were iron, arsenic, and manganese. The bioavailability and toxicity of iron to a plant relies on the specificity of the site's soil conditions (pH, moisture, ORP) [10]. These metals and metalloids (arsenic, iron, and manganese) are redox-sensitive metals, meaning they are reduced under anoxic conditions. The reduced species are more soluble, more mobile, and more toxic. Therefore, we need to prevent the formation of reducing conditions in the soil during the land application of wastewaters.

Plants are likely to reduce the formation of such conditions due to several processes, including oxygenation, rhizostimulation, and evapotranspiration. Plant roots can leak oxygen into the soil atmosphere, thus influencing the soil redox potential and the metal fate in the soil. Additionally, plants and bacteria can form specific associations where the plants provide C as an energy source for the microbes, which in turn allows the microbes to reduce the phytotoxicity of the contaminated soils and/or to immobilize the pollutants. Alternatively, plants and bacteria can form nonspecific associations in which normal plant processes stimulate the microbial community, which, in the course of normal metabolic activity, degrades contaminants in soil. Plants' roots can provide root exudates as well as increase ion solubility. These biochemical mechanisms increase the remediation activity of bacteria associated with plant roots.

The solution to the problem of contaminant mobilization in soils can be understood if plant–soil–metal–microbe interactions are studied more intensely. This is important because the interaction of the plant, soil, and contaminant influences the success of plant-based remediation systems [11]. Therefore, the goal of this project was to evaluate the plant–soil–microbe processes during land application of food processing wastewaters. The specific objectives during land application of food processing wastewater to poplar planted soil were as follows:

I. Evaluate plant-related processes (i) evapotranspiration, (ii) plant uptake, (iii) oxygenation, and (iv) rhizostimulation;
II. Assess the combined effects of the studied processes on the mobilization of metals and nitrates.

2. Materials and Methods

Six columns (6 in. diameter × 18.32 in. length) were made of polyvinyl chloride (PVC) pipes, capped with PVC caps, and sealed with sealant in a temperature-controlled indoor laboratory. Spigots were inserted in holes drilled an inch from the bottom of the columns, and $\frac{1}{4}$ in. tubing was connected to the spigots to allow for water samples' collection during experimentation. After construction, the columns were filled with a mixture of soil and silica sand with a bulk density of 1.43×10^3 kg/m^3 spiked with heavy metal(loids) up to 16.32 inches (Figure 1). The bulk density of the sand–soil mixture and the volume of the cylinder were taken into account to determine the mass of the heavy metals needed to spike the soil.

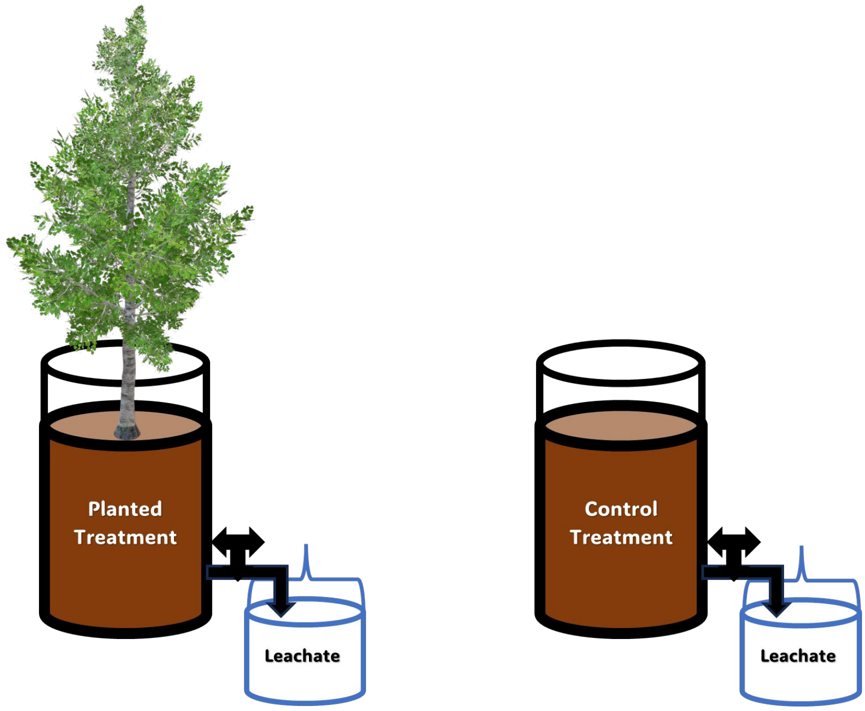

Figure 1. Experimental design of columns with containers for leachate collection.

Heavy metals and metalloids such as arsenic (As), manganese (Mn), and iron (Fe) were spiked in the soil and sand mixture to stimulate the relevant environmental concentrations of each metal in North Carolina soil, which were 40, 900, and 2×10^4 mg kg^{-1}, respectively [12]. The metal concentrations were chosen to represent a range of low, medium, and high contaminant levels in the environment (Table 1). For spiking, a standard solution of iron(III) oxide or disodium hydrogen arsenate or manganese sulfate was prepared with a known concentration, a specific volume of standard solution was pipetted and added to the soils, and soil was homogenized for even distribution. To ensure that soil–metal concentrations increased, soil samples were taken before and after spiking and then analyzed for metal concentration using ICP-OES, the results of which are presented in Figure 2.

Table 1. Concentration of spiked metals.

Level	Low	Medium	High
Metal(loid)	As	Mn	Fe
Environmental concentration (mg kg^{-1})	40	900	2×10^4
Salt used	$Na_2HAsO_4 \cdot 7H_2O$	$MnSO_4 \cdot H_2O$	Fe_2O_3

Columns were fitted with a Hanna Instruments HI3133B ORP (Smithfield, RI, USA) probe with a Fisherbrand™ accumet™ Epoxy Body Mercury-Free Reference Electrode (Waltham, MA, USA) and an Apogee Instrument (SO-110) (Logan, UT, USA) soil oxygen probe to monitor redox potential and soil oxygen levels, respectively. Data was collected from the sensors every 15 min and stored on Campbell Scientific CR1000× dataloggers. Data was collected from the dataloggers periodically throughout the experiment. ORP data was corrected and converted to a standard hydrogen electrode.

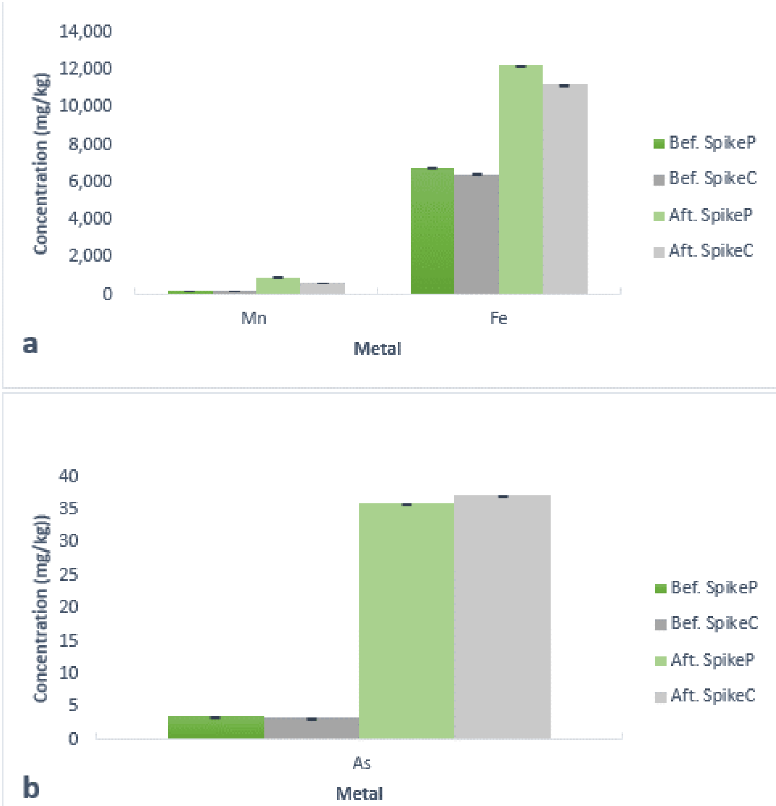

Figure 2. Soil metal(loid) concentrations before and after spiking—(**a**) Mn and Fe, and (**b**) As. P and C stand for planted and control columns.

In this experiment, there were three planted columns and three no-plant control columns. In the planted columns, tall shade 2–3 ft poplar plants *Populus deltoides* × *Populus nigra OP367* sourced from Fast Growing Trees, LLC were planted in a completely randomized design, and tap water was applied during the establishment phase. Hybrid poplar trees were chosen for this experiment because they possess the ability to achieve rapid above and below-ground biomass, have high evapotranspiration, deep roots, and are one of the most used species in phytoremediation. Additionally, poplars are able to remediate soil and groundwater through removal, stabilization, and break down of contaminants [13,14]. Synthetic, carbon-rich wastewater was prepared in the laboratory using sucrose, soluble starch, and salts, which emulated the average characteristics of factory food processing water (Table 2). The wastewater application started 14 days after planting to ensure plants were properly established. The daily local rate of application in non-coastal environments was taken into consideration when applying wastewater [15]. The synthetic wastewater was applied at a rate of 15.4 mm per day to each column for five months. Additionally, the season was taken into consideration, with WW application taking place less frequently during periods of reduced evapotranspiration when plants shed their leaves, which coincided with winter. Columns were allowed to drain freely both during and after wastewater application to avoid waterlogging. Leachate volume was measured weekly, and evapotranspiration was calculated by subtracting the total leachate volume from the volume of WW applied weekly.

Leachate water samples were collected every other week and the water quality, including COD, pH, ORP, NH_4-N, NO_3-N, and TN, was tested according to EPA-approved standard methods. 24-mL water samples were collected in glass vials and analyzed using various instruments, which included a Hach DR6000 UV/VIS Spectrophotometer (Loveland, CO, USA) with RFID Technology in conjunction with a Hach—DRB200-04 for COD; a Thermo Scientific™ Orion Star™ A211 Benchtop pH Meter (Singapore) for pH; and a Thermo Scientific™ Dionex™ ICS-6000 Capillary HPIC™ for nitrogen species concentrations. COD was measured using a HACH method 8000. As, Fe, and Mn in leachate water samples were analyzed using a PerkinElmer Optima 8000 ICP-OES (Shelton, CT, USA) with an S10 Autosampler & PolyScience Chiller. During sample preparation, the samples were filtered with a 0.45 or 0.22 µm PTFE filter when necessary. Nitrate-N and total-N were analyzed as anions and ammonium-N was analyzed as cations using a Thermofisher Scientific Ion Chromatography (IC), ICS-6000. Anions were separated using the 2 × 250 mm analytical AS11-HC-4 µm column with an AG11-HC-4 µm guard along with an EGC of 35 mM KOH at a flow rate of 0.25 mL/min and detected with a conductivity detector. Similarly, cations were separated using the 2 × 250 mm analytical CS19-4 µm column with a CG19-4 µm guard along with an EGC of 20 mM MSA at a flow rate of 0.25 mL/min and detected with a conductivity detector. For quality assurance and quality control, samples on the IC were analyzed along with replicates, anion and cation standards, and blanks. A linear calibration curve with at least six standard levels was used for calibration and R^2 for the calibration was at least 0.99.

Table 2. Synthetic FPWW characteristics and composition.

Wastewater Characteristics	Chemicals	Concentration (mg/L)
COD	Sucrose (Crystalline)	1438.5
	Starch (from potatoes)	205.5
Ca	Calcium Chloride, $CaCl_2$	66.8
Mg	Magnesium Sulfate, $MgSO_4 \cdot 7H_2O$	24.9
K	Potassium Carbonate, K_2CO_3	476.9
Na	Sodium Bicarbonate, $NaHCO_3$	35.3
Fe	Ferric Chloride, $FeCl_3 \cdot 6H_2O$	2.4
Mn	Manganese Sulfate, $MnSO_4 \cdot H_2O$	0.1
NH_4-N	Ammonium Sulfate, $(NH_4)_2SO_4$	19.7
Zn	Zinc Sulfate, $ZnSO_4 \cdot 7H_2O$	0.2
Cu	Copper Chloride, $CuCl_2 \cdot 2H_2O$	0.1
Co	Cobalt Chloride, $CoCl_2 \cdot 6H_2O$	0.02
B	Sodium Borate, $Na_2B_4O_7 \cdot 10H_2O$	0.01
Mo	Sodium Molybdate, $Na_2MoO_4 \cdot 2H_2O$	0.01
Ni	Nickel Nitrate, $NiNO_3 \cdot 6H_20$	0.07

Soil ORP was measured using an HI3133B ORP probe in conjunction with a Fisherbrand™ accumet™ Epoxy Body Mercury-Free Reference Electrode for ORP, whereas soil oxygen concentration was measured using an Apogee Instrument (SO-110).

At the conclusion of the experiment, trees were sacrificed and the columns were deconstructed. Soil samples were taken from various depths—the "topsoil" sample was taken from 0 to 4", the "root zone soil" sample was taken from 10 to 14" and the "composite soil" sample was taken from 6 to 8" below the surface. The soil samples were dried for 36 h using a Thermo Scientific Heratherm OGS60 General Protocol Oven and ground using a laboratory blender and a mortar and pestle. Triplicate soil samples from each depth and treatment were analyzed for metal and microbial populations. Microbial analysis was conducted using a 16s rRNA Illumina Amplicon Sequencing at the UNC Chapel Hill School of Medicine Microbiome Core Facility. DNA extraction and purification were performed following standard protocols and procedures. Taxonomic information at the genus/species level, calculations of alpha and beta diversity, and the basic analysis of the over or under-representation of microbial groups were determined. The data was provided by targeting the V3–V4 variable regions in the 16S ribosomal gene to analyze the complexity of the microbial communities present in the soil samples. Visualization of microbial data was conducted using the Visualization and Analysis of Microbial Population Structures (VAMPS) program to generate KRONA charts.

The trees were removed from the columns, rinsed thoroughly, separated into roots and shoots, and the mass was measured. The plant samples were dried for 24 h at 104 °C, and the dry mass was recorded. Plant tissues, water, and soil samples were analyzed by NCATSU Analytical Services Laboratory (ASL) for concentration of metals after solid samples were processed with acid digestion. ICP-OES was used to determine the concentration of metals in both soil and plant samples. For acid digestion, 0.3 g of sample was weighted, and a predetermined amount of concentrated nitric acid (67–70%, Fisher Scientific) and hydrofluoric acid (48–51%, VWR Chemicals) were added to the digestion tube. The acid-treated samples were left for 10 min, and they were subjected to automated sequential microwave digestion using the CEM Microwave Technology Ltd. (Matthews, NC, USA) MARS 6 digester.

All data was plotted in Microsoft Excel 2016, and the standard error was determined. During visualization, the replicates collected were averaged. Statistical analysis included using SigmaPlot version 14.5 to run the non-normal data test Mann–Whitney U test of medians and Kruskal–Wallis one-way analysis of variance (ANOVA) on ranks. For normal data, the Brown–Forsythe test of variance was used.

3. Results

3.1. Plant Growth and Evapotranspiration

WW application and spiked metal concentrations did not appear to hinder the growth of the trees. The poplar trees grew healthily, as expected throughout the season. The average biomass of the plants was 76.33 g $\pm$ 21.88 g (shoots, moisture content 61.3%) and 83 g $\pm$ 28.21 g (roots, moisture content 52.4%).

The rate of evapotranspiration (ET) in the planted treatments was higher than that in the no-plant controls (Figure 3a), which was expected. The rate of evapotranspiration decreased as winter approached due to plants shedding their leaves. The volume of leachate water collected from the planted columns was significantly lower than that collected from control columns (Figure 3b). The crop coefficient was calculated using the formula $ET_c = ET_o \times K_c$, where ET_o is the water use of the control treatment, ET_c is the water use of the planted treatment, and K_c is the crop coefficient. The overall crop coefficient was determined to be 1.88.

3.2. Rhizostimulation

16s rRNA amplification was used to determine the richness and diversity of the microbial communities present in the experimental systems. More specifically, the data was analyzed for the presence of denitrifying-, nitrifying-, metal-reducing-, carbon degrading-, sulfate-reducing- bacteria, and methanotrophs.

In our experiment, microbial analysis took place for six (6) sampling periods or regimes, which included before and after spiking (2), after planting but before WW application (1), and after the experiment at 0–4″, 6–8″, and 10–14″ sampling depths (3). Before spiking, denitrifiers. *Actinobacteria* and *Firmicutes* were mostly present in no-plant control samples, with *Actinobacteria* being the most prevalent. However, the presence of *Firmicutes* was enhanced after metal spiking in the no-plant controls. On the other hand, the planted soil samples comprised mainly sulfate-reducing bacteria of the *Proteobacteria* and *Firmicutes* phyla. Soils in the no-plant control treatments were more biodiverse than those in the planted treatments. However, the key difference was the observation of more microbial evenness in treatment columns than in control columns.

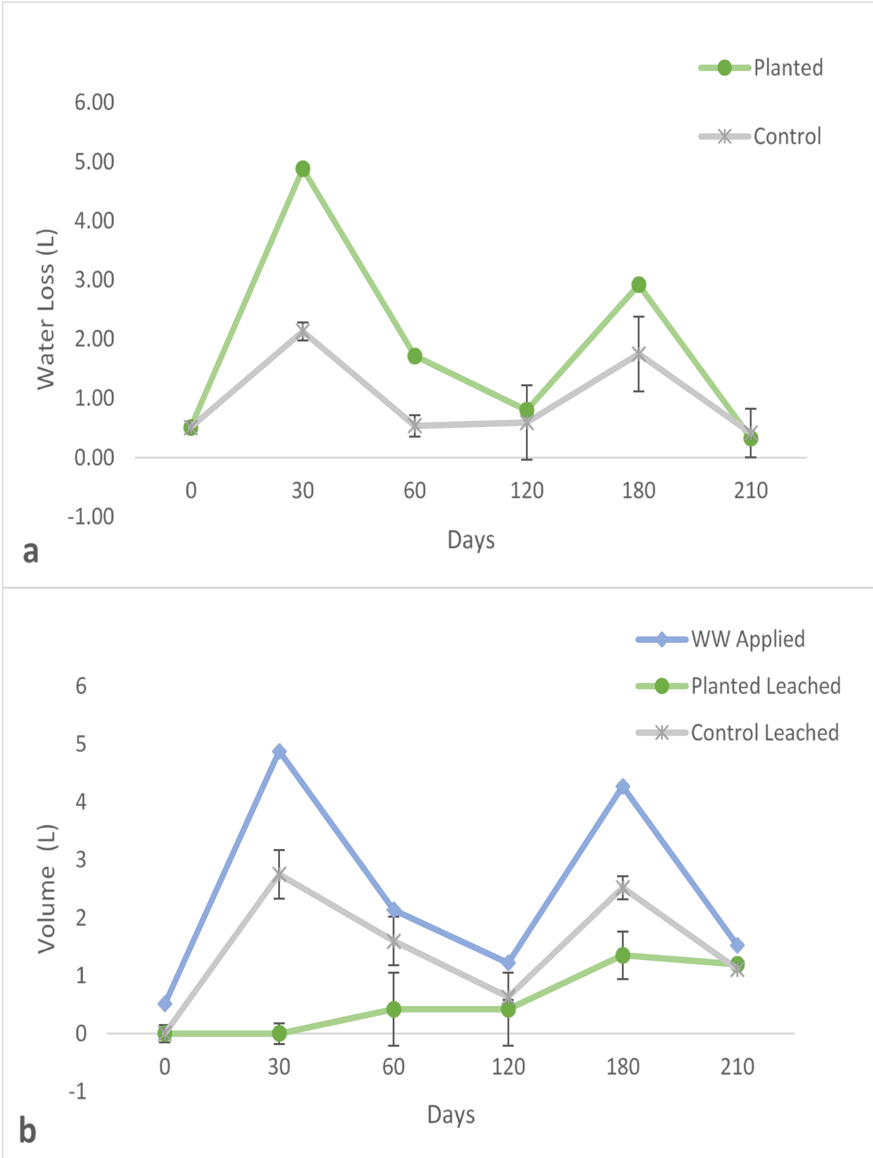

Figure 3. (**a**) Rate of water loss in treatments and controls due to evapotranspiration; (**b**) volume of wastewater applied and leachate volume collected.

Before the experiment, both treatments had microbial profiles with *Gammaproteobacteria*, a class that contains denitrifiers being the most prevalent. However, in the planted soils, metal reducers like *Clostridia* populations were present as well before WW application began (Figure 4a), whereas in the control C degraders, especially *Alphaproteobacteria* and *Firmicutes*, were found (Figure 5a). As the experiment progressed, the microbial populations became more diverse, with microbes of various functions.

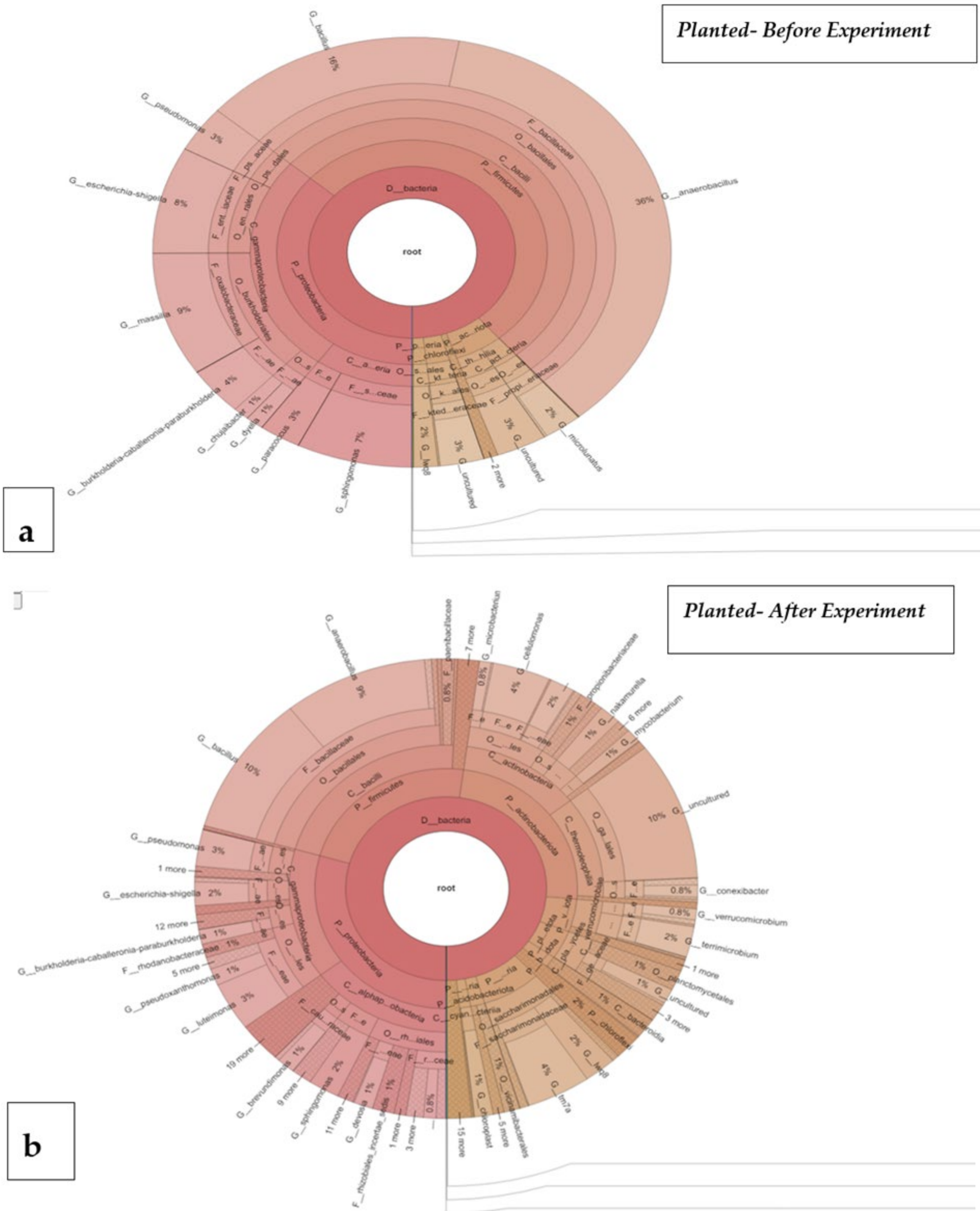

Figure 4. Microbial population in the planted column soils (**a**) before the experiment and (**b**) after the experiment.

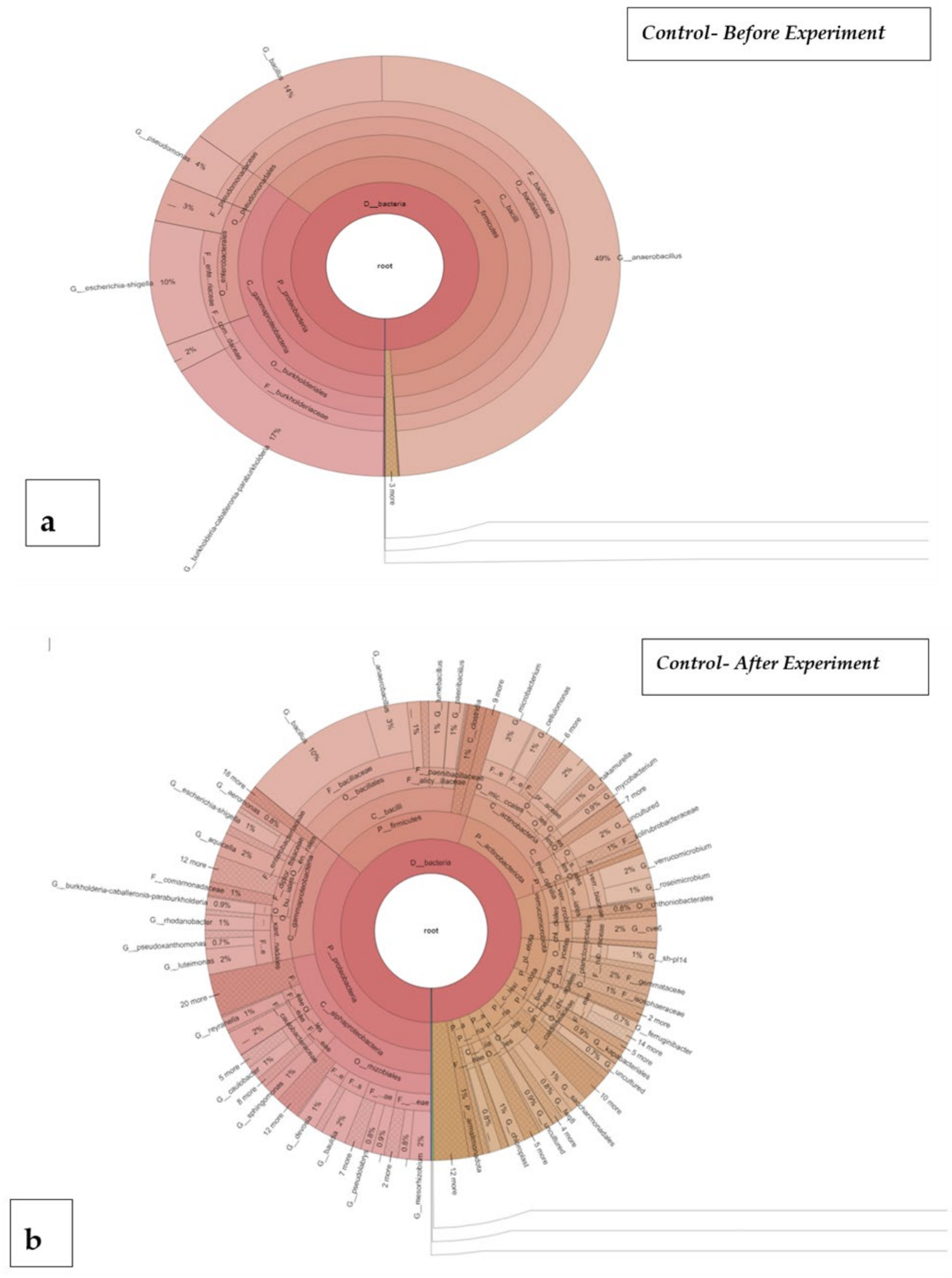

Figure 5. Microbial population in the control column soils (**a**) before the experiment and (**b**) after the experiment.

Plants were planted three days after soil and sand were mixed together. Before the poplars were planted, the presence of *Chloroflexi* (carbon-degrading bacteria) and *Plantomyceota* (an ammonium-oxidizing bacteria) increased in the planted samples. As the experiment progressed, microbes that promote plant growth, like *Pseudomonas* and *Burkholderia,* increased. The no-plant control had a higher presence of *Verrucomicrobiota*, which plays a vital role in the reduction of sulfate. As the experiment progressed, the presence of Proteobacteria like *g_Escherichia-shigella,* a Gammaproteobacteria, increased while Alphaproteobacteria (N-fixers) communities decreased. Overall, the lower layers of the soil had more microbial abundance than the top layer. The microbial population in the planted treatments was less diverse than those in the no-plant control, but certain families that facilitate the degradation of contaminants were in excess throughout the soil (Figures 4b and 5b). In each analysis, while the no-plant control appeared to be more diverse, there was a healthier (abundance and diversity) population of microorganisms in the planted treatments. This indicates that poplar presence indeed affected the microbial composition of soils (phytostimulation), which may be key in the removal of pollutants by rhizodegradation.

3.3. Soil Oxygenation

The soil oxygenation process was evaluated using data from soil oxygen sensors and oxidation–reduction potential (ORP) sensors, as plants are known to release oxygen to the soil environment through their roots. Redox potential in the soil throughout the experiment varied, with values ranging from as low as −321 mV to as high as 916 mV (Figure 6a). Similarly, O_2 concentration data also varied during the experiment (Figure 6b). The oxygen concentration of the soils usually decreased during watering periods when the soil pores were filled with water. High oxygenation took place when soils were dry and drained. The mean and standard error of O_2 concentration were 5.77 $\pm$ 0.05% for the no-plant control and 9.98 $\pm$ 0.04% for the planted treatments, while the mean and standard error of the ORP were 104.14 $\pm$ 2.13 mV for the no-plant control and 218.11 $\pm$ 2.07 mV for the planted treatments (Figure 7a,b). Non-normal statistical testing of medians revealed that the results were all significantly different.

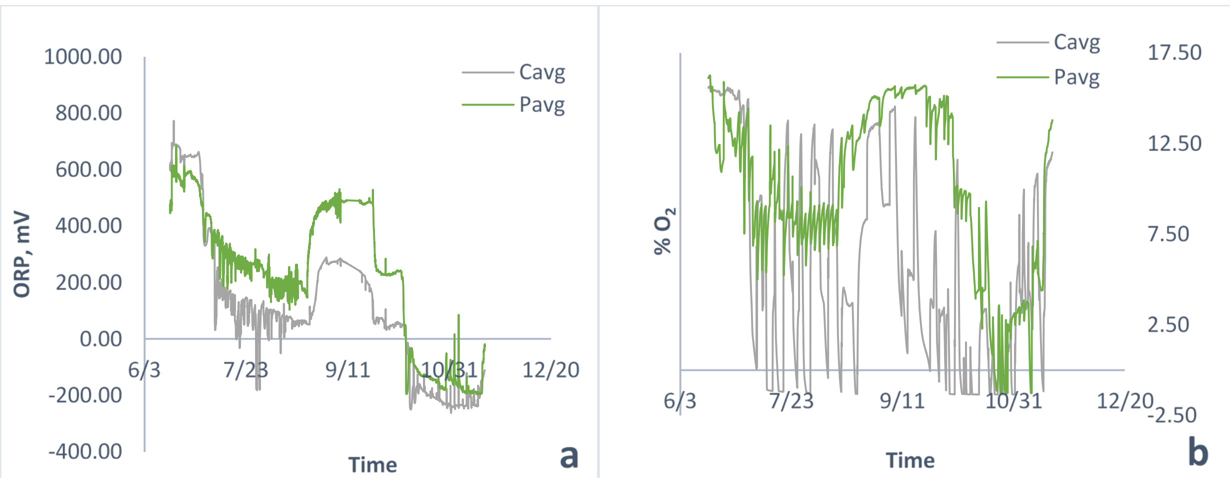

Figure 6. (**a**) Redox potential and (**b**) percentage of oxygen for no-plant controls and treatments.

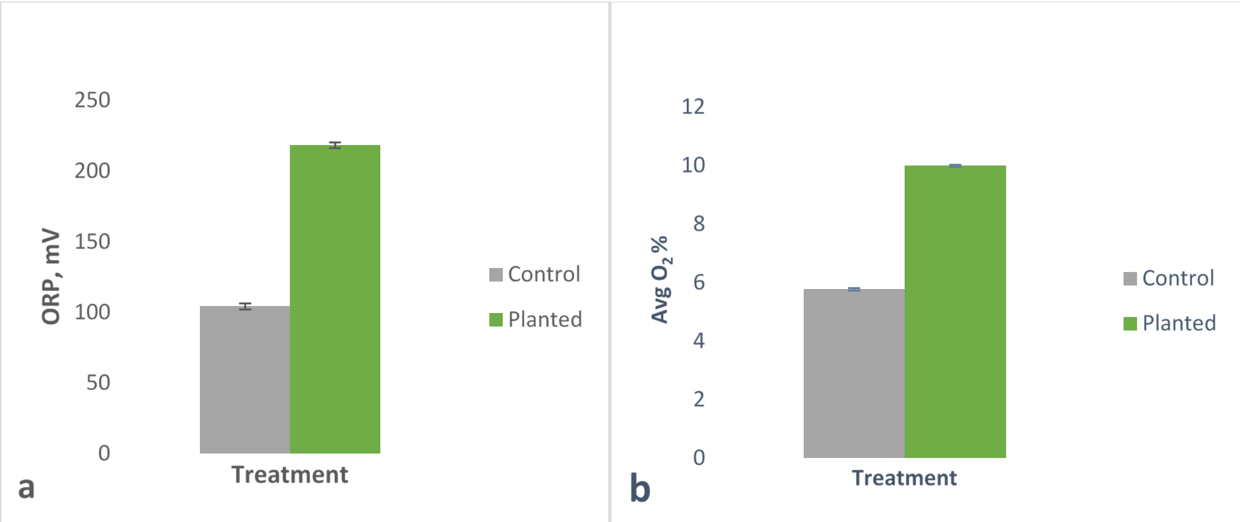

Figure 7. (**a**) Average oxidation–reduction potential (ORP) in both treatments; (**b**) average oxygen percentage in both treatments.

3.4. Plant Uptake

Plant tissue samples were analyzed for metal concentrations in the roots and shoots. Soil analyses were conducted at six (6) different sampling points to accurately measure the concentration of metals. In the plant tissue samples, the shoot tissues had a higher concentration of metals than the root tissues (Figure 8). The translocation factor (TF) was 10.62 for some metals. The translocation factor, along with the lower concentration of metals in the leachate, indicates that the poplars indeed have the ability to reduce groundwater contamination by translocating metals into their above-ground biomass. This is consistent with results from previous studies [14,15] that found that poplars have the ability to accumulate excess metals in their xylem elements [16]. When compared to the soil samples, the plant tissues had a higher concentration of Mn but a lower concentration of Fe (Figure 8). For arsenic, plant tissue concentrations were below the LOD for the instrument, which was 1 μg/L.

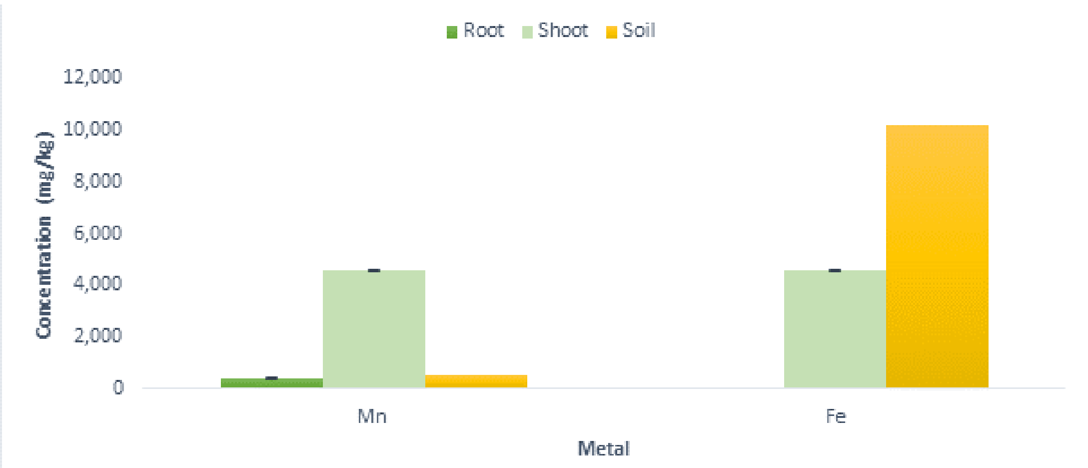

Figure 8. Concentrations of manganese and iron in soil and tissue samples.

3.5. Metal and Nitrate Mobilization

Metal and nitrate mobilization from the soils was evaluated using metal and nitrate concentrations in the leachate water, their loading released from the columns, and their soil concentrations.

3.5.1. Metal Concentrations and Loading

Results showed that less arsenic leached from planted treatments than from the no-plant controls (Figure 9). The loss of Fe was similar across both treatments. However, twice as much Mn leached from the planted treatment than from controls. This data is consistent with phytoprocesses data, i.e., ORP, O_2, and microbiome, which indicated that the planted treatments existed in the oxic zone while the controls were in the anoxic or more reducing zone.

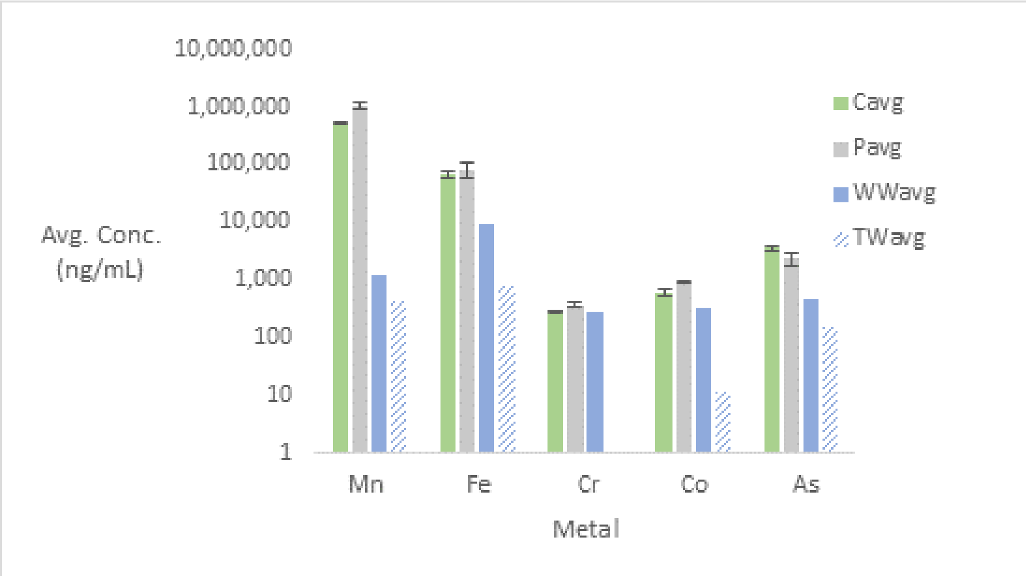

Figure 9. Concentrations of metals in leachate water (C and P), influent WW (WW), and tap water (TW).

Metal loading data (Figure 10) showed that metal loading for Co, Cr, and Mn from the leachate in the columns was higher in planted treatments than in the no-plant controls, whereas As had the opposite trend. Fe loading was similar in both treatments. These observations are consistent with redox potential or oxygen measured in the systems. Statistical testing of medians (Mann–Whitney Rank Sum Test) revealed that there was not a significant difference in metal loading between treatments ($p = 0.648$).

3.5.2. Soil Metal Concentrations

In the post-experiment soil samples, the concentration of Fe was more prevalent compared with other metals. However, there was no significant difference in the concentration of metals between various layers of the soil system in both treatments. Mn concentrations were elevated at the top layer of the soil when compared to the rhizosphere; however, there was less Fe in the topsoil samples than in the root–soil samples (Figure 11). When compared to the pre-experiment, post-experimental soil samples contained a lower concentration of metals (Figure 12). The planted treatment was successful at lowering the concentration of Mn, while the control treatment was better at reducing the concentration of Fe and As. In both treatments, the reduction of metal concentration was successfully achieved. This indicates that the presence of the poplars aids in reducing metal concentrations in the soils.

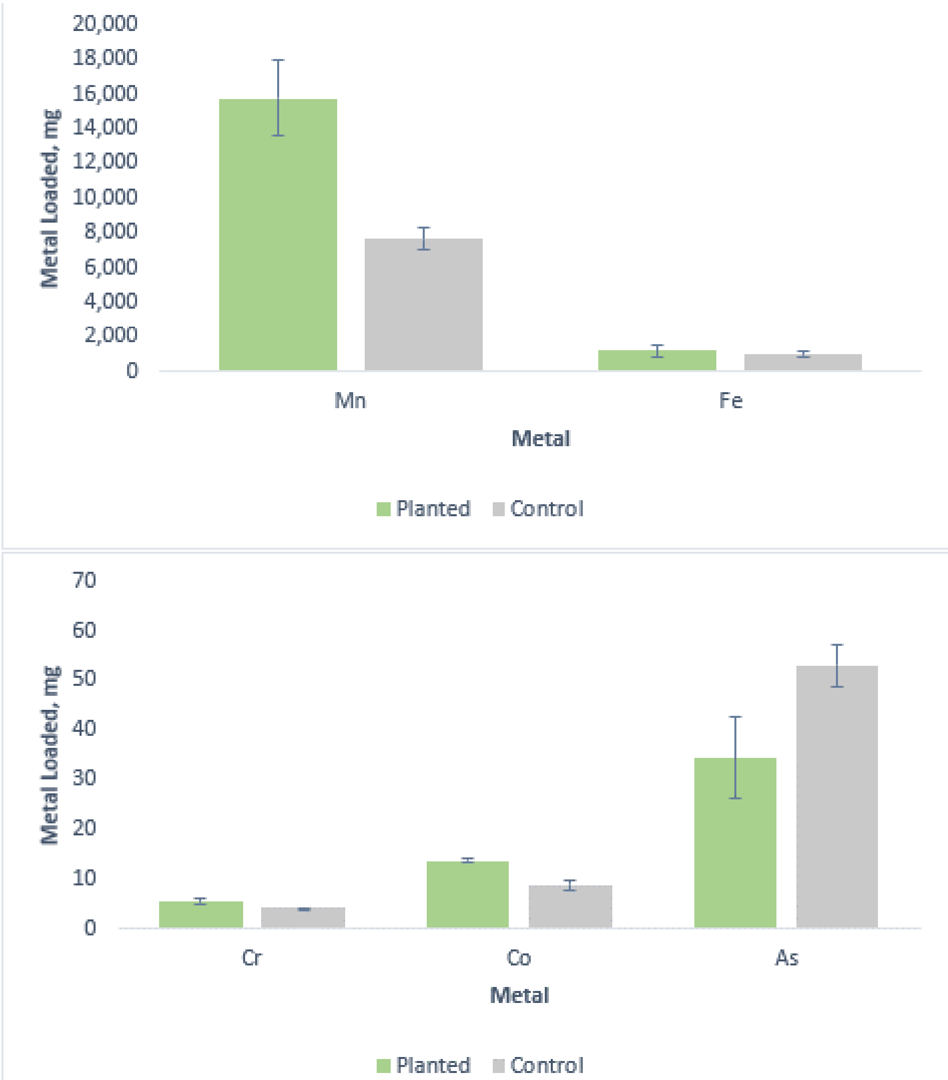

Figure 10. Mass of metals lost from each treatment through leachate water.

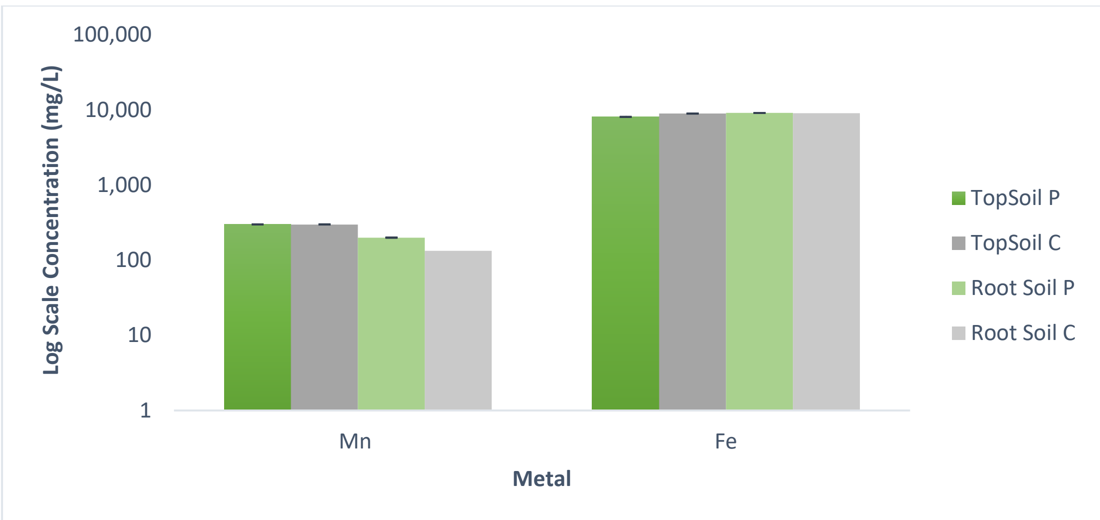

Figure 11. Soil metal comparison amongst the soil layers.

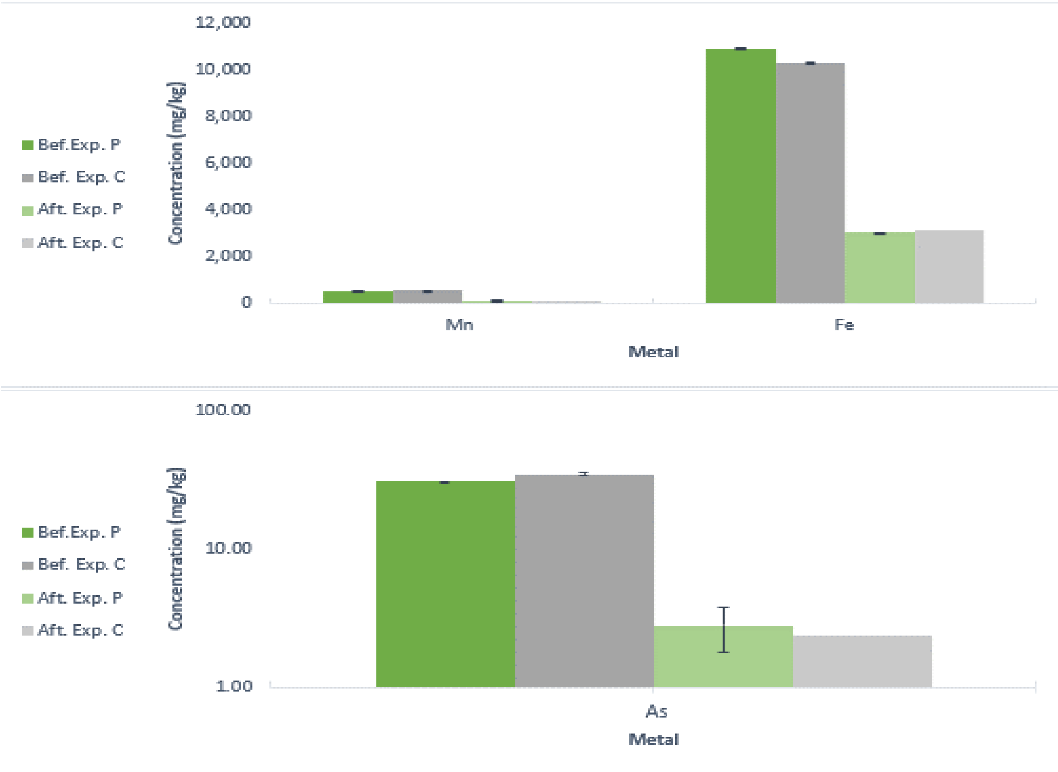

Figure 12. Soil metal comparison before and after the experiment.

3.5.3. Nitrate Concentrations and Loading

The initial application of WW yielded leachate with high concentrations of nitrate (Figure 13a). Concentrations of total nitrogen and nitrates decreased with time, while ammonium concentrations increased (Figure 13b). Overall, during extremely oxic periods, nitrate levels were high, while ammonium levels were low due to nitrification. The high concentration of ammonium is consistent with reduced conditions in the no-plant controls. In the first month of the experiment, TN was higher in the planted treatments than in the controls (Figure 13c); however, as the experiment progressed, the concentration of nitrates was higher in the controls than in the planted columns leachate (Figure 13d). TN values slowly began to fall as the experiment advanced. In all instances, the TN values were lower than 10 mg/L, which is the MCL of nitrates in drinking water set by the EPA. Non-normal statistical testing using one-way ANOVA revealed that results for NO_3-N (p = 0.004) and TN (p = 0.003) were significantly different, while NH_4-N (p = 0.963) was not significantly different.

The mass of nitrates lost from the no-plant controls exceeded that of the planted treatments (Figure 14). Statistical testing of medians (Mann–Whitney Rank Sum Test) revealed that there was a significant difference in nitrates loading from both treatments (p = 0.012).

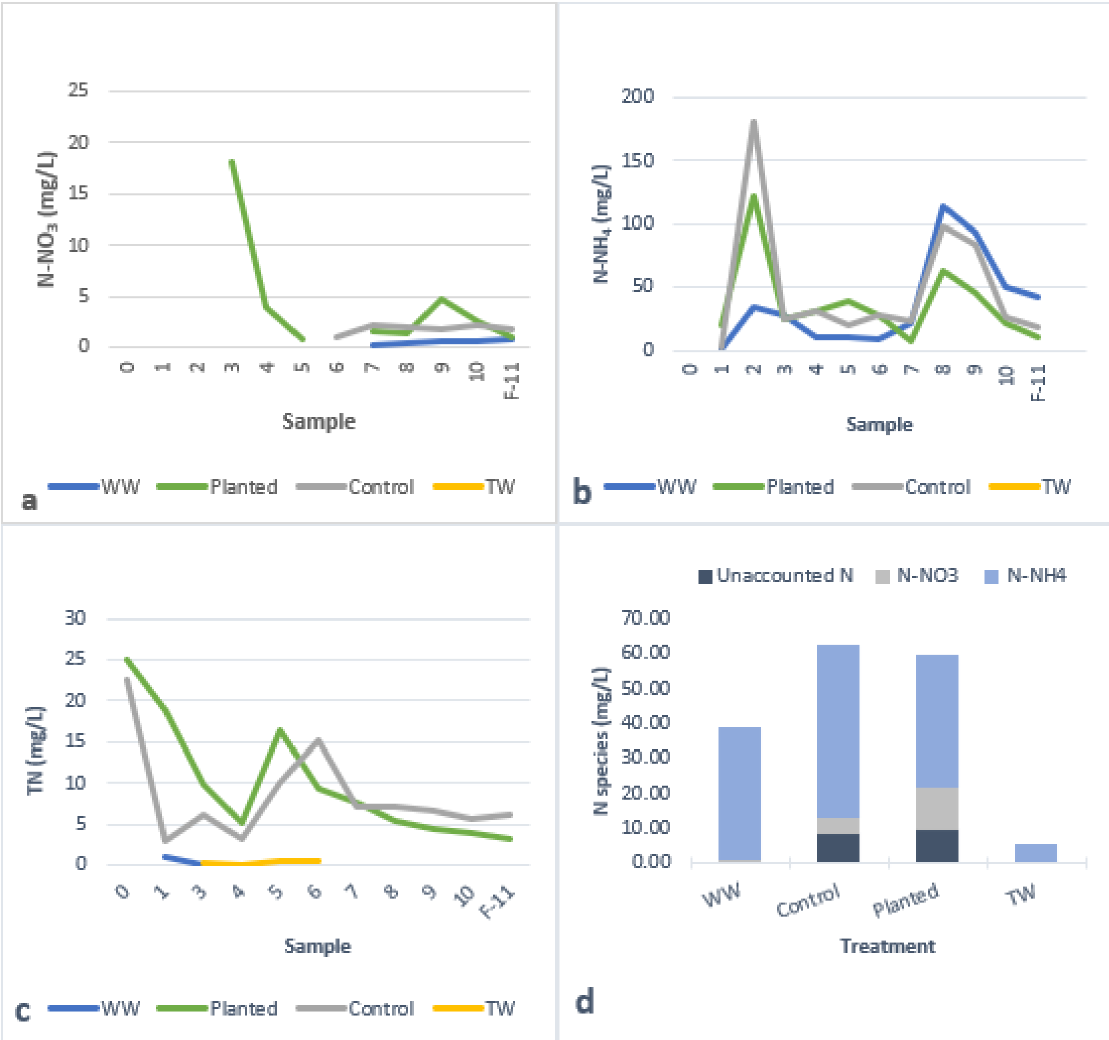

Figure 13. (**a**) Nitrate concentration in FPWW and leachate; (**b**) ammonium concentration in FPWW and leachate; (**c**) total nitrate concentration on FPWW and leachate; (**d**) total nitrogen by parts in FPWW and leachate.

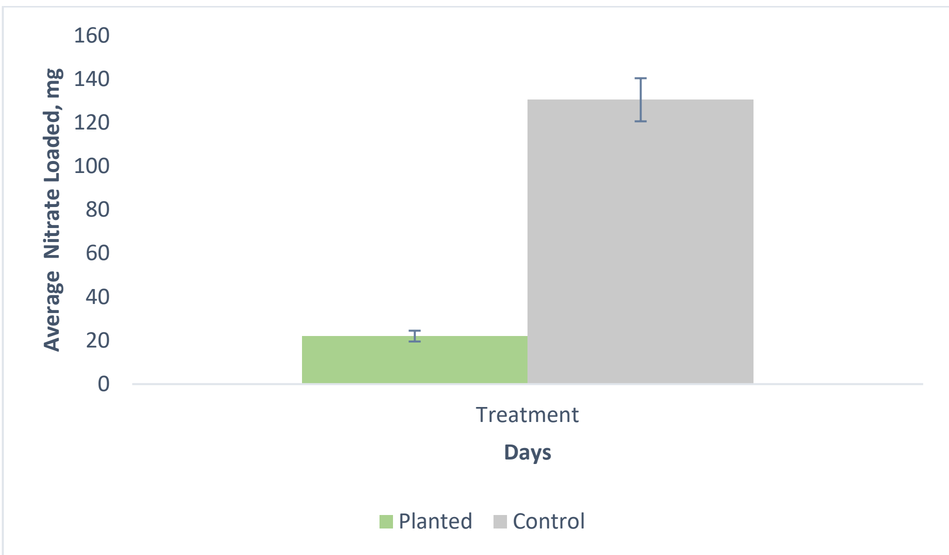

Figure 14. Mass of nitrates lost from each treatment.

3.6. pH and Carbon Treatment

Factors that affect rhizosphere pH include CO_2 evolution, root exudates, de/re-adsorption of H^+ or HCO_3^-, and production of organic acids during microbial metabolism [17]. Synthetic FPWW had a pH that was slightly basic throughout the entire experiment (Figure 15). However, the pH of all leachate samples decreased after treatment by soil and plants. Over time, with the continuous addition of WW and poplar growth, the pH of each treatment increased. In the end, the pH was still in the range (pH 6.8–8.6) that would not adversely affect groundwater based on the pH levels set by the EPA. In no-plant controls, the pH stayed close to neutral, while the planted treatment had pH values that were slightly acidic. This is consistent with studies that show both manganese and iron oxides are insoluble at a higher pH. Alternatively, arsenic is more soluble in alkaline environments [18]. Toward the end, when the pH in the planted treatment increased, there was also an increase in redox potential activity consistent with the redox potential needed for solubilizing arsenic.

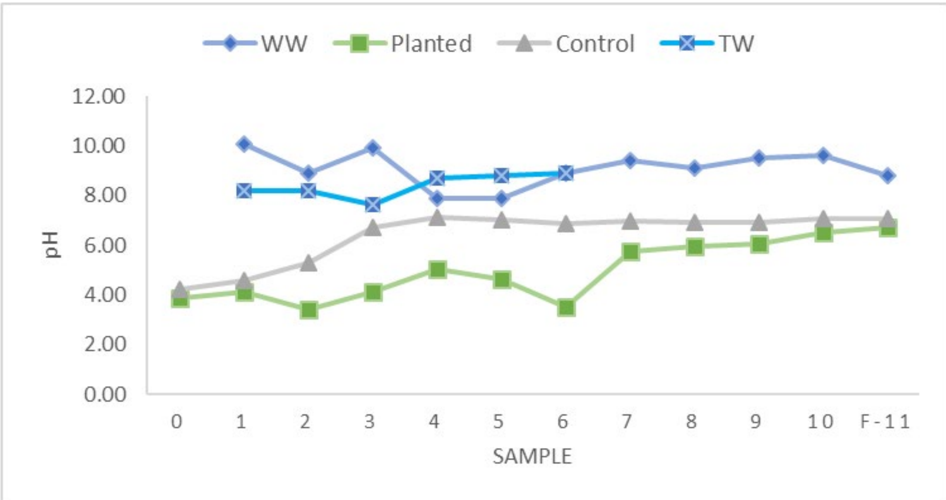

Figure 15. pH of leachate (planted and control), tap water (TW), and influent wastewater (WW) with time.

Both treatments were effective at removing C from the influent WW (Figure 16). The COD of the synthetic WW varied throughout the experiment, which is consistent with real-life production. Holistically, there was no major difference between the reduction of C in each treatment; however, individual weekly data showed that at the beginning of the experiment, the planted columns were more efficient at removing C than the no-plant controls. As the experiment progressed, the rate of C removal for the no-plant controls increased (Figure 17). On average, 65% C was removed from the planted treatment, whereas the removal rate for the no-plant controls was 71%. Non-normal statistical testing using one-way ANOVA revealed that results were significantly different ($p \leq 0.001$).

Over the duration of the experiment, the carbon loading from the no-plant control treatments exceeded the mass of carbon from the planted treatments (Figure 18). Statistical testing of medians (Mann–Whitney Rank Sum Test) revealed that there was not a significant difference in carbon loading in both treatments ($p = 0.082$).

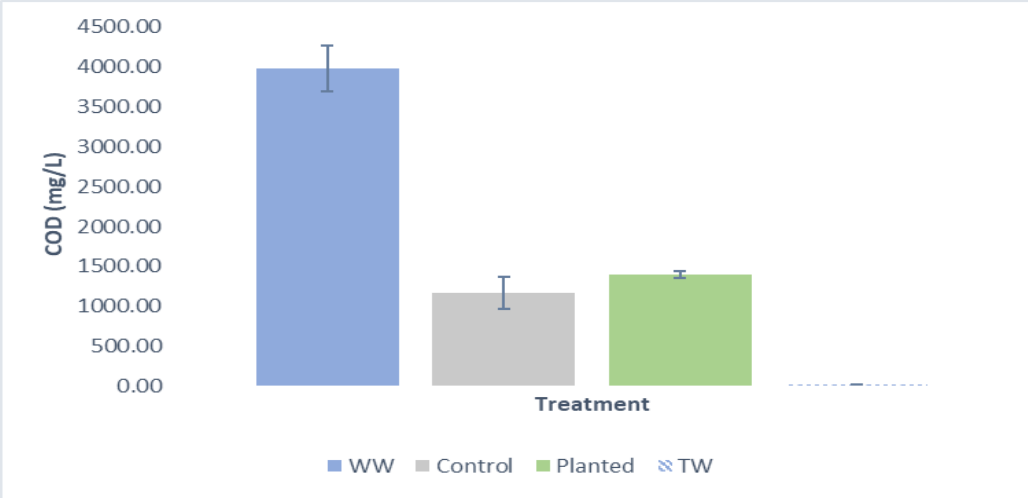

Figure 16. Concentration of chemical oxygen demand across treatments with error bars.

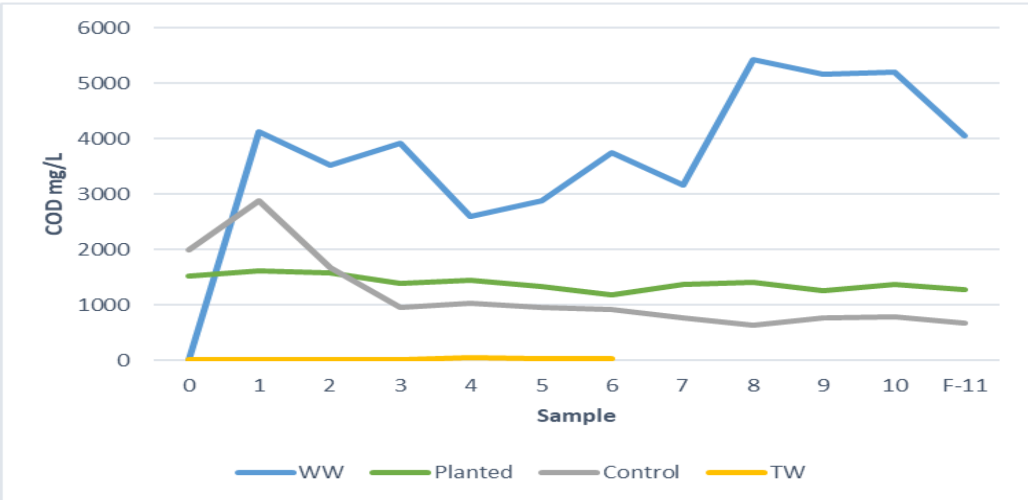

Figure 17. COD variation between treatments over the duration of the experiment, including COD of influent WW and tap water (TW).

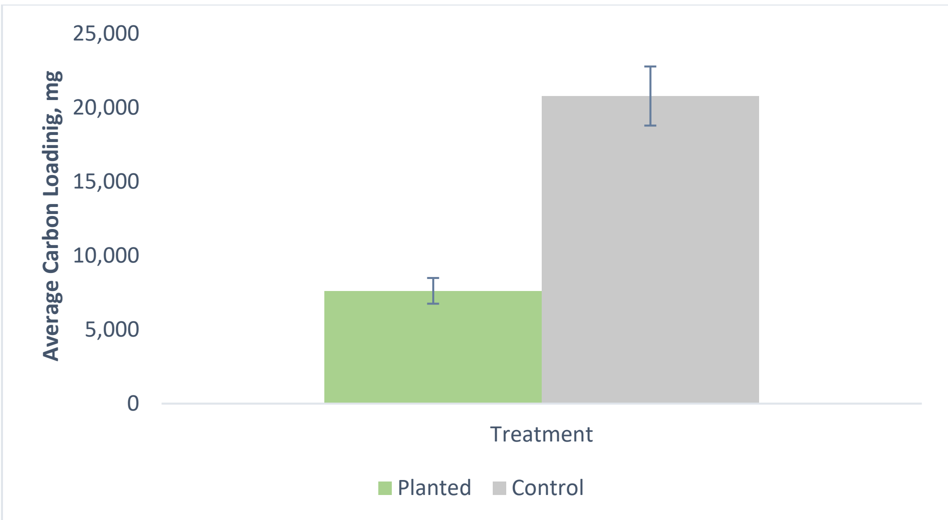

Figure 18. Carbon loading from each treatment.

4. Discussion

4.1. Phytoprocesses

Phytoremediation of land applied FPWW is successful due to the different attributes poplar plants possess, which support plant processes to remove nutrients and pollutants from the soil. The phytoprocesses that were studied during this experiment included plant uptake, evapotranspiration, root oxygenation, and rhizostimulation. These processes are facilitated by plants, soil microbes, and root exudates.

Evapotranspiration was a key process as the overall crop coefficient was 1.88, which indicated accelerated loss of water from planted columns than from controls by a factor of 1.88. Therefore, plants can act as a "pump", contributing to less leachate and less loading of pollutants to the groundwater.

Land application of FPWW can be a very rich source of carbon for soils. Extra inputs of carbon, either through plant roots or FPWW, can lead to increased microbial activity and lower oxygen availability in soils, forcing denitrifiers in the rhizosphere to find alternate energy sources [19]. The microbes present in the study included nitrifiers (ammonia-oxidizing bacteria: *Nitrosomonas* spp., *Nitrosococcus* spp., *Nitrosospira* spp., *Nitrosolobus* spp., and *Nitrosovibrio* spp.; nitrite-oxidizing bacteria: *Nitrobacter* spp., *Nitrococcus* spp., *Nitrospira* spp., and *Nitrospina* spp., and *Nitrobacteraceae*), metal reducers (*Geobacter* (*Deltaproteobacteria), Geothrix fermentans* (*Acidobacteria*), *Thermoanaerobacter* (*Firmicutes*), *Aeromonas, Escherichia, Bacteroidetes, Clostridium, Lysinibacillus, Micrococcus*), C degraders (*Achromobacter, Acinetobacter, Alkanindiges, Alteromonas, Burkholderia, Enterobacter, Mycobacterium, Pandoraea, Pseudomonas, Staphylococcus, Rhodococcus, Alkanindiges* sp., *Brevundimonas olei, Xanthomonas* sp.), denitrifiers (*Actinobacteria, Pseudomonas, Bacillus, Alcaligenes,* and *Rhizobium*), methanotrophs (*Methylobacter, Methylomonas*) and, sulfate reducers *(Chromatiaceae, Chlorobiaceae, Ignavibacterium*).

Overall, the most abundant microbes responsible for each function differed by treatment (Table 3). There were microbes that were found in the planted treatments but were not present in the control treatments and vice versa. Planted treatments included *Nitrospira* spp., *Pseudomonas*, *Actinobacteria*, and *Acidobacteria*, whereas the no-plant control treatments included *Rhizobium*, *Desulfobacterota*, and *Methylomonas*. The most abundant carbon-degrading populations differed in both treatments. While the no-plant control treatments appeared to be more diverse, there were healthier (abundance and diversity) populations of microorganisms in the planted treatments. This indicated that poplar presence indeed affects the microbial composition of soils (phytostimulation), which may be key in the removal of pollutants by rhizodegradation. The poplar plant uses microorganisms in the rhizosphere, which is enhanced via rhizostimulation, to reduce the metal(loids) and take up the reduced form of metal(loid)s by the roots into the plants, or the immobilization of these metal(loid)s in the soil via phytostabilization.

Table 3. Most abundant microbes by function.

Function	Planted	Control
Nitrifiers	*Nitrosomonas* spp. *(AOB)*, *Nitrosococcus* spp., *Nitrospira* spp. *(NOB)*	*Nitrosomonas* spp. *(AOB)*, *Nitrosococcus* spp. *(NOB)*
Denitrifiers	*Bacillus*, *Pseudomonas*, *Actinobacteria*	*Bacillus*, *Rhizobium*
Metal Reducers	*Firmicutes*, *Acidobacteria*, *Escherichia*	*Escherichia*, *Firmicutes*
Sulfate Reducers	*Proteobacteria*	*Desulfobacterota*
Carbon Degraders	*Clostridia*, *Enterobacter*	*Xanthomonas* sp., *Burkholderia*, *Alphaproteobacteria*
Methanotrophs	*Methylobacter*	*Methylobacter*, *Methylomonas*

Plants are known to leak oxygen to the rhizosphere. In this experiment, significantly higher ORP and oxygen concentrations in planted columns compared to control columns indicated oxygenation by poplar trees. The results demonstrated the ability of plants to oxygenate the rhizosphere, which can be engineered for the purpose of contaminant remediation. Oxygen is needed by nitrifying bacteria to produce NO^{2-} and NO^{3-} during the denitrification process. On the other hand, the metals of interest are more mobile or soluble in reducing conditions. At a pH of 6–8, Mn is soluble at as high as +500 mV [20], Fe is soluble at +300 to +100 mV [21], whereas lower redox potentials are conducive with the more water-soluble form of arsenic, As^{3+} [22]. The more soluble the metals are, the more bioavailable for plants during phytoremediation and the higher the potential to leach to groundwater.

Accumulation of metals is another way the poplar trees affect the bioavailable metals in the rhizosphere. The accumulation factors (Table 4) confirm the uptake of the metal from the soil to the root to the shoots of the plants and storage in their tissues.

Table 4. Accumulation factors of metals in plant tissue samples.

	Accumulation Factor (AF)		
Metals	**Mn**	**Fe**	**As**
Root	3.81	<LOD	<LOD
Shoot	40.40	1.51	<LOD

4.2. Effects of Phytoprocesses on Metal and Nitrate Mobilization

Phytoremediation is a natural, cost-effective way to remediate soils and prevent groundwater contamination. Jadia and colleagues (2009) noted that the success of phytoremediation relies on plants that can (1) uptake large quantities of heavy metals in their roots, (2) translocate absorbed heavy metals into surface biomass, and (3) rapidly produce large plant biomass. It is also very important that the plants used for phytoremediation have mechanisms that allow them to detoxify the high metal concentrations accumulated in their shoots [23]. Various plants, such as Indian mustard [24], water hyacinth, channel grass [25], herbal plants, and poplars [26], etc., are used for phytoremediation. Poplars have the ability to reduce the amount of nutrients that are leached from soil systems using phytoprocesses like evapotranspiration, oxygenation, plant uptake, and rhizostimulation.

First, the poplar trees need to grow healthily for any phytoremediation project to be successful. Indeed, the growth of both above and below-ground biomass of the poplar plants was not inhibited by synthetic FPWW application in this experiment.

Second, the primary purpose of FPWW treatment is to reduce carbon loading from the wastewater. The removal of 65–71% C concentration-wise was achieved in both treatments. Although removal in the no-plant controls was higher concentration-wise, the loading was lower from planted treatments. Overall, carbon and nitrogen loading were higher from the no-plant controls than from the planted treatments. The reduction in leachate volume contributed to lower carbon and nitrogen loading. The nitrate concentration as the experiment progressed indicated that the poplars were successful in preventing the mobilization of nitrates from the WW to the leachate, thus protecting ground water.

Third, metal leaching varied greatly between treatments. Redox potential and pH greatly affect the speciation of metals in soil, as observed in the data. Solubility and mobility of metals and metalloids were only favorable during the periods where the pH and ORP were in a specific range. The most prevalent metal varied between samples, with Mn being prevalent in the topsoil and tissue cells of the shoots, whereas Fe was prevalent in the root tissue cells and root–soil samples. Additionally, less As was leached from the planted treatments than the no-plant controls, consistent with oxygen and ORP data. Oxic conditions occurred in the planted treatments more often than the no-plant treatments, resulting in significantly higher ORP, O_2 concentration, and nitrates, while ammonium levels were low, indicating that reducing conditions occurred more frequently in control columns than in the treatment columns. Translocation of metals from the soil–root–shoot occurred throughout the experiment. Metal(oid) concentrations were reduced in the planted treatments with a translocation factor of 10.62 and an accumulation factor of ~1.5 for some metals.

Therefore, all the data strongly indicate that poplar plants helped increase oxic conditions in the rhizosphere, increasing available nitrate and manganese. However, control columns were in more reducing conditions and had more bioavailable iron or arsenic. Thus, the highly reducing species like arsenic leached less from the planted columns, while the metals that reduce in slightly reducing conditions, such as manganese, leached higher from the planted columns. This is consistent with our other data that shows that under oxic conditions, the planted leachate contained higher nitrates and lower ammonium due to nitrification.

5. Conclusions

This study evaluated the usefulness of poplar plants in preventing groundwater contamination during land-applied food processing wastewater application and the phytoprocesses during the treatment. While evapotranspiration and plant uptake directly reduced the metal and nitrate loading, rhizostimulation and oxygenation positively affected the metal and nitrate removal by changing the soil conditions and microbial population. The results indicated that N was removed at greater rates in the planted treatments compared to the no-plant treatments, likely due to oxygenation, rhizodegradation, and plant uptake, which increase the bioavailability of N. This allows the plants to absorb nitrates in their roots and use the degraded carbon as an energy source. The rate of evapotranspiration was 1.88× higher in the planted treatments than the controls, indicating that poplars have the ability to decrease the leaching of pollutants to groundwater by decreasing the leachate volume. The fact that the planted treatments retained more WW than the no-plant controls indicates that there is a chance of less environmental impact during storm events (through runoff) if poplars and similar plants are used to treat wastewater by land application. Through phytoextraction, the poplar plant can further accumulate high concentrations of soil contaminants in the plant tissues. The presence of poplar trees increased the diversity and richness of microorganisms that can perform diverse microbial functions in the environment. Overall, with these phytoprocesses working together, poplar plants achieved the observed remediation of contaminants mobilized in the native soil during the land application of FPWW. Thus, the results of this study revealed that planting poplars is beneficial for reducing metals and nitrates contamination of groundwater from the soil through the various plant–soil–microbial mechanisms. Engineers can utilize plants and wastewater loading to create conditions suitable for less metal and nitrate mobilization.

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