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Article

A novel bioreactor system to purify contaminated runoff water

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SUMMARY

Water scarcity has become a global crisis and an economic burden. Environmental challenges in climate change, population growth, and urbanization have raised serious concerns for safe drinking water, food security, social stability, and public health. Current physicochemical purification techniques are costly, chemically-invasive, or ineffective. The aim of this study was to engineer an eco-friendly and cost-effective water purification system using an ex-situ bioremediation approach. The main objective was to use limestone, denitrifying bacteria, and sulfate-reducing bacteria present in the soil as natural resources. Organic compost was added as a carbon source to enhance activities of intrinsic soil bacteria. All samples were collected from mining and industrial sites in Eastern Pennsylvania. We evaluated and verified the feasibility of a novel modular bioreactor system for the effective removal of nitrate, sulfate, and heavy metals from runoff water while increasing its alkalinity. The impacts of pH and temperature on the bioremediation efficiency were evaluated, revealing ideal temperatures to be above 16°C and pH above 7. Nitrate levels dropped from 80 ppm to 0 ppm, and pH increased from 4 to above 7 consistently. Combining neutralization with bacterial bioremediation proved to have synergistic benefits within 30 minutes of treatment. Results showed a successful removal of nitrate (NO_3^-), sulfate (SO_4^{2-}), sulfite (SO_3^{2-}), Zinc (Zn), Copper (Cu), Aluminum (Al), and Lead (Pb) from contaminated wastewater. This system is a cost-effective, energy-efficient, and practical tool that opens numerous avenues for generating sustainable, portable, and fast water purification options for low-income communities. A large-scale system could be adapted for commercial or industrial purposes.

INTRODUCTION

Environmental sustainability is directly linked to food and water security, quality of life, and socio-economic developments in every community. According to the World Health Organization, half of the globe's population may face water scarcity within the next decade (1). Although 70% of the earth's surface is covered by water, only 2.5% of it is drinkable, and just 1% of this drinkable freshwater is accessible (2). The world's population is expected to reach 10 billion by 2050, with an increase of an additional 20% for the global water demand. (3) Unfortunately, more than 30% of people living in the least-developed countries do not have access to safe drinking water (4, 5). The World Bank has announced the high cost of safe drinking water is estimated to be more than $4 billion a year for private and public sectors in the US, and many countries need to quadruple their spending budgets to deliver safe water by 2030 (6).

Aside from its scarcity, water quality is at risk by increased contaminants from human activities. Nitrate, sulfates, and heavy metals are highly water-soluble compounds that could enter the water as well as the food chain and pose serious health issues to humans (especially infants), livestock, and aquatic life (7-9). Nitrate could enter the food chain via ground and surface water through a variety of sources. The major causes of nitrate contamination are runoff or seepage from fertilized agricultural lands, municipal and industrial wastewater, and urban drainage. Elevated levels of acidity and toxic metals in drinking water are also results of anthropogenic activities such as mining for coal, metal ore, and other industrial operations within the last century. Acid mine drainage (AMD) is one of the primary surface-water pollutants in the mid-Atlantic region where sulfur-containing rocks, such as pyrite, get exposed to oxidizing conditions. (10) As water flows through these mining areas, it reacts with pyrite to form sulfuric acid and iron oxides. Iron oxides precipitate as red/orange sediments, and sulfuric acid dissolves toxic metals in the earth's crust, resulting in contamination of creeks and rivers (10). Untreated AMD affects environments around both active and abandoned mines, creating ecological and economic concerns. Acid rain is another source of elevated water pollution. It is defined as precipitations with pH levels less than 5.0 as a direct result of human influence on atmospheric CO_2 levels. These acidic precipitations result in lowering the pH of runoff water (11).

The world's demand for safe water and food has brought greater scientific attention to innovation in water management and purification. The future research will primarily focus on eco-friendly, cost-effective, and energy-efficient innovations in water technology. The goal of this study was to design a device that could eliminate toxic contaminants and increase the pH of polluted acidic water. The specific pollutants found in collected samples were nitrate (NO_3^-), sulfates (SO_4^{2-}), and toxic metals, such as lead (Pb), copper (Cu), zinc (Zn), and aluminum (Al). Our purification method was focused on using materials found in nature, minimizing energy consumption, reducing the cost, and making the process environmentally-friendly. Recent trends in bioremediation techniques show promising results for the treatment of AMD (12). Denitrifying bacteria (DNB) and sulfur-reducing bacteria (SRB) capable of removing contaminants are present in the soil sample used in this study (13). Limestone ($CaCO_3$) is also used as

an alkaline agent naturally available to reduce the acidity of water. Accordingly, We hypothesized that we can eliminate contaminants after neutralizing the acidic, polluted water with limestone chips in the first module, and then subsequently introducing this water to the soil bacteria in the second module.

The strategy in this project was to use ex-situ bioremediation as a sustainable, chemical-free, and economical approach. These techniques used the catabolic capabilities of microorganisms to degrade biohazardous contaminants into harmless byproducts. The ability of bacteria to degrade pollutants depends on the environmental conditions for their growth and metabolism, which include suitable temperature, pH, and moisture (14,15). These bacteria were in their optimal efficiency at pH values above 7 and temperatures above 16°C. *Pseudomonas stutzeri, Pseudomonas aeruginosa*, and *Thiobacillus denitrificans* were the most common anaerobic bacteria involved (16,17). The bacteria used in this study are ubiquitous to Eastern Pennsylvania and have not been specifically identified. Naturally occurring carbon from organic decays are the source of energy to feed these bacteria, so there is no need for added external source of energy. We were able to construct a functional bioreactor that allowed neutralization and purification of contaminated runoff water in short period of exposure time. The contaminant levels were significantly reduced into DEA acceptable levels (18). We feel that this device and technique presented here can be useful tool for practical uses as well as a methodological approach for other water purifications studies in the future.

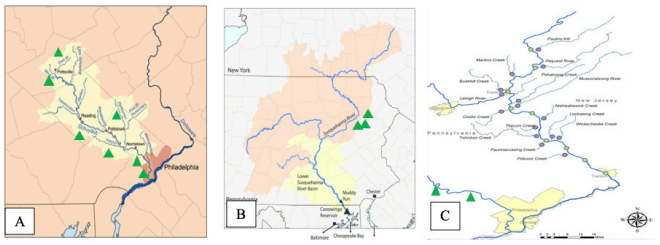

Figure 1: Map of the locations where water samples are collected. Approximate collection sites are marked with green triangles.
(A) Schuylkill River and its tributary creeks (30).
(B) Susquehanna River and its tributary creeks (31).
(C) Delaware River and its tributary creeks (32).

RESULTS

Water samples from 12 different locations (**Figure 1A-C**) demonstrated presence of various levels of nitrate,

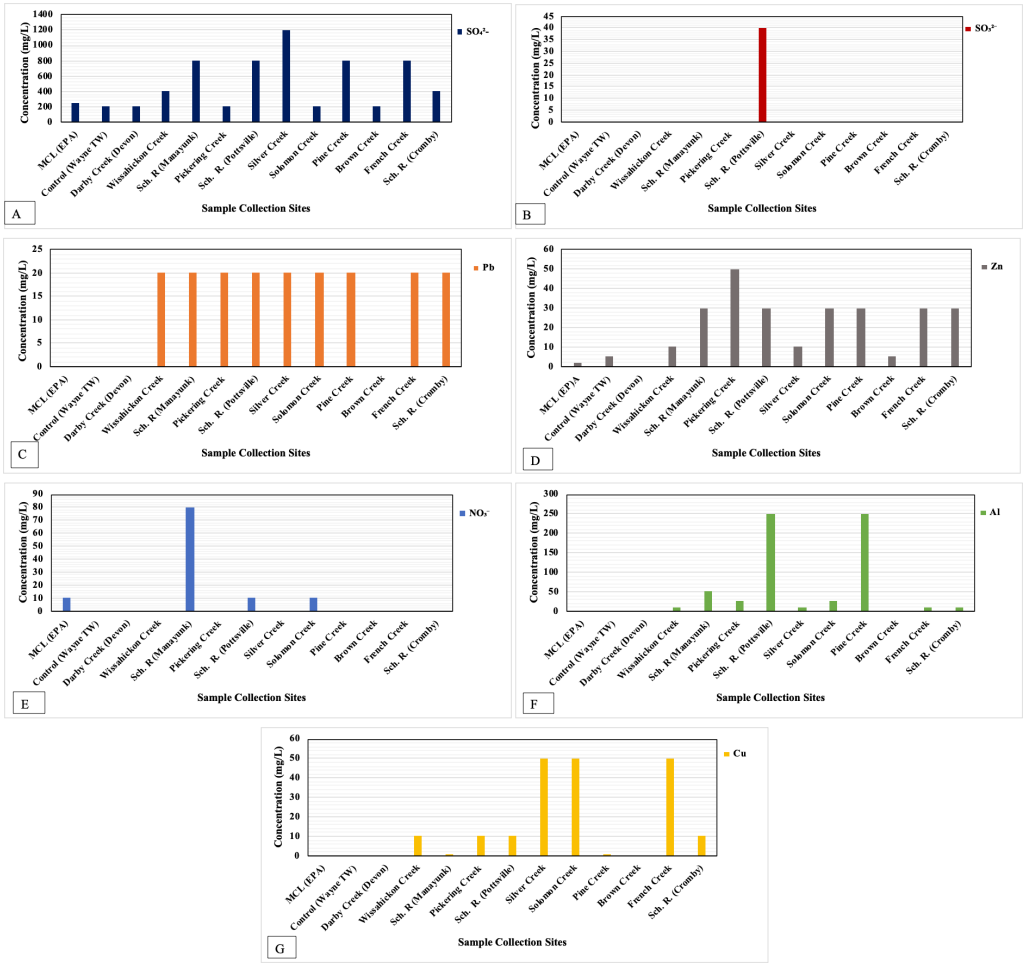

Figure 2: Initial concentrations of contaminants (mg/L) at 18°C and acidic pH before bioremediation. Seven different pollutants in 12 different locations were studied and compared. Contamination levels (mg/L) were measured for: (A) SO_4^{2-}, (B) SO_3^{2-}, (C) Pb, (D) Zn, (E) NO_3^-, (F) Al, and (G) Cu. Control samples of tap water from Wayne, PA (Wayne TW) and EPA approved MCLs are used as reference for comparison. The absence of a bar means zero level for that contaminant in that particular sample.

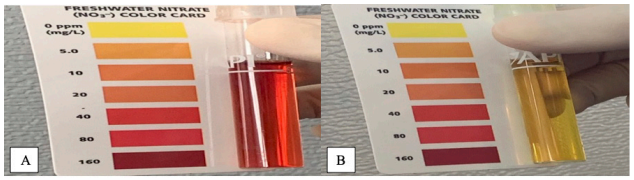

Figure 3: Nitrate concentrations in Manayunk water were reduced after neutralization and bioremediation. Colorimetric analysis for nitrate concentration in water collected from Manayunk. (A) Nitrate levels before neutralization and bioremediation were about 80 ppm (mg/L). (B) After samples were neutralized with limestone for 5 minutes and subsequently exposed to the bioreactor for 30 minutes, nitrate concentration was reduced to zero.

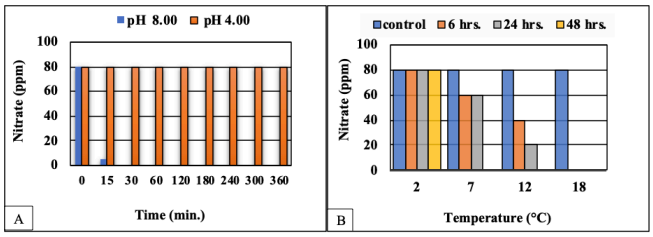

Figure 4: Low pH and low temperature reduced the bacterial denitrification rate in Manayunk water. (A) Nitrate levels (ppm) after denitrification over time (0¬–360 minutes) at two different pH values — 4.0 (orange) and 8.0 (blue). The water temperature was kept constant at 18°C. In lower pH bacterial denitrification was ineffective. (B) Nitrate levels after denitrification measured in different time intervals (0-, 6-, 24- and 48- hrs.) in varied temperatures below 18°C (~room temperature) while pH was kept constant at 8. Bacterial denitrification was slower or not present in colder temperatures below 18°C.

sulfates, lead, aluminum, copper and zinc (**Figure 2A-G**). Denitrification was the initial focus of the study. The pH of Schuylkill River water that contained highest concentration of nitrate at 80ppm (**Figure 3A&B**) rose from 4.2 to 8 in just 5 minutes after exposure to $CaCO_3$. The pH stabilized around 8 regardless of time of exposure to $CaCO_3$. We were able to completely remove nitrate after the pH increased to 8.0 at temperatures above 16°C within 30 minutes of exposure to the DNB bacteria of the soil. The nitrate levels dropped less effectively at lower pH levels (**Figure 4A**) and did not change in cooler temperatures (**Figure 4B**). These results confirmed the role of the water temperature and its pH level in bioremediation efficiency. At temperatures around room

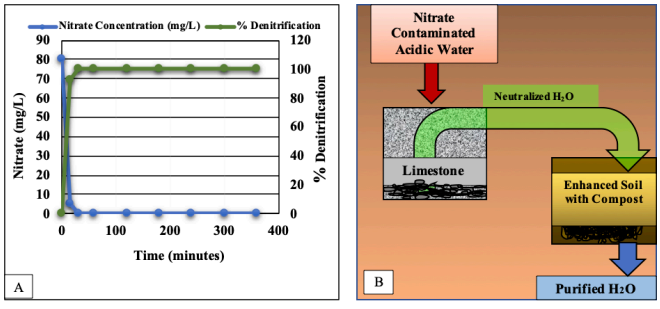

Figure 5: Efficiency and schematic view of bioreactor. (A) Denitrification efficiency of the modular bioreactor was sustained at 100% after 30 minutes of bioremediation while keeping pH 8 and 18°C constant. (B) The schematic diagram for the design of a bi-modular bioreactor for ex-situ bioremediation showing the sequence of bioremediation steps.

Table 1: Water Samples with contaminant concentrations. Concentration of contaminants (mg/L) in five rivers and creeks before bioremediation. Tap water from Wayne, PA is used as a control and MCLs from EPA are also shown for comparison.

Contaminant	French Creek (mg/L)	Pine Creek (mg/L)	Silver Creek Mine (mg/L)	Schuylkill R. Pottsville (mg/L)	Schuylkill R. Manayunk (mg/L)	Wayne Tap Water (mg/L)	Safe MCLs by EPA (mg/L)
(SO_4^{2-})	800	800	1200	800	800	200	250
(Al)	10	250	10	250	50	0	0
(Zn)	30	30	10	30	30	5	2
(Cu)	30	1	30	10	1	0	0
(Pb)	20	20	20	20	20	0	0
(NO_3^-)	0	0	0	10	80	0	10
(SO_3^{2-})	0	0	0	40	0	0	0

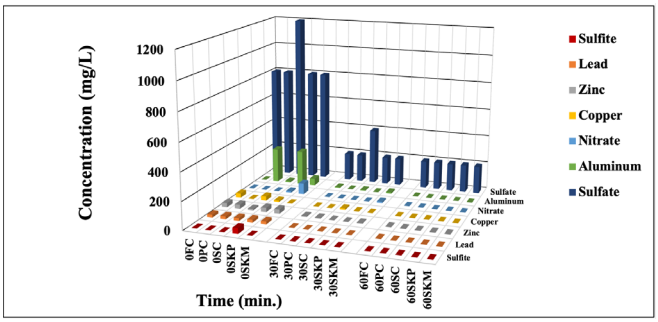

Figure 6: Heavy metals and other contaminants were effectively reduced starting at 30 minutes of exposure. Bioremediation of contaminants (Zn, Pb, NO_3^-, SO_4^{2-}, SO_3^{2-}, Al, Cu) in water samples obtained from FC (French Creek), PC (Pine Creek), SC (Silver Creek), SKP (Schuylkill River Pottsville), SKM

temperature (18°C), denitrification was 100% successful; however, trials in lower temperatures of about 2°C did not show any changes in nitrate concentration even after 48 hours. We were also able to demonstrate that neutralization preceding the decontamination is essential and they have synergistic relationship in the process. (**Figure 5A&B**).

Similarly, 5 of our collected water samples French Creek, Pine Creek, Silver Creek Mine, Schuylkill river at Pottsville, and Schuylkill river at Manayunk demonstrated higher concentrations of NO_3^-, Pb, SO_4^{2-}, Cu, Zn, and Al (**Table 1**). The pH values were low in all the collected samples, except for the tap water from Wayne, indicating the acidic nature of polluted waters. The levels of contaminants dropped after neutralization for 5 minutes with rise in pH and 30 minutes of exposure to the soil containing DNB and SRB at room temperature of 18°C (**Figure 6**). Our experiment demonstrated noticeable reduction in sulfate (down to 200 mg/L) to the levels below that of EPA and WHO accepted levels of 250 mg/L and 500 mg/L, respectively (19). Sulfite levels were detected in only one sample (Schuylkill River Pottsville) at 40 mg/L, which was reduced to zero after 30 minutes of bioremediation.

DISCUSSION

Our hypothesis was based on a two-step process of neutralization and bioremediation necessary for water purification. In the neutralization step, the acidic runoff water reacted with the alkaline agent causing a rise in pH level. Subsequently, the anaerobic bacterial respiration reduced oxygen-containing compounds such as nitrates and sulfates. Under optimized

conditions of temperature and pH, the soil facilitated the bioremediation of several hazardous contaminants. A modular bioreactor was designed to facilitate the two-step process, and experimental results supported the hypothesis. Seven contaminants of interest (NO_3^-, SO_4^{2-}, SO_3^{2-}, Pb, Cu, Zn, and Al) were effectively eliminated in a short period of time, in a portable device with no external energy needed. Bacteria used the carbon readily available in nature, and the limestone's reaction with water was exothermic, which releases energy in the form of heat. Initially, a small pump was used to transfer water from one bucket to the next, but this could be replaced by a solar-powered pump or be eliminated by using gravitational force. Therefore, the study succeeded in designing an effective, eco-friendly, sustainable, low cost, low-maintenance, portable, and grid-independent system for treating contaminated water. The fact that our bioreaction process does not depend on any external energy, such as fossil fuel or electricity, makes this process energy- and cost-efficient.

Water pollution by nitrate, sulfate, toxic metals, and acid rain are widespread problems in the world and pose serious public health issues and environmental degradation of ecosystems (20). Furthermore, contaminations in local waters are an inescapable reality. Overuse of fertilizers in croplands with an increased desire for larger and greener lawns in residential and recreational areas have introduced large quantities of nitrates into our creeks and rivers (21). This nitrate ultimately ends up in our food and drinking water. Field studies in this examination demonstrate the presence of nitrates at higher concentrations in Schuylkill River in Manayunk, at least 80 ppm (or mg/L), above the EPA MCLs of 10 ppm (18). Moreover, runoff of acidic waters from abandoned mine sites in Pennsylvania showed the presence of SO_4^{2-}, Zn, Cu, Al, and Pb in high concentrations. Long-term exposure to these contaminated waters could be highly hazardous to humans, especially infants, and aquatic life. This research study and modular bioreactor offer a practical solution to remedy this critical environmental concern.

Bioremediation techniques are emerging as promising methods for water purification in the last decade (12). This research project explored a novel approach and described valuable modifications in designing a modular bioreactor to eliminate specific contaminants. Although numerous technologies have emerged for water purification (22), not much has been reported about the bioremediation of nitrates. The presence of DNB and SRB bacteria were reportedly in specific locations such as water treatment sites (23). Similarly, in our study, it was evident that such bacteria were ubiquitous to upper layers of soil and could be found in backyard soil at 5" in depth. Resources used here were all chosen to be readily available in nature, and the design was made to simulate what takes place naturally. This system demonstrated the synergistic effect of neutralization and bacterial bioremediation. The reactions were optimized at a higher pH (8) and higher temperatures (16°C). The portable system could effectively carryout multiple biological and chemical reactions simultaneously.

This simple, versatile, and portable purification system would allow access to clean water in disaster-stricken communities or other self-sustainable camps or communities. Our bioreactor could be adopted for other remediation experiments and further research. This system could be scaled up to address in-situ experiments with larger bodies of water. Modifications could be implemented in the passages of the runoff waters so that they can be exposed to the pools of limestone and gradually drain through a bio-barrier with bacteria-rich soil so that natural decontamination takes place prior to their entrance into rivers. The fast and portable bioreactor system could be used as an educational tool for demo purposes in school science labs, demonstrating an interdisciplinary approach for problem-solving in environmental studies.

The presence of microplastics, particularly poly-fluoroalkyl substances (PFAS), in water and their impact on health and environment is a new area of interest for my research (24,25). Interestingly, there are common soil bacteria such as Acidimicrobium bacterium A6 capable of breaking down PFAS (26). Exploring the possibility of using the bioreactor designed here with additional bacterial source to look at effectiveness of PFAS breakdown would be a good target for the future.

METHODS

Experimental Design

This experiment was based on two sets of chemical reactions: acid-base neutralization reaction followed by an anaerobic redox reaction. In the first step, limestone chips ($CaCO_3$ rocks, US Plastic Corp.) were used as an alkaline agent to neutralize the acidic water. In the second step, anaerobic bacteria in soil were used to reduce oxidized forms of nitrogen and sulfur in the water (16,27). The bacteria use decomposed organic material in nature as their energy source. We used compacted moist backyard soil and mixed it with compost (Nature's Care "Really Good Compost") as the source of organic carbon in order to replicate conditions in nature. The de novo device for this study was designed such that neutralization precedes denitrification. Parameters were set so that the study would simulate what occurs spontaneously in nature. After many trials and resolving engineering errors, we designed a bioreactor that could test our hypothesis. A bi-modular system allowed increasing the pH (measured by PASCO wireless pH sensor) of the water sample in the first module then transferred using Everbilt Mobile Pump to the second module exposing to the anaerobic bacteria in the soil for desired periods of time.

Site Selection and Location of Water Samples

In this study, the levels of seven contaminants (NO_3^-, SO_4^{2}, SO_3^{2-}, Pb, Zn, Cu, and Al) were analyzed in runoff water. These analyses were done using both API Test KIT (Individual Aquarium Water Test Kit for Nitrate) and Comprehensive Water Test Kit (waterteststrip.com). All samples were collected from tributary creeks and rivers near coal-mining regions in northeastern PA, as well as urban and suburban areas of Philadelphia. Samples from Schuylkill River: This river drains major parts of coal regions in eastern PA. Selected locations were: 1) Silver Creek Mine, New Philadelphia, Schuylkill County, 2) Schuylkill River, Pottsville, Schuylkill County, 3) Schuylkill River, Manayunk, Philadelphia County, 4) Wissahickon Creek, Philadelphia County, 5) Pickering Creek, Phoenixville, Chester County, 6) French Creek, Phoenixville, Chester County, 7) Schuylkill River, Cromby Generating Station, a retired coal-fired power station, *Phoenixville*, Chester County. Samples from Susquehanna River: This river was considered "America's Most Endangered River for 2005" because of the excessive pollution it received (28). Selected locations were: 8) Solomon Creek, Wilkes-Barre, Luzern County, 9) Pine Creek, Hanover Township, Luzern County,

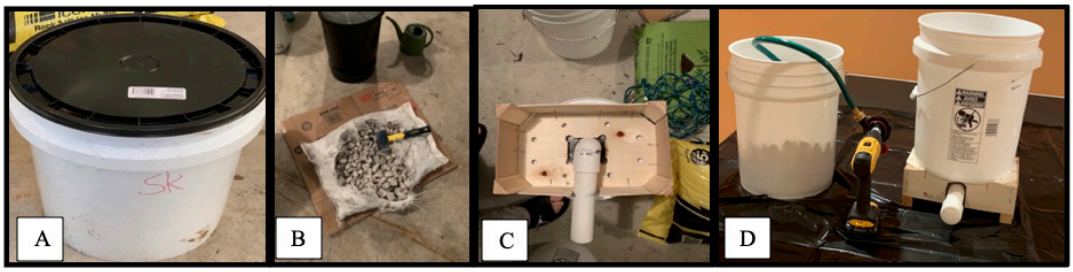

Figure 7: Design of the modular bioreactor. (A) Each module in bioreactor is made with a 5-gallon bucket. (B) Limestones ($CaCO_3$) are crushed to increase their surface area for reaction and are placed in the first module. (C) Extruding elbow and drain pipe from the bottom of the second module is stabilized on a wooden box. (D) completely assembled bioreactor showing 2 modules connected with a hose and small pump to transfer water from first module where neutralization occurs to second module where enhanced soil (soil mixed with compost as a carbon source for bacteria) is present. This soil sample contains DNB and SRB bacteria. The drain is capped to only collect samples for testing.

10) Brown Creek, Larksville, Luzern County. Samples from Delaware River: This river was named the 5th most polluted river in the United States in 2012 (29). The sample was collected from: 11) Darby Creek, Devon, Chester County. The control water sample was tap water from 12) Wayne, PA, Chester County. At each location, a five-gallon bucket was submerged into the river and filled with approximately 3 gallons of water. We sampled these sites two times between November 2018– January 2020. More than one sample was often taken to assure that the flow of river water would not create sampling variations. This also would allow adequate amount of water samples in case accidental loss of samples occurred. In the experiment, all the samples consistently gave the same values of contaminants at the time of measurement. Therefore, only results from one sample from each site was reported.

Measuring the Contamination Levels in Water Samples

Water samples were collected from 12 locations (tributary creeks of Schuylkill, Susquehanna, and Delaware rivers) and screened for the presence of specific contaminants (**Figure 1 A–C**). The Comprehensive Water Test Kit and API Test KIT were used according to the manufacturer's instructions to measure the concentrations of NO_3^-, SO_4^{2-}, SO_3^{2-}, Pb, Cu, Zn, and Al (**Figure 2 A–G**). The tap water from Wayne, PA met safety maximum contaminant levels set by EPA and was used as the control (18).

A calibrated Vivosun digital pH-meter was used to measure the acidity level of the water. The water strip test used here measures ionic forms of the metals but does not quantify each specific ion. For instance, Pb^{2+} and Pb^{4+} is reported collectively as lead level. This form of reporting is consistent with other similar published papers and EPA reporting (18).

Design of the Modular Bioreactor: A Two-bucket System

One five-gallon bucket (Encore Plastics 50640 Industrial Plastic 70-Mil with Handle, 5-Gallon, Pail White) was filled with 28 lbs. (12.7 kg) of limestone chips to approximately ¾ the height of the bucket (**Figure 7A & B**). We cut a hole approximately 1.25" in diameter in the center of a second 5-gallon empty bucket. Using PVC cement, we attached a PVC elbow with a diameter of 1.25" directly beneath the hole, so the water would drain from the bucket through the hole and enter the PVC elbow. To extend the drainage pipe, we then cut a 1.25" diameter PVC pipe to 7" in length, fitted the pipe into the elbow, and used a PVC cap to cover the exposed end of the pipe. We laid a steel woven mesh (Stainless Steel Woven Mesh Sheet 0.001" thickness) as a filter at the bottom of the bucket to prevent mud from entering the elbowed pipe. To stabilize this module, we modified a wooden box as a stand for the bucket (**Figure 7C).** The complete bioreactor is shown in (**Figure 7D**).

Testing for Viable Denitrifying and Sulfate-reducing Bacteria in the Soil

A (2' x 2' x 5") area of backyard soil was excavated for the bioremediation experiments. For each experiment, 80.5 lbs. (36.5 kg) (using digital scale) of soil were mixed in with 11 lbs. (5 kg) of compost as a source of carbon for the bacteria. The mixture was kept moist with 2 L of tap water (from Wayne, PA) and left in sealed buckets with airtight lids to reduce the oxygen content of the soil. Buckets were left at room temperature (18°C measured with digital thermometer) for seven days to acclimate the bacteria in the soil. After a week, 15 mL of water from each bucket of soil was tested with the BARTTM Kit (Bio-detector for Denitrifying Bacteria (Hach (DN-BART) kit) for denitrifying bacteria (DNB) according to the manufacturer's directions (**Figure 8A**). The presence of DNB was assessed

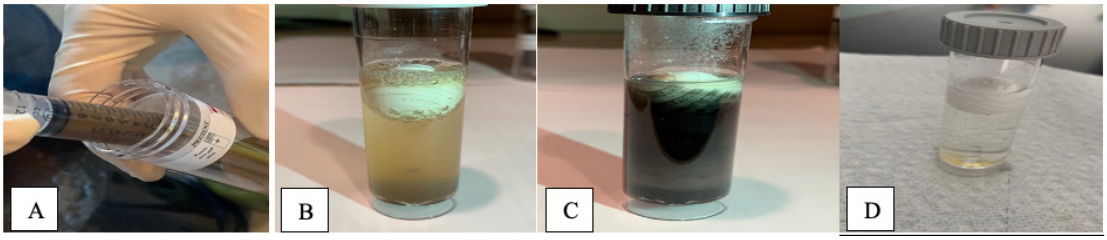

Figure 8: The soil sample was tested for the presence of DNB and SRB prior to use in the bioreactor. Testing for viable denitrifying and sulfate-reducing bacteria was performed with BARTTM Kit. The backyard soil sample demonstrated to be rich in its DNB and SRB microorganism; therefore, was decided to use this soil for bioremediation experiments.

for four days and monitored by the appearance of bubbles and foams in the vial. (**Figure 8B**). Similarly, the presence of sulfate-reducing bacteria (SRB) was tested in separate vials (using Bio-detector Hach (SRB-BART) kit), and the growth of bacteria was monitored for eight days. A black slime ring beneath the ball and a black slime growth at the base of the tube indicated the presence of SRB (**Figure 8C**). The bacterial content of at least three soil samples were evaluated (river bed, near the mining runoff water, and our backyard) using BARTTM kit. Interestingly, our backyard soil dug at about 5" in depth had the best presence of active bacteria. Further testing was repeated on compost alone and purified water. There was not any noticeable growth of DNB and SRB in either of these samples although presence of other bacteria here cannot be ruled out with BART test Kit. (**Figure 8D**). Therefore, we cannot make a safe recommendation for drinking this water until further testing that includes other parasites and bacteria is performed on the water, which is beyond the scope of this experiment.

Neutralization and Denitrification Procedures

Denitrification was the initial focus of the study. Schuylkill River water collected in Manayunk was the only sample with very high nitrate levels of around 80 ppm, where the EPA's permitted Maximum Contaminant Levels (MCL) are 10 ppm (18). To neutralize the sample, 200 oz. (6 L) of Manayunk water was measured and poured into the bucket with limestone chip and we waited 5 minutes for the reaction to be complete. The pH of water rose from 4.2 to 8 in just 5 minutes after exposure to limestone. The pH stabilized around 8 regardless of time of exposure to $CaCO_3$. The pH was tested periodically as the experiment continued over multiple days. Once a pH of 8 was achieved, it did not change significantly with time. Using a simple water pump with attached hoses, we transferred 48 oz. (1.5 L) of neutralized water from the bucket with a graduated glass container and then transferred it into the bucket with a fixed amount of soil (15 lbs. or 7 kg) to begin the denitrification process. The presence of nitrate was measured in 5 mL of water collected at different time intervals by an API Test KIT (**Figure 3A & B**). The results showed complete removal of nitrate in 30 minutes. Shorter time intervals did not allow adequate removal and longer time intervals were unnecessary as the level dropped to 0ppm in 30 minutes.

Optimizing pH and Temperature for Bioremediation

The effects of pH and the temperature of polluted water and the efficiency of bacterial bioremediation were studied in the next series of experiments. We observed the effect of acidic pH (4.0) vs. basic pH (8.0) on nitrate removal as measured in different time intervals where the temperature was kept at 18°C (**Figure 4A**). Complete nitrate removal occurred only after the pH increased to 8.0 and within 30 minutes of exposure to the DNB bacteria. Temperatures above 16°C were favorable in these bioremediation studies where pH was kept at 8.0 (**Figure 4B**).

We evaluated the possibility of a synergistic effect between neutralization followed by a denitrification reaction. Control studies were as follows: a) contaminated acidic water sample was exposed to limestone only but not to the soil at 18°C. Its pH increased but the contaminants remained unchanged, and b) similarly, contaminated water sample was exposed to soil only but not to limestone for 60 minutes at 18°C. There were no changes in the nitrate levels and pH remained acidic indicating that neutralization was an essential part of the process. c) Wayne tap water containing no contaminants was added to the soil sample for 60 minutes at 18°C and then tested. There was no trace of contaminants affirming that soil was devoid of any contaminants and did not contribute to the presence of contaminants in the water. **Figure (5A & B)** summarizes the observations above for denitrification efficiency and the design of a bi-modular bioreactor. For the remainder of experiments, specific parameters such as temperature, the mass of limestone, the mass of enhanced soil, and the volume of water samples were kept constant.

Bioremediation of Sulfate, Sulfite, and Toxic Metals

After successful denitrification using soil bacteria, we evaluated other contaminants. We established that the backyard soil sample enhanced with a compost also showed the presence of anaerobic sulfur-reducing bacteria (SRB) suitable for remediation of sulfates, sulfites, and heavy metals. Initial evaluations of samples showed five locations with high levels of SO_4^{2-}, SO_3^{2-}, and toxic metals such as Pb, Cu, Zn, and Al. Samples were first pH-neutralized and then transferred to the bioreactor containing soil with SRB at a constant temperature of 18°C. (**Table 1 & Figure 6**) show untreated samples (not neutralized or not exposed to the bioreactor with SRB; 0 minutes) as compared to treated samples (first neutralized with limestone, then sat in the bioreactor for 30 or 60 minutes). The concentrations of contaminants dropped after 30 minutes of exposure to the soil with SRB. We examined samples from French Creek (FC), Pine Creek (PC), Silver Creek Mine (SC), Schuylkill, Pottsville (SKP), and Schuylkill, Manayunk (SKM).

Our experiment demonstrated a noticeable reduction in sulfate (down to 200 mg/L) to the levels below that of EPA and WHO accepted levels (250 mg/L and 500 mg/L, respectively) (19). Sulfite levels were detected in only one sample (Schuylkill River Pottsville) at 40 mg/L, which was reduced to zero after 30 minutes of bioremediation.

Accuracy of Measurements and Bioremediation rate

The repeated bioremediation of different water samples revealed near complete removal of all the targeted contaminants in this experiment. Furthermore, the values of the contaminants after the process of bioremediation are the same as the minimum measurable quantities in water strip testing kit. These values also match or exceed the values obtained from the control (Wayne tap water) and MCLs

Table 2: Bioremediation Rates. Calculation of bioremediation rates for each contaminant in water sample from Schuylkill River at Manayunk location. Initial concentration – Final concentration / time = Rate.

Schuylkill R. Manayunk Contaminants	Initial Concentration (mg/L)	Final Concentration (mg/L)	Shortest time required in Minutes	Bioremediation Rate (M/s)
(SO_4^{2-})	800	200	30	$3x10^{-6}$
(Al)	50	10	30	$8x10^{-7}$
(Zn)	30	5	30	$2x10^{-7}$
(Cu)	1	0	30	$8x10^{-9}$
(Pb)	20	0	30	$5x10^{-8}$
(NO_3^-)	80	10	30	$7x10^{-7}$
(SO_3^{2-})	0	0	N/A	N/A

from EPA. Therefore, the percent error calculations yield zero percent in our experiment. Overall, satisfactory levels of contaminants were achieved in 30 minutes. Individual contaminant bioremediation rates can also be calculated in each sample by dividing change in concentration over the shortest time required. We have demonstrated these rates for the sample taken from Manayunk location (**Table 2**).

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