

THE OPTIMAL CONSORTIA OF MICROBIAL BIOREMEDIATION AGENTS FOR THE
REMOVAL OF HEAVY METALS FROM THE SUPERFUND SITE AT NEWTOWN CREEK
IN BROOKLYN, NEW YORK

by

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of

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DEDICATION

This paper is dedicated to my family and friends who have supported me throughout my progress in the Montana State University LRES program.

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ABBREVIATIONS AND ACRONYMS

Ag: silver
As: arsenic
C: Celsius
Cd: cadmium
CFU: Colony Forming Units
Cl: chlorine
Cr: chromium
Cu: copper
DNA: Deoxyribonucleic Acid
GEM: Genetically Engineered Microorganism
g: gram
Hg: mercury
kg: kilogram
L: liter
mg: milligram
mmol: millimole
mV: millivolt
Na: sodium
NYCDEP: New York City Department of Environmental Protection
Ni: nickel
Pb: lead
PSU: Practical Salinity Unit
rRNA: Ribosomal Ribonucleic Acid
Se: selenium
UCL: Upper Confidence Limit
USEPA: United States Environmental Protection Agency
USGS: United States Geological Services
Zn: zinc
 μ g: microgram

ABSTRACT

Bioremediation is a technique that introduces a microbial population capable of destroying, transforming, or immobilizing a pollutant of interest in a manner that is both safe to the environment and cost-effective. Bioremediation agents are effective in sequestering heavy metals, and are capable of functioning at their highest capacity when environmental conditions are complimentary to microbial growth, the indigenous microbial population favorably interacts with the introduced microorganisms, and nutritional requirements are satisfied either by the ecosystem or via the addition of amendments. Additionally, genetically engineered microorganisms can be utilized when necessary within especially challenging conditions. This study determines the ability of a consortia of microbial bioremediation agents to remove heavy metals from the Superfund site at Newtown Creek in Brooklyn, New York. The remediation of heavy metals at Newtown Creek can be accomplished using *Rhodopseudomonas palustris*, *Saccharomyces cerevisiae*, and *Cupriavidus metallidurans*. These microorganisms possess a high degree of potential and are promising tools in the reduction of Earth's ever-increasing pollution levels. Heavy metal concentrations, nutrient levels, pH, temperature, salinity, oxidation reduction potential, and the indigenous microbiome were analyzed to determine the expected benefit of microbial bioremediation in the reduction of heavy metal concentrations. Although high initial heavy metal concentrations and salinity may negatively impact this effort, all other variables are expected to be conducive to microbial growth. The introduction of amendments is expected to further benefit microbial bioremediation potential. The selected consortia of microorganisms introduced have the capacity to sequester all the chromium, lead, mercury, selenium, and silver found at Newtown Creek. The impact of arsenic, cadmium, copper, nickel, and zinc concentrations can also be decreased due to microbial bioremediation efforts. The use of genetically engineered microorganisms within a bioreactor is expected to further benefit remediation efforts for heavy metals sequestered at low rates within the natural environment. Microbial bioremediation is not expected to fully remove heavy metals from Newtown Creek, yet these microorganisms are capable of greatly improving conditions.

INTRODUCTION

Heavy Metal Bioremediation Overview

Bioremediation is a creative and promising approach to environmental pollution that involves reducing or eliminating pollutant levels in contaminated water and soils using microorganisms. Commonly, during the process of bioremediation, a microbial population uses a specific pollutant of interest as a source of nutrients [1]. By metabolizing pollutants, microbes can destroy, transform, or immobilize environmental contaminants and provide a more cost-effective alternative to conventional treatment processes [2]. Heavy metals are a very serious and highly toxic pollutant that can be treated through bioremediation [3]. Microbial heavy metal bioremediation occurs through several mechanisms. Heavy metals can be sequestered on the cell's exterior via biosorption, brought into the cell and stored in the cytoplasm by intracellular sequestration, incorporated into the extracellular polymers via the creation of a biofilm, or reduced to less toxic forms via precipitation [3]. Through these processes, microorganisms utilize heavy metals and reduce their toxicity.

In biosorption, metal ions and metal functional groups on the cell surface interact and rapidly bind together within only a few minutes time. This process occurs independently of metabolism, and therefore both living and non-living cells can participate in this bioremediation method [4]. In fact, it may be beneficial to use pre-treated non-living biomass instead of living microbes to enhance bioremediation capability. Pre-treatment methods break apart the cell membrane and expose additional binding sites, as well as reduce the positive charge of cell walls to enhance the capability of biomass for heavy metal uptake and thereby potentially bind with more heavy metals than living cells [5]. This method of bioremediation is especially useful in

harsh environmental conditions such as high heavy metal concentrations that may be toxic to living organisms.

Living microorganisms are also capable of transporting heavy metals into the cell and complexing them with the cell cytoplasm during the process of intracellular sequestration, which reduces the toxicity of heavy metals [3]. In addition, metal ions can be ejected from the cytoplasm and sequestered in the periplasm in the process of extra-cellular sequestration. Once sequestered, heavy metals can be used by microbes to synthesize useful cellular components. For instance, toxic silver ions can be taken up by bacteria that bind the silver with proteins to form nanoparticles [6]. During extra-cellular sequestration, metals may be reduced via precipitation, in which microbial cells seeking electron donors or acceptors use heavy metals and thereby reduce their toxicity. Heavy metals may also be sequestered within biofilms, which are extracellular polymeric substances that surround and guard a community of microorganisms [7]. Biofilms show great potential for binding ability with various heavy metals and a capability for heavy metal degradation [1]. Additionally, biofilms offer strong protection to microbes from toxic materials, and offer highly increased metal removal efficiency via biosorption [3]. These combinations of mechanisms allow microorganisms to survive and thrive in environments that contain toxic heavy metals.

Study Objectives and Necessity of Investigation

Newtown Creek is severely polluted by heavy metals, which negatively impact the local wildlife and human population. My goal in this study is to evaluate the capability of a consortia of bioremediation agents in the treatment of metals present at Newtown Creek in Brooklyn, New York, and gain insight to how this bioremediation effort could be accomplished. Additionally, I investigated limitations (e.g. environmental constraints) to the potential ability of this consortia

to bioremediate heavy metals in Newtown Creek and identified potential solutions to these issues. To accomplish this, I evaluated the capabilities of various microbial bioremediation agents based on their likelihood of success at Newtown Creek. I measured likelihood of success by tolerance of high heavy metal concentrations and rate of efficiency in bioremediation. I also intended to minimize microbial interactions, so I placed additional emphasis on the use of microorganisms capable of sequestering multiple heavy metals from the environment. I then assessed the properties of selected bioremediation agents versus the heavy metal concentrations, physicochemical conditions, and indigenous microbiome present at Newtown Creek. Finally, I explored the additional potential contributions of using amendments and genetically modified organisms within this study. My exploration of microbial bioremediation at this site can provide a cleaner and healthier environment for the community.

Newtown Creek Site Overview

Newtown Creek is an approximately 3-mile-long waterway located in Brooklyn, New York (Figure 1) [8]. This location was declared a Superfund site by the U.S. Environmental Protection Agency (USEPA) in 2010 and is currently one of the most polluted waterways in the country [9]. This extreme contamination is due to a history of industrial facilities lining the banks of Newtown Creek that included oil refineries, tanneries, petrochemical plants, and fertilizer factories, all of which contaminated the waterway (Figure 2).

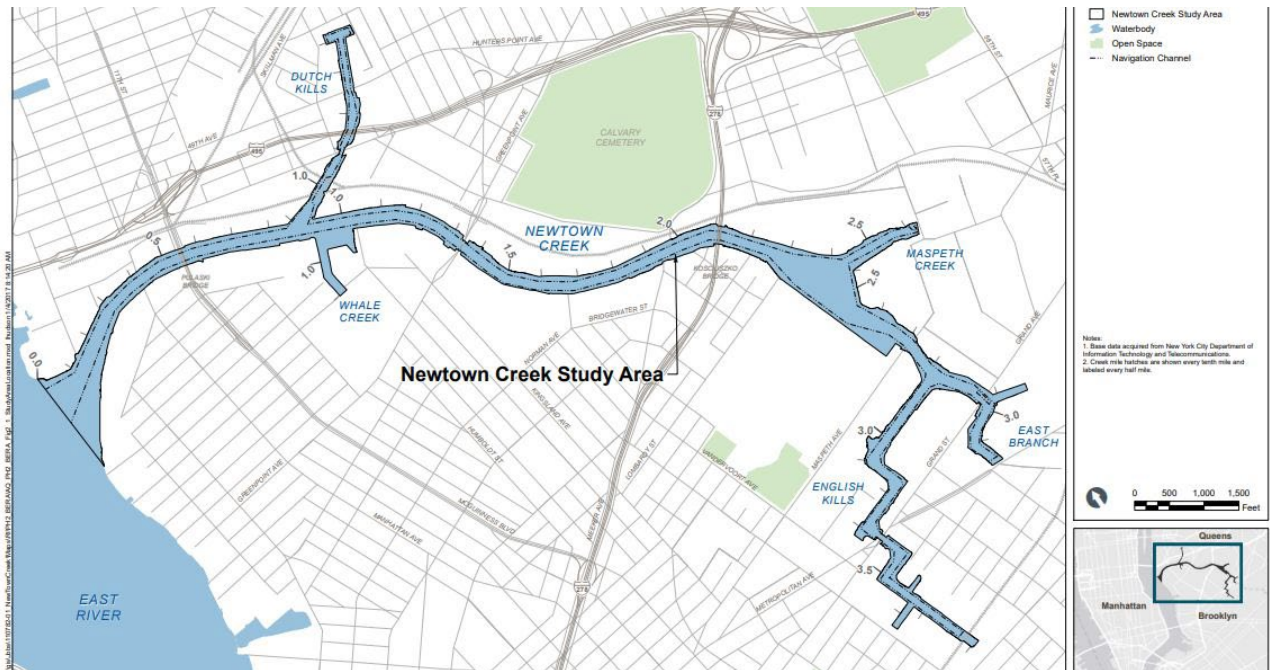


Figure 1: Map of Newtown Creek [8]



Figure 2: Aerial photograph of Newtown Creek taken in 1960 [9]

The water in Newtown Creek is mostly stagnant, and therefore many of the pollutants that have been introduced in the past still remain at this location [9]. Currently, Newtown Creek contains biological contaminants, volatile and semi-volatile organic chemicals, pesticides, excess nutrient loads, hydrocarbons, and heavy metals [3]. Clearly, this waterway requires a great deal of attention to be rehabilitated.

Overview of Selected Bioremediation Agents

Many different microorganisms have been successful in heavy metal bioremediation efforts and show great promise in the removal of heavy metals from Newtown Creek. In addition to environments with high pollutant concentrations such as heavy metals, microbes are also able to survive at temperatures below zero and in extreme heat, in high oxygen or anaerobic conditions, and within a large range of pH values [10]. These capabilities provide microbes incredible versatility and adaptability into an almost unlimited amount of potential applications. I selected *Rhodopseudomonas palustris*, *Cupriavidus metallidurans*, and *Saccharomyces cerevisiae* for this effort as these microorganisms have been found especially effective in the treatment of a variety of heavy metals in various environmental conditions. *Cupriavidus metallidurans* has successfully colonized industrial sediments and soils that contain high concentrations of heavy metals [11] and is well adapted to bioremediation within extreme environments [10]. The baker's yeast *S. cerevisiae* has been intensively studied for its potential in both passive biosorption and heavy metal sequestration for many heavy metals, and has been proven as a preferred bioremediation agent due to its safety, low cost, and effectiveness [12]. Additionally, *S. cerevisiae* can sequester silver, cadmium, chromium, copper, nickel, lead, and zinc that can potentially be recovered and recycled [13]. *Rhodopseudomonas palustris* is incredibly metabolically diverse, and can utilize sunlight, organic, and inorganic matter as energy sources to fuel bioaccumulation processes [14]. This microorganism can lower a variety of heavy metal levels via biosorption and bioaccumulation, and concurrently functions to reduce carbon dioxide levels [1]. Based on these various abilities, I considered each of these microorganisms an excellent candidate as a bioremediation agent.

Additional Factors Influencing Microbial Bioremediation

The environmental conditions present will highly influence microbial growth and bioremediation capacity. Generally, to maximize the effectiveness of each microorganism as a bioremediation agent, an environment composed of an approximately neutral pH, moderate temperatures, adequate nutrients, electron donors or acceptors, and appropriate light and oxygen levels is highly beneficial [15]. To further optimize growth, amendments can be added which provide nutritional assistance to the microorganisms of interest. Moreover, the genetic material of a microorganism can be altered by recombinant DNA technology, which involves the exchange of genes between different microbes. These genetically engineered microorganisms have been effective in various bioremediation efforts [1]. All these factors affect the efficiency of microbial growth and therefore must be considered in the evaluation of their success.

Challenges Affecting Bioremediation Efforts

Although there are many benefits to microbial bioremediation, there are also challenges that must be considered. Microorganisms can protect themselves from heavy metals and can concurrently reduce these pollutant levels. However, at high concentrations, these same metals can become toxic to microorganisms and weaken their bioremediation capacity [16]. Various heavy metals present different problems to microbial growth and function depending on concentration and microorganism (Table 1) [3].

Table 1: Heavy metals and potential problems from toxicity (adapted from Igiri et al [3]).

Metal	Problem Caused by Toxicity
Arsenic	Deactivation of enzymes
Cadmium	Denature proteins, destroy nucleic acid, hinder cell division and transcription, damage cell membrane, induce oxidative damage and denature microorganisms
Chromium	Growth inhibition, elongation of lag phase, inhibition of oxygen uptake, induce oxidative damage and denature microorganisms
Copper	Disrupt cellular function, inhibit enzyme activities, growth inhibition, cause severe injury to cytoplasmic molecules, DNA, lipids, and proteins
Selenium	Inhibits growth rate
Lead	Destroys nucleic acid and protein, inhibits enzyme actors and transcription, damage cell membranes and destroy the structure of DNA
Mercury	Denatures protein inhibit enzyme function, disrupt cell membrane
Nickel	Upset cell membrane, hinder enzyme activity and oxidative stress
Silver	Cell lysis, inhibit cell transduction and growth
Zinc	Decrease in biomass and growth, cell death

Some microbes resist heavy metal toxicity through the process of methylation, which increases the volatility of mercury, selenium, arsenic, and lead. Through the methylation process, these metals create gaseous methyl mercury, volatile dimethyl selenide, gaseous arsines, and dimethyl lead [3]. All these compounds increase the initial toxicity of heavy metals and present serious hazards to the environment. Microbial processes can also enhance the solubility of heavy metals, which increases their potential toxicity and availability to the surrounding aquatic wildlife [17].

High salinity levels can provide an additional stress to microbial growth and survival. As extracellular concentrations of Na^+ and Cl^- are brought into the cell, this can disrupt equilibrium and cause cell damage [18]. Therefore, water characterized as highly saline (10-35 parts per thousand or 1-3.5% salinity) may not be habitable for some microorganisms [19]. Tolerance of microorganisms to high salinity greatly varies and is another important variable to consider in bioremediation efforts.

Microbial bioremediation can also be challenging due to the multitude of potential environmental interactions that may take place. Microorganisms may deplete preferred nutrition sources, influence oxygen concentrations, and are capable of mutation and horizontal gene transfer [15]. Therefore, these variables must be closely monitored.

Heavy Metal Mobility at Newtown Creek

Heavy metals can be found both within the water and the surface sediment at Newtown Creek. As heavy metals are introduced into bodies of water, approximately 85% of these metals accumulate into surface sediment [20]. While the fixation of metals within sediment does offer protection to the surrounding aquatic life, heavy metals will not remain in the sediment forever. Once released back into the surrounding aquatic environment, heavy metals can become bio-accessible and negatively impact the larger ecosystem (Figure 3) [3].

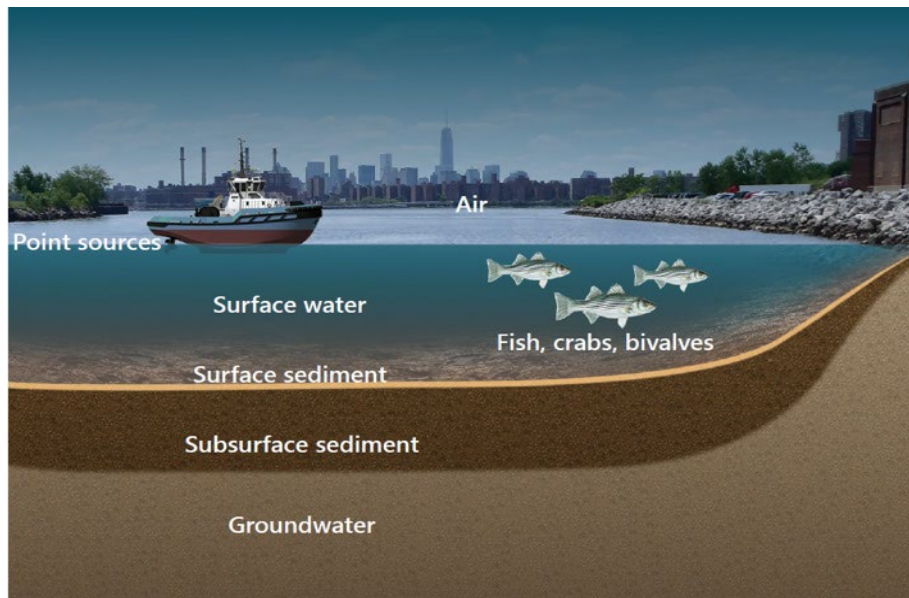


Figure 3: Surface water and surface sediment within an aquatic ecosystem [9]

To become bio-accessible, heavy metals must become desorbed from sediment [21]. Desorption from surface sediment is primarily caused by fluctuations in pH level and changes in

reduction-oxidation potential [20]. Heavy metal mobility is highest at low pH levels and an increased redox potential. Secondly, heavy metal mobility increases with temperature and salinity, decreases with retention time in sediment, and varies with heavy metal type (Table 2) [20]. Furthermore, microorganisms interact with their surroundings and affect bio-accessibility of heavy metals [21].

Table 2: Variables and their effects on Heavy Metal Mobility in sediment (adapted from Peng et al. [20]).

Variable	Effect on Heavy Metal Mobility in Sediment
pH	As pH decreases, heavy metal mobility increases
Oxidation Reduction Potential	As ORP increases, heavy metal mobility increases
Temperature	As temperature increases, heavy metal mobility increases
Salinity	As salinity increases, heavy metal mobility increases
Retention Time	As retention time increases, heavy metal mobility decreases

Heavy metals are non-biodegradable and tend to bioaccumulate, resulting in devastating effects to the functions of the brain, lungs, and kidneys [1]. Heavy metals not only possess significant toxicity to aquatic organisms, they also pose a risk to human health through the food chain [3] (Figure 4). Due to the dangers that heavy metals present, the surrounding aquatic environment must be shielded from these pollutants to protect both the wildlife and human population local to this waterway.



Figure 4: Newtown Creek ‘Do Not Eat’ Warning Sign [22]

METHODS

Site Evaluation

I consulted the USEPA ‘Final Baseline Ecological Risk Assessment Remedial Investigation/Feasibility Study for the Newtown Creek Site’ [8] (completed in 2018) for the identification of metals present in surface waters and surface sediment with hazard quotients greater than 1.0 to be targeted for bioremediation. Hazard quotients were defined as the ratio of estimated heavy metal uptake vs. the concentration at which adverse health effects are expected [8]. Therefore, hazard quotients greater than 1.0 were indicative of harmful effects due to heavy metal exposure. I also used this study to identify local wildlife with hazard quotients greater than 1.0 in their tissue. Heavy metal concentrations were reported as the 95% Upper Confidence Limit (UCL) of the mean [8]. This value represents that greatest possible value of the true mean at a certainty of 95%. Sediment concentrations were reported by the USEPA as mg/kg, and I converted these values to mg/L for comparison purposes in some instances. I used the average sediment density of 1.23 kg/L as reported in ‘Sedflume Analysis- Newtown Creek’ from the United States Geological Services (USGS) [23] to convert heavy metal concentrations.

Additionally, I analyzed New York City Department of Environmental Protection (NYCDEP) Harbor Survey Monitoring Program data records collected from 2003 to summer of 2020 to find values and ranges of fluctuation for bottom water temperature, pH, redox potential, average nutritional availability, salinity, light transparency, and oxygen saturation at Newtown Creek [24]. Although the data collected by the NYCDEP was taken from several different locations along Newtown Creek, I compiled these values together to provide a general overview of the area.

Microbial Capabilities and Potential Interactions

I completed a literature review to determine which microorganisms had previously been found effective in bioremediation efforts as well as the conditions in which these studies occurred. I evaluated potential bioremediation agents based on their tolerance of high heavy metal concentrations and rate of efficiency in bioremediation. Furthermore, to minimize additional microbial interactions, I selected microorganisms capable of removing multiple heavy metals from the environment. Finally, I avoided the use of microorganisms that may increase heavy metal toxicity in the environment through processes such as methylation. Based on these criteria, I identified *S. cerevisiae*, *R. palustris*, and *C. metallidurans* as the three bioremediation agents most likely to benefit Newtown Creek. Although other microorganisms may also be capable of contributing to this bioremediation effort, these three microbes show the strongest promise based on the criteria I set for this study.

To further assess the expected effectiveness of the selected bioremediation agents, I analyzed the indigenous microbiome present at Newtown Creek based on the study ‘Bacterial Diversity Impacts as a Result of Combined Sewage Overflow in a Polluted Waterway’ from 2017 that used 16S rRNA data to determine the indigenous microbiome [25]. I then investigated interactions between indigenous and introduced microbial populations, as well as the potential of microbial bioremediation agents already present at Newtown Creek. Finally, I evaluated the enhancement of microbial capabilities via the introduction of amendments and genetically modified organisms (GEMs) to determine the effectiveness and feasibility of incorporation into this study.

RESULTS

Site Evaluation

I documented the following heavy metal concentrations and hazard quotients within Newtown Creek surface water and surface sediment (Table 3).

Table 3: Heavy metals, concentrations, and hazard quotients found in Newtown Creek surface water and surface sediment [8]

Surface Water			Surface Sediment		
Metal	Hazard Quotient at Max Conc.	95% UCL Value (µg/L)	Hazard Quotient at Max Conc.	95% UCL Value (mg/kg)	95% UCL Value (mg/L)
Arsenic			5.2	38	47
Cadmium	9.2	0.3	39	27	33
Chromium			4.3	220	270
Copper	1.3	4.1	100	1900	2300
Lead			18	540	660
Mercury	17	0.0085	17	2.2	2.7
Nickel			17	270	330
Selenium			2.1	4.3	5.3
Silver			14	10	12
Zinc			15	1800	2200

Within the surface water at Newtown Creek, cadmium, copper, and mercury were identified as heavy metals with hazard quotients greater than 1.0. These hazard quotients at maximum concentrations observed ranged from 1.3 for copper to 17 for mercury. Additionally, methyl mercury is currently present at Newtown Creek at a concentration of 0.00010 µg/L (hazard quotient: 0.0025) [8]. Within the surface sediment, many heavy metals were found to contain hazard quotients greater than 1.0. Of these, hazard quotients at maximum concentrations observed ranged from 5.2 for arsenic to 100 for copper. Barium, antimony, and tin were also identified as metals present in surface sediment with a hazard quotient greater than 1.0 (barium

hazard quotient: 8.5, antimony hazard quotient: 3.6, and tin hazard quotient: 14), but microbial bioremediation did not appear feasible for these heavy metals after I completed an exhaustive literature review. Therefore, I excluded these heavy metals from this investigation.

Additionally, I documented hazard quotients for heavy metals accumulated within the local wildlife population at Newtown Creek (Table 4) to determine the impact of heavy metals on these animals [8].

Table 4: Hazard quotients for local wildlife at Newtown Creek [8]

Metal	Mummichog Tissue Hazard Quotient	Striped Bass Tissue Hazard Quotient	Blue Crab Tissue Hazard Quotient	Sandpiper Tissue Hazard Quotient
Arsenic				11
Cadmium				2.0
Chromium				6.2
Copper	12	4.9	5	79
Lead	1.7		2.5	42
Mercury		2.8		
Nickel				3.7
Selenium	1.1	1.6		14
Silver	1.2			
Zinc	1.2			3.1

The type of heavy metal found within the animal tissue varied among species. Hazard quotients found for a small fish such as the mummichog were generally small (apart from copper), but generally increased along the food chain to birds such as the sandpiper.

Factors affecting heavy metal mobility within the ecosystem were evaluated based on records for the creek bottom (Figure 5). Ranges of pH were generally found between 7.0 and 7.5, while all collected values apart from three outliers (5.68, and 6.49 recorded twice) were between pH 6.5 and 8.5. Redox potential was mainly found approximately between 100-200

mV, and salinity levels were mainly found within a fairly tight range between 21 and 24 Practical Salinity Units (PSU). Salinity is reported as PSU, and this directly converts to parts per thousand [26]. Bottom temperatures ranged from 0 to 28 °C but were generally between 16-23 °C.

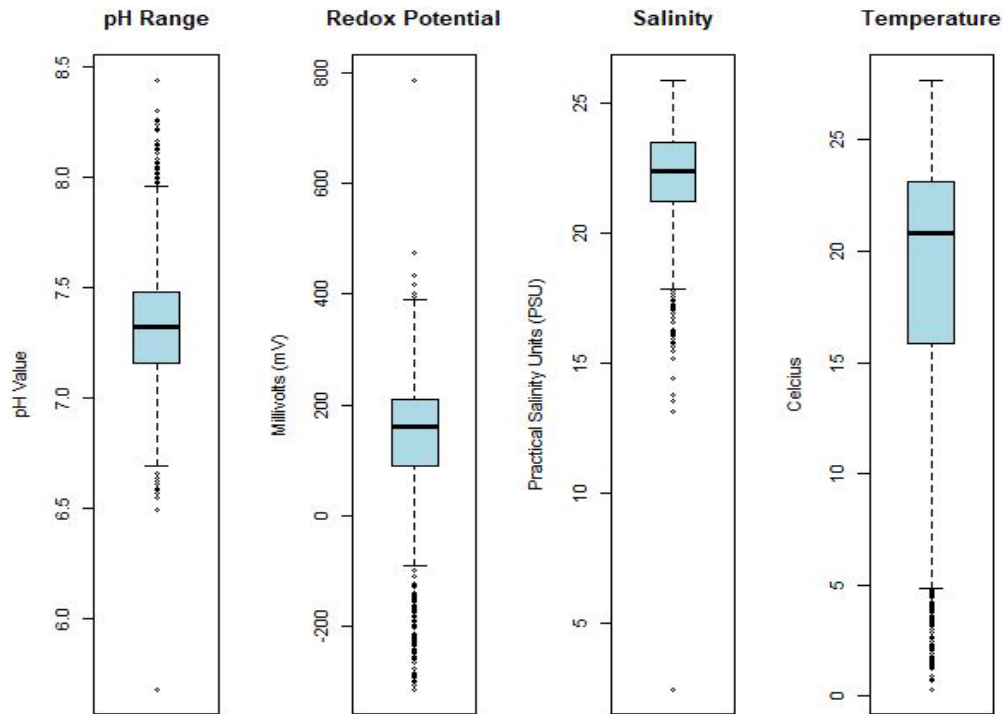


Figure 5: Factors influencing microbial growth and metal mobility at Newtown Creek.

I compiled nutrient levels for nitrate/nitrite, ammonium, and total phosphorus found at the bottom of Newtown Creek to determine availability to microbial bioremediation agents (Figure 6). The median nitrate/nitrite level was 0.22 mg/L, median bottom ammonium level was 0.46 mg/L, and median total phosphorus was 0.22 mg/L. I then evaluated these values compared to natural background levels for these nutrients. Phosphorus levels are naturally expected to be <0.03 mg/L but can range from 0.005 to 0.05 mg/L [27], ammonia is naturally <0.2 mg/L [28], and nitrate levels at ≤ 2 mg/L are considered appropriate background levels [29].

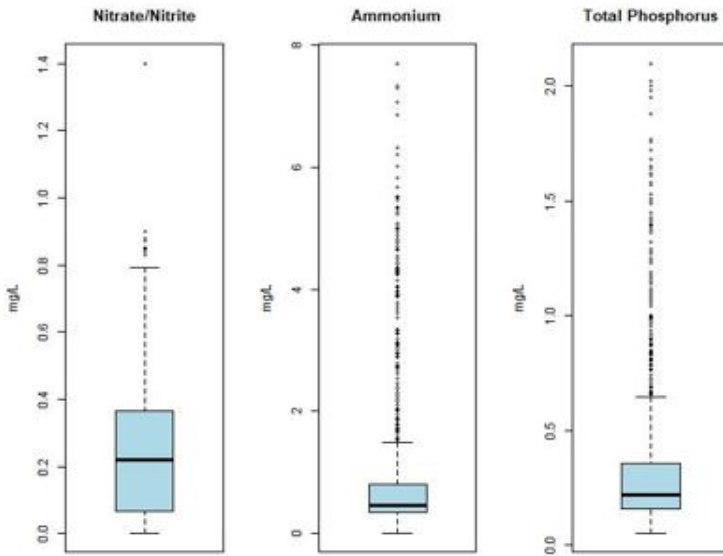


Figure 6: Nutrient levels found at the bottom of Newtown Creek

Additionally, I investigated light transparency and oxygen saturation at the bottom of Newtown Creek to determine effects on microorganism growth (Figure 7). The median light transparency at the bottom of Newtown Creek was 67.4%, while median oxygen saturation was 44.5%.

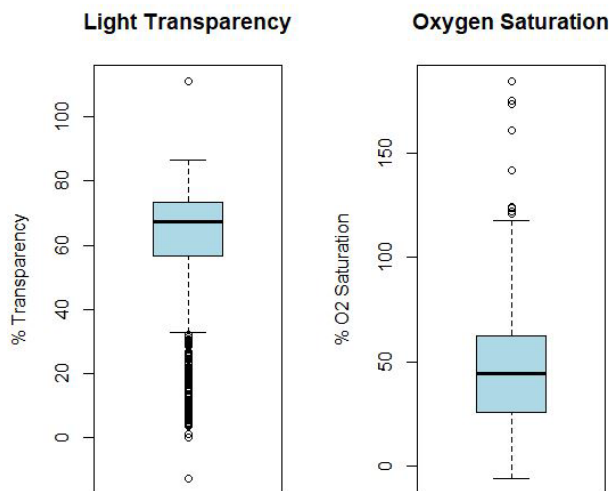


Figure 7: Light and oxygen levels found at Newtown Creek bottom

Photosynthetically active species (plants, algae, etc.) are common additional sources of dissolved oxygen in many bodies of water. Therefore, it is possible for oxygen saturation levels to exist outside of the 0-100% range, which is a scale based on oxygen levels in the air [30]. However, bottom light transparency is expected to range from 0-100%, and values outside this range may be indicative of equipment errors [31].

16S rRNA sequencing found over 1,000 bacterial species present within the Newtown Creek microbiome. Benthic bacteria, pathogenic bacteria, hydrocarbonoclastic bacteria, and bacteria indicative of proximity to the Newtown Creek Wastewater Treatment Plant were all identified by this study [25]. Additionally, the bacterium genus *Pseudomonas* was observed in the local Hudson River, with its presence attributed in part to pollution from Newtown Creek Wastewater Treatment Plant [32]. Of the many microorganisms identified, I noted *Yersenia*, *Escherichia coli*, *Rhizobium*, and *Pseudomonas* for their potential for microbial interactions

Literature Review Findings

Cupriavidus metallidurans

Cupriavidus metallidurans is a highly resilient bacterium capable of growing in the presence of many environmental stressors including high heavy metal concentrations, a lack of nutrients, and extreme temperature and pH fluctuations. This microorganism has been isolated in locations ranging from South American copper mines to industrial sites with high abundance and regular replenishment of toxic materials [10]. Due to these abilities, *C. metallidurans* is considered an excellent bioremediation agent and has had a large amount of success in previous cleanup efforts.

Cupriavidus metallidurans is metabolically diverse and capable of growth in a variety of conditions. This microorganism is capable of both aerobic and anaerobic growth and can utilize a wide variety of nutrients [33]. When growing chemolithotrophically, inorganic materials are utilized and carbon dioxide is reduced to synthesize organic molecules [33].

Chemolithoautotrophic growth allows this microorganism to use hydrogen produced from other bacteria and carbon dioxide from the air, providing a greatly beneficial ability to grow in nutrient-poor environments as a biofilm or within soil [11]. When carbon sources are available, *C. metallidurans* can use citrate and d-ribose, but is unable to use glucose, galactose, lactose, or maltose [11]. Additionally, this microorganism can utilize the hydrocarbons benzene, toluene, and phenol as energy sources [33]. Under experimental conditions, growth for this microorganism was optimal at 37 °C, a pH range from 3-11 was tolerated, and a salt tolerance up to 1% NaCl was observed. *Cupriavidus metallidurans* growth did not occur at the next highest salinity concentration of 3%, and 2% salinity was not tested [11].

Cupriavidus metallidurans functions as a bioremediation agent for heavy metals via bioprecipitation, the solubilization and subsequent adsorption of metals, or adsorption to outer membrane proteins [10]. Dead *C. metallidurans* have a larger biosorption capacity than living cells [6]. For living cells maintained in a pH of 7.2, zinc, lead, copper, and cadmium concentrations were all considerably decreased within the course of 20 hours (Table 5) [10]. This microorganism was also found to remove nickel at a 10% reduction [11]. However, this percentage reduction for nickel is much lower than other metals and is expected to not be preferred over other metals in higher concentrations.

Table 5: Metal Reduction attributed to *Cupriavidus metallidurans* (adapted from Diels et al [10])

Heavy Metal	Initial Amount (mg/kg)	Final Amount (mg/kg)	Total Reduction (mg/kg)	Percent Reduction
Zinc	500	63	437	87
Lead	384	47	337	88
Copper	66	4	62	94
Cadmium	5.0	0.5	4.5	90

Saccharomyces cerevisiae

Saccharomyces cerevisiae sequesters dissolved metal ions from complex solutions with high speed and efficiency [4]. This yeast has been well-studied in many different forms including living/dead cells as well as raw material/pre-treated cells [4]. The process of biosorption per unit biomass can be improved by pre-treatment of cells using a variety of methods. The treatment of *S. cerevisiae* with the organic acids tetrahydrofuran, acetone, acetonitrile, dimethylsulfoxide, and ethanol all increase the overall amount of metal uptake [5], and heat treated *S. cerevisiae* was found to have an increased metal-binding ability for cadmium, lead, and copper biosorption vs. caustic treated and ground biomass [34]. These processes break down the cell membrane which offers a larger surface area containing additional accessible binding sites. As heavy metal toxicity and other challenging environmental variables may limit the use of living cells, biosorption is an excellent option in these instances [36]. This microorganism can be cultured easily and inexpensively, or biomass from food and beverage production may be used as *S. cerevisiae* is best known as a baker's yeast. Due to this supply from the food and beverage industries, large quantities of *S. cerevisiae* are easily and cheaply accessible [4]. Furthermore, strains of *S. cerevisiae* used by the brewing industry generally settle into multicellular groupings called flocs. Although concentrations of uptake were not available, these flocculent yeast cells were found to have stronger affinity for copper removal than other

non-flocculent cells. Additionally, flocs that have accumulated heavy metals can be removed from the environment and these metals can be recycled for future uses [13]. Heavy metal biosorption by *S. cerevisiae* can reduce several heavy metal concentrations from the environment (Table 6).

Table 6: Heavy Metal Biosorption Attributed to *Saccharomyces cerevisiae* [4]

Heavy Metal	Initial Amount (mg/L)	Final Amount (mg/L)	Total Reduction (mg/L)	Percent Reduction
Lead	260	26.3	233.7	89.9
Silver	118	20.3	97.7	82.8
Arsenic	1.5	1.4	0.14	90.5

Saccharomyces cerevisiae also adsorbs heavy metals via bioaccumulation. Although bioaccumulation is more environmentally dependent and requires a greater amount of time, there is also a large benefit to the implementation of this process. Living *S. cerevisiae* can remove heavy metals from the environment within a pH range of 5 and 9, with optimal pH values for bioremediation generally between 5 and 6. At pH 6, the preference of bioaccumulation was determined to be Hg>Pb>Zn>Cd>Ni>Cu [36]. Temperature changes from 5-40°C were found to have a minimal effect on heavy metal accumulation [4] and in 2% salinity, neither *S. cerevisiae* growth nor morphology was significantly impacted [37]. *Saccharomyces cerevisiae* is capable of both aerobic and anaerobic growth, and functions well at temperatures ranging from room temperature (~20°C) to 30°C [12]. Additionally, wild type *S. cerevisiae* strains were found to be capable of adapting to high levels of copper by introducing increasingly greater copper concentrations at the mid-log phase of growth. Using this method, *S. cerevisiae* was able to grow in concentrations up to 400 mg/L of copper [35]. Based on these abilities, *S. cerevisiae* is expected to grow well in challenging conditions.

This microorganism has been found to remove large quantities of heavy metals, with reduction percentages as high as 99.6% at an initial concentration of 570 mg/L when working with the microbial partner *Pseudomonas* [36]. Although initial concentrations were not available in all experiments, *S. cerevisiae* was able to remove a certain amount of a given heavy metal (measured in mg) per gram of *S. cerevisiae* biomass. This is noted as mg of heavy metal collected by *S. cerevisiae* per gram of microorganism introduced as the final amount of heavy metal sequestered (Table 7).

Table 7: Metal bioaccumulation attributed to *Saccharomyces cerevisiae* (adapted from Al-Saraj et al [36])

Heavy Metal	Amount Bioaccumulated (mg/g)
Mercury	56.97
Lead	48.89
Zinc	18.96
Cadmium	17.54
Nickel	7.98
Copper	6.35

Rhodopseudomonas palustris

Rhodopseudomonas palustris has been found to be highly effective in the treatment of a variety of heavy metals at high concentrations. This microorganism lives in many different environments such as lakes, oceans, rivers, and soils [38]. This ability to live in a wide variety of locations is based upon the large metabolic flexibility of *R. palustris*. Normally, these bacteria are photoheterotrophic, but is also capable of photoautotrophic, chemoorganotrophic, photolithotrophic, and chemoautotrophic growth [16]. This ability of *R. palustris* to use many different energy sources provides not only incredible versatility but can also be adapted to an almost unlimited amount of potential applications. This microorganism can mineralize heavy metals into end-products or the metabolic intermediaries to be primary substrates for cell growth

[1]. Based on these abilities, *R. palustris* is an extremely useful and promising microbe for bioremediation purposes. This microorganism can tolerate conditions where very low nutrition is available, as well as in a large range of pH values, temperatures, light, and oxygen levels [16]. *R. palustris* growth was negatively impacted at a 2% NaCl concentration, but the degree of inhibition was somewhat low. Pinoargote found that the initial growth phase lasted 24 hours in a control group vs. 48 hours in 2% NaCl. Additionally, the control group reached stationary phase after 24 hours with a population of $9.05 \pm 0.22 \log_{10} \text{CFU ml}^{-1}$ while the culture grown in 2% NaCl did not reach stationary phase during the 120 hour experimental time frame and reached a lower population of $7.23 \pm 0.22 \log_{10} \text{CFU ml}^{-1}$ [37]. Therefore, salinity does affect *R. palustris* growth, but the microorganism is still able to grow within a high salinity environment. Experimental conditions were completed in anoxic conditions at a temperature of 30 °C, a pH of 8, and with high light levels [7].

Rhodopseudomonas palustris was able to lower nickel, zinc, and copper concentrations during bioremediation [16]. Additionally, at a concentration of 1 mmol/L, selenite was eliminated from a solution within 5 days' time [39]. Lastly, *R. palustris* was able to adsorb high concentrations of lead, copper, cadmium, and zinc and irreversibly incorporate these metals into *R. palustris* at a rate of 5-15% when pH value in solution was dropped as low as 2 [14]. Based on this, *R. palustris* has a great deal of potential as a bioremediation agent (Table 8).

Table 8: Metal Reduction Attributed to *R. palustris*: adapted from Pokrovsky et al [14]

Heavy Metal	pH	Percent Reduction
Lead	6.5	100
Copper	6.5	100
Cadmium	8	~65
Zinc	8	~65

Indigenous Microbiome and Microbial Interactions

Microbial species are linked together via many complex interactions that influence the structure and function of the overall community. These interactions between organisms are powerful and may provide positive, neutral, or negative influence on one another [41]. Once introduced, bioremediation agents will interact with the indigenous biome in many ways that affect their ability to become established within Newtown Creek

Microorganisms may engage in cross-feeding between species, a mechanism that may assist in the establishment within an ecosystem. During this process, microorganisms reciprocally exchange metabolites between one another, a process that facilitates a stable co-existence and coupled species growth [41]. *R. palustris* and *E. coli* have been found to obligately cross-feed carbon in the form of organic acids and nitrogen in the form of ammonium between one another. In the environment, *E. coli* consumes glucose while *R. palustris* metabolizes nitrogen. Therefore, neither organism will compete for this primary nutrition source of the other [41] (Figure 8).

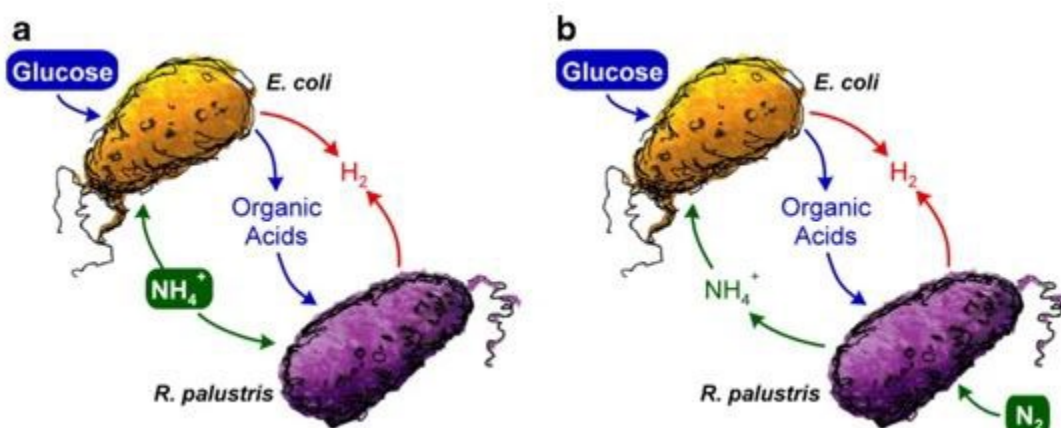


Figure 8: *R. palustris* and *E. coli* cross-feeding [41]

When working in combination, *S. cerevisiae* and *Pseudomonas* possess excellent rates of heavy metal biosorption, and have been effective in cadmium, lead, chromium, nickel, copper, and zinc bioremediation efforts [36]. Additionally, antimony removal via bioremediation has not been well studied, but *Pseudomonas* has been found to oxidize antimony, although specifics on experimental conditions and concentrations of antimony removed were not available [42]. *Pseudomonas* sp. was also found to bioremediate zinc at an initial concentration of 1 mg/L with a sorption efficiency of 49.8% [3].

The probiotic microbes *R. palustris* and *S. cerevisiae* have been found to reduce instances of disease and increase the growth of aquatic life such as fish and crustaceans [37]. *Saccharomyces cerevisiae* may also interact with the local aquatic life to reduce pathogenic effects on the fish population. *S. cerevisiae* var *boulardii* has been found effective in the reduction of *Yersenia* infection in rainbow trout [43]. All of these interactions would provide some form of benefit to the greater ecosystem.

However, microbial interactions can be challenging to predict and may change along with environmental shifts. *Saccharomyces cerevisiae* and *Rhizobium* are not known to have a previously established relationship but were cultured together in a laboratory to determine the interactions between the two microorganisms. Early in the experiment, the yeast improved bacterial growth vs. the yield of *Rhizobium* grown in pure culture while *S. cerevisiae* growth was unaffected. However, a day later, *S. cerevisiae* began to excrete a metabolite that inhibited bacterial growth [41]. Therefore, although specific interactions are challenging to predict, *S. cerevisiae* demonstrates it can influence the larger microbial community.

Introduction of Amendments and Review of Genetically Engineered Organisms

To further benefit introduced microorganisms and stimulate metabolic processes, the introduction of amendments is expected to provide an added benefit [15]. The amendments of choice vary with the nutritional needs of each microorganism. For *S. cerevisiae*, the addition of glucose will enhance growth and benefit metal biosorption. *Saccharomyces cerevisiae* treated with glucose increased the heavy metal removal rate of cadmium, chromium, copper, zinc, and nickel [12]. To optimize biomass yield of *R. palustris*, the agro-industrial by-products corn steep liquor and molasses were identified as amendments able to replace carbon and nitrogen sources, respectively. Using a corn steep liquor concentration of 39.41 mL/L and molasses at 32.35 g/L at a temperature of 39.7 °C, a pH of 7, and a dissolved oxygen level of 30%, biomass yield increased by almost 8 times as much than without the incorporation of amendments [22]. *Cupriavidus metallidurans* can utilize citrate and d-ribose for growth [11]. Therefore, the addition of these energy sources is expected to provide an added benefit to this microorganism. The use of these amendments provides an excellent opportunity to increase the growth of a microorganism and provide an advantage in its establishment.

Genetic manipulation of microorganisms may also provide an added benefit to bioremediation efforts that would not normally be available from an individual microbe. GEMs provide a great deal of potential for bioremediation purposes and are an excellent tool for the targeting of a specific heavy metal.

Nickel has been found to be especially challenging to remove from the environment, as many microorganisms have low nickel binding capabilities. This is observed in *E. coli* strain SE5000, which normally has an uptake capacity of 1.62 mg/g of Ni. Once genetically engineered, a binding capacity of 7.14 mg/g Ni was found at the optimum pH of 8.6 [44].

However, nickel biosorption was severely reduced in the presence of mercury, chromium, and copper at high concentrations. Mercury and chromium provided an especially large challenge, as nickel uptake decreased up to 85% at a concentration of 10 mg/L of these other metals [44]. In another experiment, the protein ArsR, a high affinity arsenic-sensing protein that regulates arsenic metabolism was overexpressed in *E. coli* leading to increased arsenic bioaccumulation, and 98% removal of arsenite from water at an original concentration of 50 ppb. While cadmium and zinc were also present, these metals did not appear to negatively impact the targeted removal of arsenic [45]. Therefore, this GEM demonstrates an extraordinary selectivity and does not appear to be affected by the presence of other heavy metals. *S. cerevisiae* strain NY4743 was integrated with recombinant genes to increase copper bioremediation potential and was able to sequester copper when incubated at 30 °C at an initial concentration of 330 mg/L [34]. Although the heavy metal concentrations found at Newtown Creek may require dilution before bioremediation could be initiated due to high heavy metal concentrations, bioremediation appears to be feasible by using GEMs.

Although GEMs can provide a great deal of benefit, there are also challenges associated with their use. Two major concerns of introducing GEMs to the environment are the occurrence of horizontal gene transfer with indigenous microorganisms, as well as the possibility of hazardous side or end products being created that may potentially harm the ecosystem [46]. Due to these concerns, the approval of GEM use may be withheld by governing regulatory bodies. The use of GEMs is also expected to add to the overall expense of a remediation effort. Although microbial bioremediation is generally lower in cost than traditional methods, the use of GEMs may reduce or eliminate this benefit. Before approving the use of GEMs, regulators may require expensive and time-consuming risk assessment studies which are generally considered not to be

worth the effort unless a large reward is available [46]. Furthermore, a niche for GEMs must exist within the ecosystem for a microorganism to establish itself within for as long as a pollutant of interest is present. If this is not available or the targeted pollutant is only a minor part of the environment, repeated inoculations with GEMs will further increase the expense of a bioremediation effort.

As a potential solution to some of these challenges, bioremediation may also take place inside a bioreactor. Bioreactors are tanks where contaminated materials are mixed with microorganisms and amendments for ex-situ treatment [15]. As these tanks are separated from the environment, the potential for horizontal gene transfer and toxic side and end-product development is contained. Bioreactors also allow further control over the artificial ecosystem in which bioremediation takes place [15]. Due to this, bioremediation conditions may be optimized to remove greater quantities of pollutants that would not be possible in natural conditions.

DISCUSSION

Site Evaluation

Heavy metal content in Newtown Creek surface sediment was much higher than concentrations found in surface water, and not all heavy metals observed in sediment seemed to be at levels hazardous in the surface water. This is expected as these pollutants were first introduced many years ago and heavy metals generally accumulate in surface sediment as retention time within a system increases [20]. Interestingly, only cadmium, copper, and mercury were identified as heavy metals with hazard concentrations above 1.0 in surface water while many heavy metals with hazard quotients above 1.0 were identified within the sediment.

Variations in surface water vs. surface sediment heavy metal levels may partially due to the higher surface sediment hazard quotients. Copper and cadmium had the first and second highest hazard quotients in sediment (100 and 39 respectively), while the hazard quotient for mercury in surface sediment (17) was tied for fourth. Lead and nickel also had high hazard quotients in surface sediment (18 and 17 respectively), but these metals were not observed in surface water at dangerous levels. Heavy metal variations between these two layers may also be due to differences in mobility due to heavy metal species [20]. Bondarenko found that soil spiked with zinc then introduced in water to create a soil-water suspension remained at a very low rate of bioavailability (0.31-0.61% of total value). Conversely, mercury and cadmium were found to be highly extractable from soil and able to easily become bioavailable. Cadmium bioavailability ranged from 2.6-5.1% of total heavy metal present, while mercury ranged from 2.6-38.9% of total metal present [21]. Therefore, the heavy metals found in sediment at Newtown Creek are not fully bioavailable to microorganisms and other wildlife present. Additionally, bioavailability is largely dependent upon heavy metal, and this may impact lead and nickel mobility.

Regarding local animal life, threatening heavy metal concentrations were observed in animal tissue for all metals identified as hazardous in both water and sediment, although different animals were threatened by different heavy metals. This may be attributed to varied sensitivity levels, environmental exposure, or from the bioaccumulation of heavy metals through the food chain [3]. However, based upon this finding, both surface water and surface sediment heavy metal accumulation seem to negatively impact local wildlife.

While heavy metals accumulate within surface sediment and become immobile for a given time, they can be released back into the surrounding area by changes in environmental conditions [20]. Environmental physicochemical conditions influence the extent of this immobilization. At Newtown Creek, pH generally remains between 7.0 and 7.5, which is not expected to greatly influence heavy metal mobility. Mobility is considered highest at low pH values [20], while Newtown Creek generally remains near a neutral pH. *Cupriavidus metallidurans* can tolerate a pH range from 3-11 [11], *S. cerevisiae* can tolerate a pH range of 5-9 with optimal pH values for bioremediation between 5 and 6 [36], and *R. palustris* is capable of growth within a large range of pH values, but has been observed as a highly effective bioremediation agent at pH 8 [2]. Therefore, the pH values observed are considered hospitable to all potential bioremediation agents in this study

The bottom water temperature of Newtown Creek was between 16-23°C, which is suitable to microbial growth and successful heavy metal bioremediation. *Saccharomyces cerevisiae* functions best at temperatures ranging from room temperature (~20°C) to 30°C, and temperature changes from 5-40°C were found to have a minimal effect on heavy metal accumulation [4]. *Rhodopseudomonas palustris* experimental bioremediation was completed at 30°C [7], and *C. metallidurans* growth was optimal at 37°C [11]. While Newtown Creek

temperatures are lower than those optimal for the introduced microorganisms, they should be suitable for microbial bioremediation to occur.

Redox potential at Newtown Creek varied over a large range, and highly influences heavy metal mobility [20]. Therefore, as heavy metals become more mobile, environmental conditions are expected to also become increasingly beneficial to microorganisms that require electron transfers [15]. Optimally, as heavy metals become additionally mobile in the environment, this will stimulate the growth of the introduced microbial consortia to function at a higher capacity. Additionally, *C. metallidurans* was found to bio-precipitate heavy metals to reduce their toxicity [10], so the presence of high heavy metal concentrations may assist in microbial colonization during times of decreased redox potential.

Newtown Creek normally ranges from 2.1-2.4% salinity, and this is classified as highly saline water [18] which is expected to maintain a high degree of heavy metal mobility [20]. The effects on microbial growth and capacity for bioremediation in a highly saline environment were varied for the microorganisms investigated. *Saccharomyces cerevisiae* growth was unaffected at a salt concentration of 2% [37], while *R. palustris* and *C. metallidurans* appear to be affected by this high degree of salinity. *Rhodopseudomonas palustris* growth and total population size was reduced at 2% salinity [37], but this microorganism was still able to grow and reproduce at this rate. *Cupriavidus metallidurans* tolerated a concentration of 1% NaCl well but was unable to grow at the next highest experimental concentration of 3% NaCl [11]. Based upon this, it is possible the bioremediation ability of the selected microorganisms may potentially be somewhat impacted by salinity at Newtown Creek. However, *C. metallidurans* can grow within a biofilm [11] and this may provide protection from high salinity environments. If salinity does present a

serious problem, in-situ bioremediation efforts are expected to still benefit from the use of non-living biomass which does not depend on cellular metabolism [4].

The concentrations of total phosphorus, ammonium, and nitrate/nitrite present at Newtown Creek were all elevated in comparison to natural background levels. Median total phosphorus was 0.22 mg/L while background levels are <0.03 mg/L [28], the median ammonium concentration was 0.46 mg/L while expected background levels are <0.2 mg/L [29], and the median nitrate/nitrite level was 0.22 mg/L while levels <0.2 are considered background levels [30]. Elevated nutrient levels may be attributed to the Newtown Creek Wastewater Treatment Plants continued release of these materials into the waterway.

Light transparency and oxygen saturation at the bottom the Creek were also evaluated to determine effects on microorganism growth (Figure 7). Appropriate light and oxygen saturation levels are highly influential in microbial photosynthetic growth [2]. Median light transparency was 67.4%, while median oxygen saturation was 44.5%. This indicates that both light and oxygen are available along the bottom of this waterway that can be utilized. *Saccharomyces cerevisiae*, *C. metallidurans*, and *R. palustris* are all capable of both aerobic and anaerobic growth [12], [34], [16] and therefore bioremediation efforts should not be impacted by these variables.

Heavy Metal Reduction

Cupriavidus metallidurans, *S. cerevisiae*, and *R. palustris* have all proven capable of reducing heavy metal concentrations in previous experiments, and due to the resilience of these microorganisms in challenging environments, appear to be capable of bioremediation at

Newtown Creek. My estimates of bioremediation potential at Newtown Creek are expected to be rough due to the large number of variables present at this location but should somewhat accurately reflect overall expectations. To maintain conservative estimates of bioremediation potential, only final amounts of experimental heavy metal reduction values were applied to Newtown Creek heavy metal concentrations. Percent reductions were also available for some previous studies, but these values may overestimate the overall reductions possible due to high initial heavy metal concentrations at Newtown Creek. Optimally, bioremediation efforts will exceed the projections from this study. Additionally, to estimate heavy metal concentrations both within sediment and surface water, I converted heavy metal concentrations between mg/kg for surface sediment and mg/L for surface water based on sediment density. This was completed to consider bioremediation potential within both sediment and mobilized heavy metals in surface water.

Heavy metal concentrations at Newtown Creek can be damaging to microorganisms, and therefore bioremediation agents capable of handling the most extreme conditions should be introduced first. The use of non-living *S. cerevisiae* and *C. metallidurans* in the most polluted areas of Newtown Creek can sequester heavy metals [6],[4] and these cells are capable of bioremediation in very toxic conditions [4]. While specific non-living *C. metallidurans* bioremediation potential still must be evaluated, *S. cerevisiae* appears to be capable of reducing or removing lead, silver, and arsenic this microorganism encounters from Newtown Creek (Table 9). *Saccharomyces cerevisiae* is also capable of biosorption of copper [13], but specific concentrations must be determined.

Table 9: Heavy metal reduction attributed to *Saccharomyces cerevisiae* biomass at Newtown Creek [4]

Heavy Metal	Initial Concentration (mg/L)	Removal Estimate (mg/L)	New Concentration (mg/L)
Lead	660	234	426
Silver	12	12	0
Arsenic	47	0.14	46.86

As well as bioremediation, *S. cerevisiae* biomass has been used in heavy metal recovery efforts [13], and therefore it may be possible to recover and recycle these heavy metals. To further reduce Newtown Creek heavy metal concentrations and reduce potential of toxicity to other microorganisms, several rounds of *S. cerevisiae* and *C. metallidurans* biomass could be introduced to further decrease heavy metal concentrations. As biosorption is not dependent upon metabolism [4], the main consideration for introducing cells in Newtown Creek would be when pH is low and redox potential is high, as these conditions provide the greatest heavy metal mobility [20]. Therefore, under these conditions, biomass will have the greatest opportunity to encounter and sequester heavy metals.

Living *C. metallidurans* is known to tolerate high heavy metal concentrations well and is effective in the treatment of a variety of heavy metals [10]. While salinity may pose a challenge to *C. metallidurans*, this microorganism can grow within a biofilm or within the soil [11] and therefore should be somewhat shielded from salinity. Additionally, growth within soil or a biofilm should benefit surface sediment heavy metal bioremediation, and therefore heavy metal concentrations are listed as mg/kg. *Cupriavidus metallidurans* is expected to reduce lead, zinc, cadmium, and copper levels [10] (Table 10).

Table 10: Heavy metal reduction attributed to *Cupriavidus metallidurans* at Newtown Creek [10]

Heavy Metal	Initial Concentration (mg/kg)	Removal Estimate (mg/kg)	New Concentration (mg/kg)
Lead	347	337	10
Zinc	1800	437	1400
Cadmium	27	4.5	25
Copper	1900	62	1800

Living *S. cerevisiae* can reduce or eliminate concentrations of mercury, lead, zinc, cadmium, nickel, and copper [36]. Experimental bioaccumulation measurements were recorded as mg of heavy metal accumulated per gram of *S. cerevisiae* [36], and Newtown Creek bioremediation estimates were based on one gram of *S. cerevisiae* introduced per liter. Additionally, *S. cerevisiae* was found to work in tandem with *Pseudomonas* to remove chromium at an efficiency rate of 99.6% [36]. Should *S. cerevisiae* be able to access these metals from surface sediment for bioremediation, chromium, mercury, and lead could potentially be totally sequestered from Newtown Creek (Table 11). Additionally, microbial activity of *C. metallidurans* within surface sediment may increase heavy metal mobility [21]. Therefore, microorganisms in Newtown Creek surface water must be available to sequester these metals should they become bio-available.

Table 11: Heavy metal reduction attributed to living *Saccharomyces cerevisiae* at Newtown Creek [36]

Heavy Metal	Initial Concentration (mg/L)	Removal Estimate (mg/L)	New Concentration (mg/L)
Chromium	270	270	0
Mercury	2.7	2.7	0
Lead	12.7	12.7	0
Zinc	1778	20	1758
Cadmium	32	18	14
Nickel	330	8	322
Copper	2286	6	2280

Rhodopseudomonas palustris is also expected to contribute to this effort via bioremediation of several heavy metals within the environment. Experimentally, *R. palustris* was able to remove 100% of selenite for a total reduction of 126.9 mg/L [39] and is therefore expected to be capable of removing bio-available selenium from Newtown Creek. This microorganism is also capable of lead, copper, and cadmium bioremediation, although removal estimates were not available for these heavy metals [14] (Table 12). *Rhodopseudomonas palustris* can grow in both water and soil [40], and therefore can greatly benefit bioremediation efforts by sequestering heavy metals in both parts of the ecosystem.

Table 12: Heavy Metal Reduction Attributed to *R. palustris* at Newtown Creek [14], [40]

Heavy Metal	Initial Concentration (mg/L)	Removal Estimate (mg/L)	New Concentration (mg/L)
Selenium	5.3	5.3	0
Cadmium	14	TBD	TBD
Copper	2280	TBD	TBD

Through the introduction of *S. cerevisiae*, *C. metallidurans*, and *R. palustris*, all bio-available selenium, lead, chromium, mercury, and silver may be fully sequestered from the environment. Additionally, arsenic, zinc, cadmium, nickel, and copper concentrations within Newtown Creek are expected to be reduced to various degrees. Cadmium and copper estimates do not include the addition of *R. palustris* because these values still must be determined, and therefore the impact of heavy metal bioremediation at Newtown Creek may be greater than given estimates (Table 13).

Table 13: Estimated heavy metal reduction attributed to *Saccharomyces cerevisiae*, *Rhodopseudomonas palustris*, and *Cupriavidus metallidurans* at Newtown Creek

Heavy Metal	Initial Concentration (mg/L)	New Concentration (mg/L)	Estimated Percent Removal:
Arsenic	47	46.86	0.3%
Cadmium	33	14	58%
Chromium	270	0	100%
Copper	2300	2280	1%
Lead	660	0	100%
Mercury	2.7	0	100%
Nickel	330	322	2%
Selenium	5.3	0	100%
Silver	12	0	100%
Zinc	2200	1760	20%

While microbial bioremediation using these three microorganisms will not be fully capable of removing heavy metals from the environment, there is still a great deal of potential from their introduction. As heavy metals are sequestered from the environment, this provides a benefit to local animal and human life and also provides additional opportunity for the further use of microbial bioremediation agents that may be more sensitive to the heavy metal concentrations originally found at Newtown Creek.

Potential of Amendment Introduction

Although it can be challenging to quantify the impact of amendments in bioremediation efforts, the addition of these materials is expected to benefit microbial metabolism and overall heavy metal bioremediation ability [15]. *Cupriavidus metallidurans* can use citrate and d-ribose as carbon sources [11], so the addition of these two nutrients should aid in the growth of this organism. *Rhodopseudomonas palustris* growth could be further fueled by the addition of molasses and corn steep liquor [22], which is expected to also benefit the growth of *E. coli* as these two microorganisms engage in cross feeding [41]. The introduction of glucose will fuel

both *S. cerevisiae* [12] and *E. coli* [41] as both microbes can utilize this amendment. Therefore, while the addition of these amendments may benefit *R. palustris*, they may also hinder *S. cerevisiae* growth as *E. coli* will be in competition for resources. Therefore, glucose should not be introduced until *S. cerevisiae* has been provided ample time to establish itself within the ecosystem. Overall interactions between introduced and indigenous microorganisms are difficult to predict, and both positive and negative interactions are expected. Due to this, microbial composition within Newtown Creek should be closely monitored during any further investigation to better determine interactions between microorganisms and the impact of these interactions on the overall bioremediation effort. While monitoring is required to determine how amendments are utilized, these nutrients provide an excellent opportunity to further assist bioremediation agent growth and establishment in otherwise challenging environments.

Benefits of GEM Utilization and Additional Microbial Interactions

Based on heavy metal reduction estimates, arsenic, copper, and nickel sequestration is expected to occur at very low rates. For especially challenging heavy metals such as these, the use of GEMs may provide an additional benefit to a bioremediation effort. While the use of genetically engineered microorganisms is not expected to be approved for use within Newtown Creek [46] the use of GEMs within a bioreactor could further benefit this study. The use of the *E. coli* strain SE5000, which selectively binds with nickel at a greatly improved uptake rate once engineered [44], is expected to be effective in the further reduction of nickel. Nickel bioremediation by *E. coli* SE5000 is severely reduced by mercury, chromium, and copper [44], but it is expected that mercury and chromium would be removed by previous bioremediation efforts. However, the high concentration of copper still present may negatively impact the

overall capability of *E. coli* SE5000. The introduction of *E. coli* with the ArsR protein overproduced to generate increased arsenic bioaccumulation could effectively be implemented to reduce arsenic concentrations at Newtown Creek [45]. Additionally, the genetically engineered *S. cerevisiae* strain BY4743 was found capable of copper bioremediation at an initial concentration as high as 330 mg/L [35]. By using these GEMs, arsenic, copper, and nickel concentrations may be further reduced within the environment.

Of the indigenous bacteria at Newtown Creek, *E. coli* and *Pseudomonas* both are expected to have the highest degree of interaction with their environment and potential bioremediation agents. These microorganisms are both considered potentially problematic due to their methylation of mercury, selenium, arsenic, and lead, which converts these metals into toxic gaseous forms [3]. The presence of methyl mercury in Newtown Creek serves as evidence that members of the indigenous microbiome have contributed to the production of this dangerous compound. While the current concentration is very low (0.00010 µg/L; hazard quotient 0.0025) [8], increased mercury toxicity within the environment requires attention to avoid additional development. However, *Pseudomonas* has been identified as a potential bioremediation for both antimony [42] and zinc [3], two heavy metals that have been challenging to sequester from the environment. Additionally, *Pseudomonas* and *S. cerevisiae* were found to have beneficial interactions with one another [36], and *R. palustris* and *E. coli* have been found to engage in cross feeding which will assist in the establishment of *R. palustris* within the microbial ecosystem [41]. These benefits and interactions must be closely monitored to ensure effectiveness in positive reactions and that negative interactions are not taking place.

CONCLUSION

At Newtown Creek, heavy metals in both surface water and surface sediment appear to negatively impact local wildlife and require bioremediation. The conditions found at Newtown Creek are challenging for microbial bioremediation, but the microorganisms *C. metallidurans*, *S. cerevisiae*, and *R. palustris* have all been proven effective in previous studies for heavy metal bioremediation in high metal concentrations and are expected to perform well in the environmental conditions present at Newtown Creek. However, high salinity levels and high initial heavy metal concentrations may negatively impact microbial bioremediation potential. Regardless of these challenges, non-living *C. metallidurans* and *S. cerevisiae* are expected to be beneficial to bioremediation efforts. Additionally, bioavailability of heavy metal concentrations found within surface sediment is varied based on physiochemical characteristics as well as heavy metal type. Therefore, living microorganisms are expected to tolerate the conditions found in Newtown Creek and participate in this bioremediation effort. The addition of amendments as well as ex-situ bioremediation using GEMs within a bioreactor is also able to assist in the treatment of challenging heavy metals. While this effort will not fully remove heavy metals from the environment, there is clearly a great deal of potential for the selected microorganisms to greatly assist in the bioremediation of Newtown Creek.

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