COMPARATIVE FUNCTIONAL METAGENOME ANALYSIS OF THE JUKSKEI RIVER SYSTEM IMPACTED BY THE URBANIZATION PHENOMENON

Report to the
Water Research Commission

by

T. Teffo, C. Ndwandwe, A. Abrahams
Department of Biotechnology and Food technology,
University of Johannesburg

WRC Report No. 3110/1/23
ISBN 978-0-6392-0568-7

December 2023

Obtainable from
Water Research Commission
Lynnwood Bridge Office Park, Bloukrans Building
4 Daventry Street
Lynnwood Manor
PRETORIA

orders@wrc.org.za or download from www.wrc.org.za

This is the final report of WRC project no. C2020/2021-00551.

EXECUTIVE SUMMARY

BACKGROUND

Urban rivers represent a unique ecosystem in which pollution occurs regularly, leading to significantly altered of chemical and biological characteristics of the surface water. However, the impact of urbanization on the diversity and structure of the river microbial community has not been well documented. The universal problem is the environmental pollution and most important pollutants are the heavy metals in aquatic network because of their toxicity and accumulation. Domestic, industrial and anthropogenic activities may broadly become the source of natural aquatic systems contamination. In extreme environments, some microorganisms adapt and grow as they use pollutants as a source of energy, synthesis of cell components and for microbial activities. These microorganisms have been shown to have unique genes, able to express enzymes to assist them to survive and thrive in unfavourable conditions. The effects produced by environmental stressors on microbial diversity can be multifaceted, leading to significant changes in community composition and diversity, high spatiotemporal variability, and alteration of community functionality.

PROJECT AIM AND OBJECTIVES

The aim of this study was to make a comprehensive description of the taxonomic and functional profile of the microbial community, using PICRUSt in the Jukskei river impacted by the urbanization phenomena with the potential of expressing enzymes that are of industrial relevance.

The following are the specific objectives of the project:

1. The study will be screening environmental factors such as pH, dissolved oxygen (DO) and electrical conductivity.
2. Identify and quantify heavy metals and toxic secondary metabolites.
3. Perform molecular docking studies of the heavy metals and toxic secondary metabolites on selected enzymes.
4. To confirm inhibitory potential of the heavy metals and toxic secondary metabolites on selected enzymes by conducting enzymatic assays.
5. Evaluating the microbial community profile and functional potential of these microorganisms through 16S amplicon sequencing.

PROJECT APPROACH AND METHODOLOGY

An initial literature review, which focused on the reported effects of urbanization on the extent of pollution in the Jukskei river was conducted. Subsequently, laboratory studies involving physicochemical analysis, metal and secondary metabolite analysis, enzyme assays and microbial community profiling was conducted. The portable HI9813-5 Portable pH/EC/TDS meter was used to determine all physical parameters on-site. Heavy metal and secondary metabolite analysis were conducted at the spectra laboratory at the University of Johannesburg, Auckland Park. Molegro Virtual Docker (v7.0.0) was used to perform all tasks related to docking simulations. Metal and phenol induced inhibition of Phosphofructokinase, Hexokinase and Cytochrome P450 were performed as reported in literature at various metal and phenol concentrations. Genomic DNA was isolated using a modified CTAB method for 16S amplicon sequencing to assess the microbial community profile and PICRUSt was used to establish predictive functional potential.

RESULTS AND DISCUSSION

Laboratory studies on the evaluation of physicochemical data

From the physicochemical data, most pH levels in the Jukskei river were mostly within the WHO water standard guidelines. The pH values did not differ significantly from those that have been reported in literature. The mean conductivity values (µS/cm) of the Jukskei river ranged from 352 to 688 µS/cm, with an average of 418 and 479 µS/cm upstream and downstream, respectively. The highest value, 688 µS/cm, was found downstream of the river. All TDS levels were within the WHO water quality standards. The highest recorded value was 349 ppm, which were found downstream of the river.

Laboratory studies on the quantity and quality of metals and secondary metabolites

All heavy metal concentrations were well above the WHO water quality standards. This came as no surprise, given the high population density of the informal township, as well as the poor infrastructure. Chlorophenol and 2.4 dichlorophenol were the only secondary metabolites that were detected above the standard limits. These are compounds that are frequently employed in the production of fungicides, insecticides, herbicides, and dyes. Given the agricultural activities north of the Jukskei river, it is unsurprising that they are found in high concentrations. These include farms such as the Northern- and the Monaghan Farms located close to the riverbanks of the Jukskei River.

Docking analysis and inhibitory potential of metals and secondary metabolites

Amongst all the docked metals against cytochrome p450, Cd had the highest affinity to the protein with a Molscore of -22.8949. Hexokinase however had the strongest affinity of Ni with a Molscore of -21.2528. Like Hexokinase, PFK had the highest affinity to Ni, with a Molscore -48.9701. Amongst the docked metals, Cu, Cd and Ni showed the highest affinities with all proteins studied. Docked phenols against cytochrome p450 showed the highest affinity to 4-

nitrophenol with Molscore -68.967. However, cytochrome p450 had the highest hydrogen bond with pentachlorophenol (-3.88099). The highest affinity with PFK was observed with 4-nitrophenol with Molscore -75.9286. Inhibitory potential of the metals and secondary metabolites demonstrate that there appears to be link between molescore and the inhibition activity of an inhibitor on hexokinase and phosphofructokinase. In addition, the phenolic compounds exert an even greater inhibitory action and at lower concentrations compared to the metals and heavy metals. This may be attributed to the chemical structure of phenolic compounds and their interaction with either the enzyme and/or the substrates.

Assessing the microbiome of the river and their predictive functional potential

Microbial profiling analysis of the data revealed that *Proteobacteria and Firmicutes* were present and the two most dominant phylum. Given the activities in and around the Jukskei River, faecal related bacteria were expected. Bacterial community composition upstream of the Jukskei River were most dominant in the genus *Pseudomonas, Flavobacterium* and *Prevotella.* Downstream of the Jukskei, Pseudomonas was also seen to be the most abundant genus whilst, *Bacillus* and *Massilla* were second and third most abundant genus. The inferred functional analysis of the bacterial communities revealed several differences in gene abundance. The predicted downstream pathways tend to be more focused towards maintenance of regulatory processes such as the citrate cycle and the glycolysis cycles which produce energy whilst on the other hand, upstream processes were more focused towards reactions to biotic stress within the environment.

CONCLUSION

The findings of this study suggest that, generally, the Jukskei River is distinguished by a complete lack of basic sanitary amenities and a stable water supply. Because of their proximity to the river, these informal settlements have convenient access to surface water for waste disposal. As a result, Alexandra Township has been identified as a significant contributor to poor water quality at the Jukskei River. The existence of bacterial community profiles along the river indicates the presence of faecal pollution as well as other pathogenic bacteria usually associated with the gastrointestinal system. The existence of such microorganisms indicates that home, agricultural, and industrial waste have been dumped into the river. Some of these bacteria's existence may also signify acquired genetic resistance to certain environmental toxins.

RECOMMENDATIONS FOR POLICY, IMPLEMENTATION AND FUTURE RESEARCH

The monitoring of various water bodies, water treatment and sanitation systems, and the enforcement of laws protecting consumers and the environment are all made possible through a comprehensive water quality monitoring approach. However, storage and transport may result in sample changes particularly in microbiological and DNA samples producing biased results. These limitations can be circumvented by portable technologies, enabling near-real-time water quality screening for quick decision-making. These technologies should also cater for screening of waterborne perils such as virus, protozoa and helminths in order to conduct an even more thorough and in-depth assessment. Once these systems are in place, through continuous monitoring efforts and the ability to forecast the spatial distribution and temporal dynamics of the microbiome and metadata, effective process management techniques can be established to protect the public's health. Finding anomalies and invasive infections more quickly depends on understanding the steady-state operating parameters for the system.

ACKNOWLEDGEMENTS

The project team wishes to thank the following people for their contributions to the project.

Reference Group	Affiliation
Dr Hope Serepa-Dlamini	University of Johannesburg
Prof. Langelihle Dlamini	University of Johannesburg
Dr Cornelius Ssemakalu	Vaal University of Technology
Dr Isaac Dennis Amoah	Durban University of Technology
Dr Andani Mulelu	University of Cape Town
Dr Jeremy Woodward	University of Cape Town
Dr Phumza Vuyokazi Zungu	Durban University of Technology
Dr Kulsum Kondiah	University of Johannesburg
Others	
Ms Cebile Ndwandwe	University of Johannesburg
Dr Eunice Ubomba-Jaswa	Water Research Commission

This page was intentionally left blank

CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACRONYMS & ABBREVIATIONS

CTAB	Cetyltrimethyl ammonium bromide
DNA	Deoxyribonucleic acid
DO	Dissolved oxygen
EC	Electrical conductivity
EDTA	Ethylenediaminetetraacetic acid
KEGG	Kyoto Encyclopaedia of Genes and Genomes
NaCl	Sodium Chloride
pH	Acidity (potential of hydrogen)
PICRUSt	Phylogenetic Investigation of Communities by Reconstruction of Unobserved States
QIIME	Quantitative Insights Into Microbial Ecology
rRNA	Ribosomal ribonucleic acid
STAMP	Statistical Analysis of Metagenomic Profiles
TDS	Total Dissolved Solids
Tris HCL	Tris hydrochloride

GLOSSARY

Anthropogenic activities. Activities that are increasing concentrations of atmospheric pollutants.

Biocatalysts. Often enzymes that have been isolated or enzymes that still reside inside living cells to perform chemical conversions of organic compounds.

Functional metagenomics. Involves isolating DNA from microbial communities to study the functions of encoded proteins.

Metagenomics. The study of genetic material recovered directly from environmental samples.

Microbiome. The collection of all the microorganisms that live in a given environment.

Molecular docking. A method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex.

PICRUSt. A bioinformatics software package designed to predict metagenome functional content from marker gene (e.g. 16S rRNA) surveys and full genomes.

Secondary metabolites. Are generally defined as small organic molecules produced by an organism that are not essential for their growth, development and reproduction.

STAMP. A software package for analysing metagenomic profiles (e.g. a taxonomic profile indicating the number of marker genes assigned to different taxonomic units or a functional profile indicating the number of sequences assigned to different biological subsystems or pathways) that promotes ‘best practices’ in choosing appropriate statistical techniques and reporting results. It encourages the use of effect sizes and confidence intervals in assessing biological importance.

CHAPTER 1: BACKGROUND

1.1 INTRODUCTION

A river is a naturally formed channel that drains water from upstream (source) to downstream (destination) (estuary) and they can be classified according to their morphology, ecology, and anthropogenic (Juliandar *et al.,* 2021). Rivers were created by runoff flowing into low elevations and coalescing to form rivers that sculpted and eroded pathways to the earth's lowest points. Huge lakes formed, which gradually joined to form our oceans, and these processes still occur today, but in more subdued forms (Siegel and Casteel, 2018). One such river which flows in a northwards direction of Johannesburg is the Jukskei River. The river passes through several residential areas of Johannesburg and joins the Crocodile River prior to entering the Hartbeespoort dam. The water quality has degraded over time because of the release of substances into subsurface groundwater to the point that the substances interfere with beneficial water use or the ecosystems' natural functioning (Nathanson, 2020). As a result, the river is characterized by extremely high soluble carbonate alkalinity, high pH, high salt and high concentrations of hydrogen sulphide containing nutrients of various types of compounds such as fats, sugars and proteins (Leketa and Abiye, 2019).

Microorganisms adapt, grow, and use these substances as a food source for energy, synthesis of cell components and to maintain life processes. The microorganisms in extreme environments such as polluted rivers have been shown to have unique enzymes and metabolic pathways that are able to assist them to survive and thrive in these conditions (Sogin *et al.,* 2006). Microorganisms represent the richest collection in chemistry and molecular diversity in nature, providing the basis for processes such as biogeochemical cycles and food chains, as well as maintaining dynamic relationships among themselves and with higher organisms (Hunter-Cevera, 1998). These microbial networks contribute fundamentally to various support constructions of the earth (Pinheiro *et al.,* 2019). Microbial enzymes are interesting biocatalysts that have been widely studied due to their unique activities (Brahmachari, 2017). Focus has shifted to the biocatalysts from these sources because of their dynamic and stable nature (Anbu *et al.,* 2017). Majority of microorganisms cannot develop and express enzymes under such harsh conditions but due to the selective pressure exerted by these streams, some microorganisms have evolved and adjusted by expressing genes under such conditions (Khan et al., 2001). Several studies have reported on the isolation of new bacterial strains and genes that they express from harsh conditions such as extreme pH, temperature, and salinity environments (Kumar *et al.,* 2020), but little work on the network of enzymes from microbial communities in streams have been reported.

The identification of these enzymes may be broadly utilized in the biopharmaceutical, medical diagnostics, food, paper, and plastic industries (Sanchez and Demain, 2012).

In this study, a metagenomics approach (16S amplicon sequencing) was used to reveal the diversity of microorganisms that exist in a river catchment as well as their predicted functional potential that permits them to express enzymes in order to survive in such environments. Metagenomics involves genomic examination that is applied to whole networks of microorganisms (Nema, 2019; Ngara and Zhang, 2018), bypassing the need to separate and culture individual microbial species. This method exposes the numerous capacities of the microbial networks that drives cycles and bolster the strength of its occupants (Nawy, 2013). Metagenomics will inconceivably improve our insight into microbial networks and prompt significant progressions in numerous areas, including human wellbeing, agribusiness, vitality creation, and ecological remediation (Jurkowski *et al.,* 2007). Furthermore, this study will be able to construct and supply details of enzyme-related networks that may be of novelty and their metabolic pathways associated with river water microorganisms on how they are able to survive such a severe environment. These networks may also have applications in various industrial processes (production of disinfectants, detergents, and treatment of waste), pharmaceutical industries (antibiotic drugs, disease treatment drugs) and agriculture (Gurung et al., 2013).

1.2. PROJECT AIMS

To make a comprehensive description of the taxonomic and functional profile of the microbial community in an urban lotic environment. The following are the main objectives of the project:

1. Screening of physico-chemical parameters.
2. Docking of metals and secondary metabolites.
3. Confirmation of docking results by screening the inhibitory potential of metals and secondary metabolites on selected enzymes.
4. To isolate, purify and sequence genomic DNA for taxonomic profiling.
5. Predictive functional profiling to expand understanding of the structure, function, and evolution of protein catalysis through identifying pathways.

1.3. SCOPE AND LIMITATIONS

The scope of the study focusses only on the impact of the urbanization phenomena of a densely populated area of the Jukskei river and not the entire stretch of the river basin. The project comprised both laboratory and field work to assess the impact of pollution on a freshwater system. Efforts were made to ensure that no harm to animals were done and for this reason, commercial enzymes were purchased. Limitation of the study included not being able to identify the oxidation state of metals.

CHAPTER 2: LITERATURE REVIEW

INTRODUCTION

The arrival of different natural toxins from various sources is an issue of concern in numerous countries. Rivers, dams and lakes serve as immediate environmental reservoirs for all possible organic pollutants. In South Africa, many such catchments appear to be a polluted source of major concern (Msadala, 2017). One such river which flows in a northwards direction of Johannesburg is the Jukskei River. The river passes through several residential areas of Johannesburg and joins the Crocodile River prior to entering the Hartbeespoort dam (Leketa and Abiye, 2019). The water quality of the Jukskei river especially from Bertrams all the way down to the Alexandra township is heavily polluted and poses a serious health concern around these residential areas (Wu *et al.,* 2017). Sewage wastewater from the Alexandra township continually spill into the river as well as alloy companies from the Johannesburg CBD that spill industrial effluent into the river (Matowanyika, 2010). As a result, the river is characterized by extremely high soluble carbonate alkalinity, high pH, high salt and high concentrations of hydrogen sulphide containing nutrients of various types of compound such as fats, sugars and proteins.

Microorganisms adapt, grow and use these substances as a food source for energy, synthesis of cell components and to maintain life processes. These microorganisms have been shown to have unique enzymes and metabolic pathways that are able to assist them to survive and thrive in these conditions (Sogin *et al.,* 2006). Microorganisms represent the richest collection in chemistry and molecular diversity in nature, providing the basis for processes such as biogeochemical cycles and food chains, as well as maintaining dynamic relationships among themselves and with higher organisms (Hunter-Cevera, 1998). These microbial networks contribute fundamentally to various support constructions of the Earth (Pinheiro *et al.,* 2019).

Microbial enzymes are interesting biocatalysts that have been widely studied due to their unique activities (Brahmachari, 2017). Focus has shifted to the biocatalysts from these sources because of their dynamic and stable nature (Anbu *et al.,* 2017). Majority of microorganisms cannot develop and produce enzymes under such harsh conditions but due to the selective pressure exerted by these streams, some microorganisms have evolved and adjusted by expressing genes under such conditions (Khan *et al.,* 2001). Several studies have been reported on the isolation of new bacterial strains and genes that they express from harsh conditions such as extreme pH, temperature and salinity environments

(Krishnaveni *et al.,* 2018), but little work on the network of enzymes from microbial communities in streams have been reported. The identification of these enzymes may be broadly utilized in the biopharmaceutical, medical diagnostics, food, paper and plastic industries (Sanchez and Demain, 2012).

In this study, a metagenomics approach will be used to reveal the diversity of novel microorganisms that exist in river catchments and the functional potential of their genes – that is, the proteins/enzymes they express at a given point in time. Metagenomics involves genomic examination that is applied to whole networks of microorganisms (Nema, 2019; Ngara and Zhang, 2018), bypassing the need to separate and culture individual microbial species. This method will expose the numerous capacities of the microbial networks that drives cycles and bolster the strength of its occupants (Nawy, 2013). Metagenomics will inconceivably improve our insight into microbial networks and prompt significant progressions in numerous areas, including human wellbeing, agribusiness, vitality creation, and ecological remediation (Jurkowski *et al*., 2007). Furthermore, this study will be able to construct and supply details of enzymes that may be of novelty and their metabolic pathways associated with river water microorganisms on how they are able to survive such a severe environment. These enzymes may also have applications in various industrial processes (production of disinfectants, detergents and treatment of waste), pharmaceutical industries (antibiotic drugs, disease treatment drugs) and agriculture (Gurung *et al*., 2013).

2.1 Influences of water quality

Water quality is influenced by 2 major aspects, anthropogenic activities such as industrial, agricultural and domestic activities, as well as natural activities such climate change. Water quality requirements can either follow national and international guidelines but most countries have their own set of guidelines in addition to the WHO (World Health Organisation) international guidelines, as seen in Table 1. Generally, all guidelines evaluate the odour, pH turbidity, taste, colour, conductivity, and concentration of organic and inorganic compounds. These values differ for each country, based on their chemical use and pollution (Barbosa, 2015). South African water standards are governed by the South African National Standards (SANS). Some water quality criteria are developed for drinking-water supply, recreation, agriculture or as a habitat for biological communities. Additionally, criteria may be developed with regards to the functioning of aquatic ecosystems. Therefore, water quality criteria are different for different intended uses.

2.1.1 Anthropogenic impacts on water quality

Different anthropogenic activities such as industrial, agricultural and domestic activities produce different types of wastes which cause water pollution. Industrialization is one of the leading causes of environmental degradation such as soil and air pollution, which inevitably finds their way into water bodies. Inexorably, pollution is the price everyone pays for economic development (James *et al.,* 2019).

2.1.1.1 Industrial activities

Industrial activities such as mining and manufacturing have been at the forefront of water pollution problems worldwide. As industrial wastes from industries flow into water bodies, the repute of the eco-system including human, plant and animal health are compromised (Abdelfattah *et al.,* 2018). Industrial wastewater from various industries have high concentrations of heavy metals, organic pollutants, pesticides, petrochemicals, dioxins, phenolic components, etc. Water quality degradation induced by human pollution discharge exacerbates the water scarcity since contaminated water may have little or no useful value.

2.1.1.2 Heavy metals

Heavy metals are metallic elements mostly found in nature. They have higher densities than water. There is an unproven assumption that heaviness and toxicity are related. There are over 50 elements that are classified as heavy metals, of those 50, 17 of them are considered toxic (Seema *et al*., 2011). Metals such as copper (Cu), cobalt (Co), zinc (Zn), chromium (Cr), magnesium (Mg), iron (Fe), manganese (Mn), nickel (Ni), and selenium (Se) are essential nutrients that are required for various physiological and biochemical and functions in the body. However, these are only required in very minute quantities. Heavy metals are one of the highest inorganic pollutants. They have a negative effect on human beings, plants and aquatic organisms. Other toxic heavy metals that are of high importance to the human body are Arsenic (As), lead (Pd), cadmium (Cd), and mercury (Hg). Since heavy metals are none-biodegradable, it becomes a threat in environmental pollution and human health upon consumption. When heavy metal contamination occurs, biomagnifications (also known as biological magnification) takes place through the food chain. Translocation through plants and subsequent consumption by humans is the major pathway from soil to human exposure (Ebrahem *et al*., 2016). Studies in toxicology has revealed that excessive accumulation of heavy metals causes developmental retardation through the damage of the central nervous system. As these heavy metals enter the body, they bind to proteins, nucleic acids and disrupt cellular functions. They also cause immunological disorders in humans (autoimmunity kidney damage, endocrine disruption), cancer, and subsequently death (Qing *et al*., 2015).

Table 1: Water quality standards (WHO (2011)) for heavy metals mg/L

Metals	WHO
Arsenic	0.01
Cadmium	0.003
Chromium	0.05
Copper	2
Aluminium	100
Lead	0.01
Mercury	0.006
Selenium	0.04
Zinc	100

In different biological networks, heavy metals have been revealed to affect cellular organelle as well as cellular membranes, lysosomes, mitochondria, endoplasmic, nuclei, and some enzymes involved in repair, metabolism and detoxification (Selina *et al*., 2017). Toxic metals have been and continue to be a universal pollution problem. China and India are one of the countries hardest hit by this type of pollution, due to the high numbers of different industries (Wei and Yang, 2010). Because HMs are the most common contaminants in our food supply, especially vegetables, heavy metal contamination poses a threat to our ecosystem. Vegetables become contaminated by absorbing heavy metals from polluted air, soil, and water (Kacholi, 2018). The contamination of heavy metals in soil and water has many harmful consequences and is therefore of great concern to public health, agricultural development and environmental health (Hemson, 2016). These changes in the physical and hydrological dynamics of the habitat modify the structure of fish species and the trophic relationships of river ecosystems, contributing to a decline of their ecological health.

Aluminium

Aluminium (Al) is a macro-element that occurs naturally in the environment (Alloway, 1990). Aluminium is the most common metallic element in the Earth's outer crust. However, it is very insoluble in freshwater. Aluminium is formed through the mineral weathering of feldspars such as orthoclase, albit, micas, and bauxite and eventually ends up in clay minerals. Aluminium may be found in a variety of gemstones, including ruby and sapphire.

Due to aluminium's natural availability in soils, it exists in water as water-insoluble hydroxide. Solubility rises below pH 4.5. This can also happen at very high pH levels. Plants are poisonous to dissolved Al^{3+} ions. They damage roots and reduce phosphate uptake. This explains the link between acid rain and soil aluminium levels. The quantity of aluminium increases with increased nitrate deposition, but it decreases on big heather and agricultural surfaces. It rises on forest soils. Most aquatic species have not yet evolved to tolerate significant intracellular accumulations of Al (Allin and Wilson, 2000). Only a minor amount of Al is absorbed into the bodies of animals and humans.

Aluminium is mostly ingested through food and water. The most recent standards ranged from 50 to 200 µg/L (WHO, 2010). Aluminium particles have been linked to functional lung disorders. However, Al shortages have not been connected to any recognized illnesses. Aluminium chloride can erode the skin, irritate the eyes' mucous membranes, and induce sweating, shortness of breath, and coughing. Alum promotes blood coagulation.

Arsenic

Like most heavy metals, As occurs naturally in the environment. It is released naturally into through the discharge of geothermal water and volcanic eruptions. Arsenic is the 20th most abundant element on earth. It is mostly available in small concentrations of between 0.5 and 5000 µg/L (Sun *et al*., 2014). However, due to industrial processes such as burning of fossil fuels, mining and iron smelting, this has led to higher concentrations of As in water, air and the environment. Studies performed by Abbas *et al.* (2015) on the toxicity of As revealed that the presence of As in groundwater was affecting over 200 million people worldwide (Abbas, 2015). Countries such as China, Germany and Australia are amongst the most affected by As water contamination.

In China, the industrial contribution to high As levels was through coal burning and other industrial activities. Additionally, arsenic-containing pesticides has been one of the mechanisms in which As has leeched into water sources. This was the causative factor in the huge number of instances of liver-cirrhosis between 1940 and 1950s. This also led to lung cancers. In Germany, workers in a winery that used arsenic-containing pesticides were seen to have symptoms of chronic As poisoning. Approximately 25% had signs of vascular disorders (Butzengeiger, 1940). Australia is rich in minerals that contribute greatly towards arsenic contamination to the environment, also as a result of mining and pesticide usage. In Australia, survey was conducted in 1991 on groundwater in Victoria. Unavoidably, the information uncovered significant levels of As in surface and groundwater around gold mines (Smith *et al*., 2003).

Cadmium

Just like As, Cd is also a toxic element in small qualities, it also bio-collects in life forms and the environment. It has a long half-life in human beings, ranging between 10 to 33 years. Long term exposures to Cd have been proven to cause renal damage. Therefore, Cd is regarded as one of the important metal pollutants in most countries as well as international environmental organizations (Okiei, 2012). Known fatal effects of Cd associated with drinking polluted water include lower energy levels, as well as reduced or damaged central nervous system as well as experiencing energy level. They have additionally been believed to cause inconsistency in blood creation and gravely affecting crucial organs, for example, the liver and kidneys. (Sher Ali Khan *et al*., 2011).

Chromium

Chromium is classified as a transition metal. Cr (VI) pollution in the environment is caused by its various applications in the chemical industry, chrome plating, dye manufacture, wood preservation, alloy fabrication, leather tanning, and other anthropogenic activities (Zhitkovich, 2011; Brasili *et al*., 2020). Mining and metalworking, steel and metal alloy manufacture, paint manufacturing, wood and paper processing, dyeing, and increasing the Cr concentration in wastewater are examples of anthropogenic activities that largely contribute to high Cr concentrations in water (Jones *et al*., 2019). Furthermore, the ash fallout from the burning of coal or municipal waste for electricity generation, as well as the manufacture of second-generation fertilizers, contribute to the higher Cr (VI) concentration (Tumolo *et al*., 2020)

Copper

Copper is a heavy metal with a high electrical and thermal conductivity. It is also corrosive-resistant, but metal oxidizes slowly in the presence of oxygen. In comparison to other non-ferrous heavy metals such as arsenic, cadmium, zinc, and lead, it has an extremely high melting point (1083°C) and a very high boiling point of 2595°C. Biologically, copper has a high affinity for water, soil, and organic ligands that include nitrogen and sulphur. Oxygen level, pH, and alkalinity are all factors that influence copper precipitation.

At elevated concentrations, copper can be dangerous to human health, wildlife and microorganisms. A metallic taste in the mouth, abdominal pains, difficulty breathing, vomiting, male infertility, leg cramps or spasms, insomnia, diarrhoea, jaundice, severe headaches, allergies, hair loss, attention deficit disorder, asthma, candida, prostatitis and fibromyalgia are all symptoms of copper toxicity (Ashish eta al., 2013).

Lead

Mining and manufacturing processes involving Pd has widely contributed to the water pollution. Processing of lead-bearing ores involves physical separation. However, some of these ores may be chemically processed, such as cyadiantion (the process used to treat Pd-bearing gold deposits). During the manufacturing and processing stages, the refinery wastes may still have traces of metals, depositing them onto the environment, which then wash into rivers. After smelting and mining, Pd is distributed to other regions for further processing and manufacturing of products. This widespread distribution then leads to higher risks of Pd contamination in other areas far from the mining plants (Vignes, 2013).

Magnesium

After sodium, magnesium is the second most common cation in the ocean. Magnesium is found in several minerals, such as dolomite (calcium magnesium carbonate) and magnesite (magnesium carbonate; $MgCO_3$). Magnesium gets eroded by rocks and ends up in water sources. Magnesium serves numerous anthropogenic functions, and as a result, it may wind up in water in a variety of ways. Magnesium is added to plastics and other products by the chemical industry as a fire retardant or as a filler. It also enters the environment as a result of fertilizer application and livestock feed. Magnesium sulphate is used in beer breweries, and magnesium hydroxide is used in wastewater treatment facilities as a flocculent (Woonyen *et al*., 2018).

Manganese

Manganese is a transition metal that ranks seventh in abundance in the Earth's crust (Krauskopf & Bird, 1995). Mn, like other transition metals, exists in a variety of states in nature. The most common states are Mn (II), which is typically soluble, and Mn (III), which is more insoluble (IV). Manganese is released into soils through mineral weathering and enters runoff when circumstances enable the mobile Mn (II) state to develop.

Drinking manganese-rich water may impair brain development in babies and young children. Manganese is most easily absorbed in the body orally, according to Health Canada (Dorman *et al*., 2006). Manganese's health effects are determined by factors such as the route of exposure, the chemical form, the age of exposure, and an individual's nutritional state. Infants, the elderly, and those with liver illness are among those who are particularly susceptible to manganese (Yoon *et al*., 2011).

Nickel

Nickel is an essential nutrient for many species, but at high enough concentrations, it may be poisonous. Metallic nickel and certain nickel compounds are carcinogenic and teratogenic to animals. Nickel functions in the conversion of ammonia to urea. However, Ni can also be toxic. Nickel inhalation poses a greater risk than nickel ingested from water. This can result in lung cancer or nasal tumours. Nickel carcinogenesis is most likely induced by Ni substituting for Zn and Mg ions on DNA-polymerase. These observations were mostly made on Ni workers (Kusal *et al*., 2019).

Nickel tetra carbonyl, for example, is water insoluble yet poisonous and carcinogenic. Higher dosages of nickel generally cause vomiting, resulting in fast elimination from the body (Novelli *et al*., 2015).

Selenium

Mining, natural deposits, refineries, or agricultural run-off all contribute to high Se concentrations in water sources. Selenium is available in nature and is required by the body in minute quantities. Experiencing prolonged exposure to Se at levels above the recommended levels specified by the WHO (2017). Selenium, even at low levels, is an essential nutrient. People exposed to levels above the WHO guidelines may experience the following short-term health effects: fingernail and hair changes, damage to the peripheral nervous system and irritability (Morris *et al*., 2019).

Anthropogenic emissions from coal burning or other industrial operations can lead to toxic exposure of humans to selenium. In locations with high geology selenium concentrations and growing industrial and mining activity, increased selenium concentrations are expected to have widespread detrimental consequences on sensitive ecosystems and human health (Okonji *et al*., 2021).

Zinc

Zinc is abundantly dispersed throughout the natural environment and is also a vital trace element for aquatic creatures and humans (Sankhla *et al*., 2019). When the dosage of zinc reaches a specific quantity, it may produce harmful consequences to the organisms. EPA water quality criteria for zinc state that zinc concentrations in freshwater aquatic organisms should not exceed 120 g/L (short-term hazardous concentration) and 120 g/L (long-term hazardous concentration), respectively, and that the concentrations in drinking water should not exceed 7400 g/L and 26,000 g/L, respectively (edible aquatic organisms only) (US EPA, 2009). Evidently, aquatic creatures are far more sensitive than humans to zinc concentrations in water.

Zinc's aquatic toxicity has been the subject of extensive investigation. European and American researchers, for example, have concentrated on the harmful effects of pesticides on fish, shrimp, flea, hydroids and phytoplankton. Chinese scientists examined the harmful effects of zinc on common Cyprinid fishes and other species showing regional peculiarities of China. In most investigations, zinc toxicity was studied exclusively in single species, such as fish, benthic animals or phytoplankton (Wu *et al*., 2012).

2.1.1.2.1 Metal tolerant microorganisms

Microbes require some heavy metals for metabolic functions and redox reactions. However, microbes often the first biota to be affected by high levels of heavy metal contamination. Thus, microorganisms have developed a variety of methods to deal with high concentrations of heavy metals. Microbial communities are under to selection pressure when exposed to high concentrations of heavy metals, which results in populations that are less diverse than those found in a clean environment (Singh *et al*., 2011). Most bacteria have metal ion efflux outside the cell, and genes for tolerance mechanisms have been discovered on both chromosomes and plasmids (Hookoom *et al*., 2013).

Thus, microorganisms from heavy-metal contaminated environments acquire resistance to harmful metal ions through a variety of mechanisms, including membrane transport, trapping in extracellular capsules, biosorption on cell walls, and oxidation-reduction processes (Yao *et al*., 2012; Roohi *et al*., 2014).

Furthermore, some microorganisms have the ability to tolerate multiple environmental stresses, a phenomenon known as co-tolerance or cross-tolerance. Issazadeh *et al*. (2013) reviewed lead resistance in microorganisms and reported that lead-binding regulatory proteins regulate the expression of various Pb resistance mechanisms. Because Pb has no biological significance, it enters bacterial cells via the absorption routes for important metals such as Mn and Zn. It is hazardous even at low concentrations, and its toxicity is determined by its bioavailability, which is influenced by environmental variables such as clay minerals and organic molecules (Ojuederie *et al*., 2017).

2.1.1.3 Other pollutants

Phenolic compounds

Phenolic compounds are a type of refractory organic matter with chemical structures similar to alcohol. They have an aromatic ring with one or more hydroxyl functional groups. Their hydroxyl groups allow them to have intermolecular hydrogen bonding with other molecules (Magdalena, 2017).

Phenolic compounds are naturally occurring compounds in the environment through the decay of natural compounds. However, due to industrialization, phenols have been used as raw materials in pesticides, dyes and pharmaceutical products. They are toxic and bio-accumulate in the environment, therefore cause a threat to animal and human health (Wang *et al*., 2012; Wolff *et al*., 2015).

Phenolic compounds in water have a high proclivity to interact or react with other components of the aquatic environment such as organic compounds, as well as microorganisms. Bacteria have the ability to convert complex phenols into various intermediate forms under particular environmental and chemical conditions (Montgomery-Brown & Reinhard, 2003). Phenols react with inorganic compounds with the help of UV radiation. Sunlight causes a reaction in the aquatic environment with phenolic hydroxyl group. For example, in water. The interaction of hydroxyl radical with phenol in water results in the formation of 2-nitrophenol (Magdalena, 2017).

The US Environmental Protection Agency (EPA) identified 11 phenols as priority pollutants (EPA, 2014). There are some phenolic chemicals are known to disturb the endocrine system, such as 2,4,6-trichlorophenol (2,4,6-TCP), 2.4,5-TCP and pentachlorophenol (PCP) were listed as 2B pollutants by the World Health Organization in 2011 (WHO, 2011). Additionally, more recently, because of the environmental and human health consequences of phenols, the World Health Organization (WHO) has established a phenolic compound threshold of 1 mg/L in drinking water (WHO, 2017).

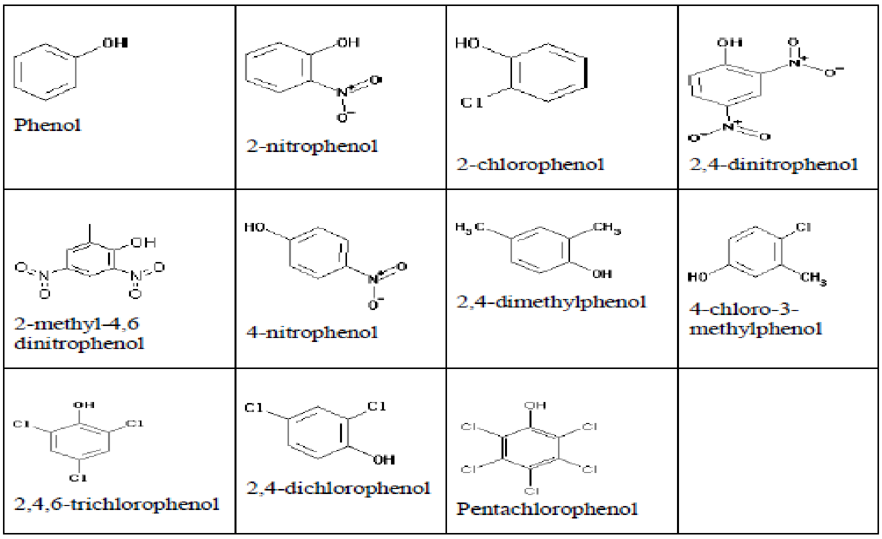

Figure 1: Structures of the eleven phenols specified in the U.S.EPA priority pollutants list (EPA, 2009)

2.1.1.4 Agricultural activities

Crop production

Pesticides are defined as substances used to control or kill insects, weeds, fungi and rodents (Stehle *et al*., 2019). They make significant contributions towards maintaining world food production. However, some pesticides are toxic and need to be managed carefully. The WHO classified pesticides according to their toxicity levels (Table 1). Due to their toxicity, these pesticides thus have health implications on humans and animals as well as environmental degradation. They are exposed to humans through ingestion, dermal, oral, respiratory and eye exposure. For example organochlorines (OC) such as dichlorodiphenyltrichloroethane (DDT). They have been widely used as pesticides between the 1950s and 1980s causing over 45,000 poisoning cases yearly between 1980 and the 1990s. However, it is believed that the number is understated because of a lack of statistical records at the time (Qiao *et al*., 2012).

Table 2: WHO Pesticide classification (WHO, 2019)

Classification	**Examples**	**Toxicity levels**	**LD_{50} (mg/kg body weight) Oral**
Class Ia	Parathion	Extremely hazardous	<5
Class Ib	Eldrin	Highly hazardous	5-50
Class II	DDT	Moderately hazardous	50-2000
Class III	Malathion	Slightly hazardous	>2000
Class IV	Carbetamide	Unlikely to be hazardous	≥5000

Kim *et al.* (2017) supported these previous findings through a review on the health effects of pesticides on humans. In their review, they looked into a study that was done in Crete, Greece, where high levels of dialkylphosphate metabolites (DAPs) were found in urine and hair samples of farmers responsible for the spraying of OP pesticides compared to the control group (Kokkinaki *et al*., 2014). Additionally, a relationship between the use of pesticides and the development of cancer has been reported in different studies, such as the Koutus (2015) study. The study was done with 57,310 pesticides in the USA. This study revealed that two imidazolinone herbicides (imazaquin and imazethapyr) were linked to colon and bladder cancer.

According to World Atlas, China is the biggest user of pesticides, with an average of 1,806 billion kilograms a year. In China, pesticide residues in foodstuffs were investigated from the year 2000 to 2009. It was discovered that in 16 provinces, 65 pesticides were found. This investigation also revealed that organophosphorus pesticides (OP pesticide), were the major products used. Organophosphorus chemicals are produced through the reaction of phosphoric acid and alcohol. The Chinese Government banned the use of OCs in the early 1980s, as well as other toxic Ops used in crop production due to their bioaccumulation in the environment and negative health effects on wildlife, environment and humans. The world has since gravitated towards less toxic OPs and pyrethroids (PYRs) because they have lower toxicity and they are biodegradable. However, more recent studies have found that the less toxic pesticides can still cause critical chronic diseases if excessively used (Wang *et al.,* 2012).

Concerns over their toxicity began in the early 2000s when an American company called 3M (the major manufacturer of these chemicals) decided to stop the manufacturing of related products. Many American companies have since stopped the production of these chemicals in 2015. Even though production was stopped, a study in Dallas, Texas on children revealed that PFC compound levels continued to increase from birth up until the age of 12. This demonstrates how children continue to be exposed to these chemicals in the environment, even years after manufacturing was banned. A recent study done by Manzano-Salgado (2019) in Spain on the prenatal exposure to perfluoroalkyl substances looked into the effects of PFC exposure and immune and respiratory health on subjects up to the age of 7. The study suggested that different PFASs affect the development of the immune and respiratory systems differently. Prenatal exposure to PFOA was found to be associated with the reduction of lung function in young children. Higher PFOA concentrations were linked to lower forced expiratory volume. Given the epidemiological studies as well as the bio-accumulative nature of PFOS, they have also been associated with immunosuppression in early life (Zeng *et al.,* 2019). Given the limited data available on the effects of PFCs on human health, more extensive studies need to be conducted in order to better understand their effects.

Livestock farming

The production of livestock accounts for approximately 70% of all agricultural land, making it one of the top three contributors to water quality degradation (Rojas-Downing *et al*., 2017). The continuous increase in population results in the increased demand for more livestock products globally, further increasing water pollution risks. This has led to more waste production, e.g. slurry, farmyard manure, silage effluents and poultry litter. The rearing of animals also produces a lot of waste. Animal waste contains nutrients such as Nitrogen which aquatic life use as sources of food (Michel *et al*., 2012). However, excess nutrients result in excessive algal blooms, which cover water bodies and cut off sunlight, increasing their oxidation requirements (for organic matter), leading to the reduction in Dissolved Oxygen (DO), posing a serious threat to aquatic life, a situation likely to pose a potential health risk to humans by dietary means (De Vries *et al*., 2013).

Livestock wastes such as manure and farm maintenance wastes contain antibiotics, hormones, organic and excess nutrients. Over-grazing accelerates the risks of soil erosion, enhancing easy flow of wastes into water bodies through rain, leading to high turbidity, trace elements and excess nutrients into water systems. This causes eutrophication when they get into water bodies, causing sunlight and oxygen depletion in the aquatic ecosystem (Wen *et al*., 2017). Livestock manure is widely used to enhance soil fertility and tilth. However, surface and groundwater pollution from manure occurs when manure applications are mismanaged.

2.1.1.5 Natural contributors to pollution

Water quality and even water ecosystems can be affected by geological activity and climate change, either directly or indirectly, via different biochemical processes.

Geology

Different types of rocks encompass different chemical properties. As the weathering of rocks occurs, different ions, metals and nutrients are released into the environment (Alloway, 2013). These chemicals are eventually washed into ground water sources through different channels such as rain and irrigation. Water quality in such instances may be poor, due to natural influences without any anthropogenic inputs. Physical properties of water quality such as alkalinity and hardness are also influenced by geology (Wimver, 2013). Studies have revealed that the waters that been responsible for the weathering of the gypsum rock had high concentrations of calcium and magnesium. In contrast, slow weathering rocks such as metamorphic rocks have very little influence on water quality (Selinus, 2013).

Climate change

The impact of climate change are increasing very fast in South Africa. More steps need to be taken to reduce greenhouse gases emissions. It is said that temperatures will rise by over 4°C in southern Africa interior by the year 2100, and by over 6°C over the western, central and northern parts of South Africa (Kaptwata *et al*., 2018).

Climate change affects water quality as well as water ecosystems. Water temperature increases with increasing atmospheric temperature. This happens through the increase of temperatures which affect different microbial biochemical reactions. Significant increases in water temperature have been shown to cause high growth rates of phytoplankton, epiphytes and macrophytes. Their fast growth results in the depletion of DO (with Oxygen levels below 2-3 mg/l), causing other beneficial microbes to suffocate and perish (Verweij *et al*., 2010).

Temperature is also significant due to its impact on water chemistry. At increasing temperatures, the rate of chemical reactions typically rises. Water at greater temperatures, particularly groundwater, may dissolve more minerals from the surrounding rock and so has a higher electrical conductivity. When a gas, such as oxygen, is dissolved in water, the reverse is true. A cold Coke, for example, has more bubbles than a warm one. Cold soda can hold more of the carbon dioxide bubbles dissolved in the liquid than warm soda, making it appear fizzier when consumed. Temperature has a significant impact on biological activity and growth. Through biochemical and physiological mechanisms, it regulates the types of species that may survive in rivers and lakes. Temperature ranges are favoured by fish, insects, zooplankton, phytoplankton, and other aquatic organisms (Xia *et al*., 2014).

Rain has been shown to play an important role in the exposure of aquatic biota to pollutants. The rainiest season in South Africa is in the summer. At this time, pollutant levels drop drastically due to dilution. However, pollutants are then suspended from the environment when flooding occurs, washing more pollutants into water bodies (Lasagna *et al*., 2013).

2.2 WATER QUALITY INDICATORS

Besides heavy metals, organic and inorganic pollutants, microbes have been used to qualify water bodies. The presence of certain toxins compromises microbial integrity, leading to the growth of pathogenic microbes and killing beneficial ones. Key microbial indicators of water pollution have been studied over the years, relating to different forms of pollution.

2.2.1 Microbial indicators of water quality

Microbes can be either beneficial or pathogenic to other organisms. The presence of harmful microbes poses a threat to water quality. Bacteria, fungi, virus and algae play a role in water safety. One of the most common tests performed to determine microbial quality is the turbidity test. Organisms like phytoplankton can contribute to turbidity in open water (Yasmin, 2015).

2.2.1.1 Bacterial factors in water quality

According to the WHO, there are three classes of bacterial identification methods used in water quality, namely: faecal indicators, general microbial indicators and index/ model organisms (WHO, 2001).

Bacterial coliforms

Previous studies regarding coliform bacteria has been focused on human health and disease. In sub-Saharan Africa and other low-income countries such as India, the limited or lack of sanitation systems and hygiene has resulted in open defecation, causing water bodies to be vulnerable to faecal contamination (Harris *et al*., 2017). Factors such as High population densities permit easy contraction and spread of waterborne diseases, such as informal settlements in urban areas as well as rural areas with polluted water. India is once again one such example, with approximately 700 million people in rural areas depending mostly on untreated groundwater (World Bank, 2017).

Bacterial coliforms are present in faeces and in warm blooded animals. The most common bacterial coliform used as an indicator for faecal contamination is *E .coli*. It is present in the gut and assists in the manufacturing of vitamin K. However, some strains of *E. coli* such as *E. coli* O157:H7 can be pathogenic. This strain causes intestinal infections (Campbell, 2011). It was discovered to be the most common cause of diarrheal disease. Since *E. coli* is a sub-group of faecal coliforms, their presence is indicative of domestic and animal waste. Like all other bacteria, the presence of faecal coliforms may be influenced by external factors such as season changes. Besides *E. coli,* other bacteria such as *Aeromonas hydrophila* (a none-coliform), faecal streptococci and *Clostridium perfringens* may also be used as indicators for faecal contamination. Accordingly, there is a need to explore South Africa's sanitation and hygiene practices and water quality at the difference sources (Kivanc *et al*., 2011).

Fungi as a water quality indicator

Fungi are ubiquitous, heterotrophic species (Gostinčar, 2011). They can be split into three classes based on the ability to colonize various environments: as mesophilic fungi, as generalists and as specialists. In drinking water, biofilms are an important habitat for fungi. Many factors, including temperature and nutrient concentration, influence their development. Water pH has been shown to play an important role in the presence of fungi, their growth and the processes of bioremediation. A positive association was observed between aquatic hyphomycetes growth and pH between 5 and 7 (Shama, 2013) and was recently verified in a deep groundwater analysis reporting the highest diversity at slightly lower pH in mixed fungal populations (Mawaz *et al.,* 2018). Acidic pH levels have beneficial effects on the binding properties of heavy metals on fungal cells.

Another form of fungal entry is through the skin. Symptoms have resulted from proximity to water-damaged buildings while showering or swimming in mycotoxin-contaminated water. Some fungi, including *Claviceps spp., Aspergillus spp., Penicillium* spp., *Fusariam* spp., and Mycotoxins such as patulin, aflatoxin and zearalenone are known to be made by these fungi. Mycotoxin concentrations in drinking water are known to be low because they are diluted. However, no records of mycotoxin-caused illnesses have been reported through ingestion of water (Paterson & Buddie, 2019).

Due to the variety of data that remain poorly understood because of the relative lack of literature on the subject of fungi in drinking water, research criteria include the need to be assessed in order to obtain the value at which fungi is a risk for drinking water. A better understanding of the risk value of ingestion as an exposure pathway for fungi in drinking water will ensure that preventative strategies are in place to avoid fungi-related health effects.

Viruses in water

Aquatic viral pathogens that are one of the major microbial concerns of the WHO (2011) are astrovirus, hepatitis A and E viruses, adenovirus, rotavirus, novovirus, caliciviruses, enteroviruses, and polioviruses. These viruses have the potential to be spread through water. While it has been proposed that other enveloped viruses, like influenza and coronaviruses, can be spread by drinking water, this has yet to be confirmed. Given the current global pandemic of Covid-19, more research on the corona virus' persistence in water is being studied. The current data available has shown that the corona virus has been found in sewage water and the data is currently being used for pandemic response and management (Michael-Kordatou *et al.,* 2020).

Enteric viruses in water cause a variety of diseases in humans, varying from asymptomatic infections to severe disease, leading to hospitalisation or death. These viruses are transmitted through faecal contamination. They are considered the greatest risk for contamination of groundwater sources because of their high infectivity, small size and persistence in the environment (Gall *et al.,* 2015).

Algae

Algae is also used as a biological indicator for water quality monitoring and protection. Algae has a rapid reproduction rate which makes them suitable candidates for water quality. As mentioned by Patrick (2010), algae is sensitive to pollutants as well as changes to its environment. Nutrient enrichment with nitrogen and phosphorus is the most common stress in lakes. When this happen, algae grows extremely fast and eventually changes the colour of water (Gökçe, 2016). There are many different species of algae. When the N; P ratio changes, most species do not survive instead there is a bloom of Cyanobacteria, causing health problems by producing toxins (Griffin, 2017).

2.3 PHYSICOCHEMICAL WATER QUALITY INDICATORS

Ground water quality depends on different chemical constituents and their concentration. Most of these are taken from the geological data of the area in question, Industrial Waste AND urban solid waste has been one of the leading sources of pollution of groundwater and surface water.

Electrical Conductivity and Total Dissolved Solids

Electrical Conductivity and Total Dissolved Solids are water quality standards used as indicators of salinity levels in water. TDS concentration is representative of inorganic salts and small amounts of organic matter in water, whilst EC is the measure of water's capacity to conduct electrical current (Sawyer *et al.,* 1994).

TDS and EC concentrations can be influenced by geological conditions, human activities such as domestic, industrial and agricultural waste (Rusydi *et al.,* 2015) .The WHO has standards that govern the levels of TDS and EC in water. However, they were last updated in 1996. According to the WHO, concentrations higher than greater than 1200 mg/litre are deemed unacceptable (WHO, 1996). The WHO has not to date reported on health effects associated with the ingestion of high concentrations of TDS in drinking-water. However, according to the United States Environmental Protection Agency (EPA) (2018), high levels of TDS are indicative of elevated levels of nitrates (which have been shown to cause accelerated heart rate, nausea, headaches, and abdominal cramps) and other contaminants (ATSDR, 2015).

Electrical Conductivity is a measurement of a liquid's ability to conduct an electric charge (Kumar et al., 2015). Water's EC is heavily influenced by dissolved ion concentrations, ionic strength, and temperature. The ionic process of a solution that allows it to transfer current is measured by EC. Because of the high quantities of dissolved ions such as chloride (Cl), magnesium (Mg), sodium (Na), sulfate (S), and calcium in saltwater, its conductivity is one million times greater than that of pure (deionized) water (Ca). Acid mine drainage can be caused by anthropogenic operations that expose minerals containing iron, sulphate, copper, cadmium, and other ions to air and water.

EC is the measure of liquid capacity to conduct an electric charge (Kumar *et al*., 2015). The EC of water is largely dependent on dissolved ion concentrations, ionic strength, and temperature. EC measures the ionic process of a solution that enables it to transmit current. For instance, seawater's conductivity is one million times higher than pure (deionized) water due to the large concentrations of dissolved ions in the seawater such as chloride (Cl), sulfate (S), sodium (Na), magnesium (Mg), and calcium (Ca). Anthropogenic activities resulting in acid mine drainage can add iron, sulphate, copper, cadmium and other ions if minerals containing them are exposed to air and water. According to WHO guidelines, the EC value should not exceed 500 µs/cm (WHO, 2011).

Temperature

Factors such as climate have an effect on thermal conditions that have an affect aquatic organisms. The solubility of oxygen decreases as the temperature of the water rises. This creates aquatic stress and may result in organism death. Some aquatic species, such as fish, require a certain temperature for spawning and the development of eggs and larvae. The eggs will not develop if this threshold is greater or lower than predicted (Adeogun *et al.,* 2016).

In water bodies, microbial biofilms are a hotspot for organic matter use and recycling. Their activity is largely affected by temperature conditions. As temperatures increase, respiration rates of bacteria and algae, bacteria, resulting in increasing the metabolism of organic carbon (Díaz *et al.,* 2011).

BOD/COD

Industrial activities such as food manufacturing have grown over the past decades. Their contributions to water pollution is through the accumulation of by-products. For example, In India, Uttar Pradesh (a state in northern India) is the one of the major manufacturers of sugar in the world. It has 119 sugar industries that produce approximately 1.1 million tons of sugar annually (Sunil & Arora, 2012). Besides the primary item, sugar, the plants likewise creates different by-products which present critical effects on the earth. Sugar factory effluent created in different advances discharge molasses, alcohols as significant poisons for the different waterways and lakes (Moses, 2010). During sugar creation, phosphoric corrosive and sulphur dioxide are utilized during the clarification of sugarcane juice. This is the fundamental driver of algal blossoms (because of overabundance supplements). This at that point prompts the increase in the BOD/COD ratio (Chemical Oxygen Demand/Biochemical Oxygen Demand), undesirable smell and metals as well as the reduction in dissolved oxygen (DO). The increased value of COD is an indication of high organic load of chemicals that are not biodegradable (Prambudy *et al.,* 2019).

pH

Possible Hydrogen (pH) in most fresh waters are neutral (pH of 6-8) (Feng *et al.,* 2017). Different factors affect the pH levels of rivers such as the weathering of rocks. The type of rocks and minerals in a catchment usually determines the pH of a river (Lamb, 1985). The pH is an important factor in metabolism. When pH levels are too high or too low, enzymes are inactive, interfering with metabolic processes which eventually kill the organism. Microorganisms grow best at neutral pH levels, these are called neutrophiles, whilst those that grow best in acidic conditions are called acidophiles). Human-induced acidification of rivers is normally the result of industrial effluents, mine drainage and acid precipitation (Hamid *et al.,* 2020).

2.4 IMPACTS OF WATER POLLUTION

Fish

The numerous sources of water contamination cause catastrophic consequences to marine life. Fish and other aquaculture are the most exposed to elevated levels of toxins. Fish protein is the most popular source of cheap protein in impoverished areas in South Africa. South Africa has 112 freshwater fish species of which 94 are indigenous and 18 are foreign species, according to Skelton (1993).

As previously stated, acute diseases are caused by pollutants such as heavy metals, which are toxic to aquatic creatures. Bioaccumulation of various toxin species, such as heavy metals, is a major concern for fisheries. When they are ingested, they are the initial link in the food chain. Heavily metal-polluted fish have a delayed embryonic development. (Biswas *et al.,* 2011). Toxin build-up, in addition to intrinsic variables, is heavily influenced by water parameters such as salinity, pH, water temperature, and hardness. Heavy metal poisoning in fish has been shown to induce loss of balance, increased opercula movement, and erratic vertical motions, ultimately leading to death. Furthermore, metals like Ca, Pd, Hg, and As cause significant harm to fish's renal and neurological systems, as well as gill damage. The harmful effects of heavy metal pollution in the marine environment, such as mercury, are directly connected to human toxicity after ingestion. (Maurya *et al.,* 2019).

Human beings

Organophosphate poisoning affects organs such as the brain, kidneys, liver, and ovaries. Germany developed these compounds as neurotoxins during World War I. They are cholinesterase inhibitors that influence neuromuscular transmission. They induce chemical and structural alterations in these organs, as well as growth retardation. In 2004, dogs were used in a research in Egypt. They subjected the dogs to various OP concentrations. The findings indicated that 7.5 mg/kg of atrazine (an herbicide) each day resulted in a reduction in food intake. Additionally, it also resulted in the increase of heart and liver weight (Sameeh, 2004). Reports that have been conducted in the last 30 years revealed that OP pesticides were responsible for the admission of millions of patients into hospitals, as a result of accidental poisoning as well as suicide cases. It was also discovered that an estimated that 25 million of agricultural workers in third world countries suffer from poisoning every year (Alavanja, 2009).

2.5 FISH LIVER PROTEINS AND THEIR FUNCTIONS

The zebra fish (*Danio rerio*) is an essential vertebrate model for the mutational study of genes influencing developmental processes. Studying the link between zebra fish genes and mutations with those of humans would need understanding the syntenic correspondence between the zebra fish and human genomes (Clemens *et al*., 2013). Furthermore, zebra fish have been discovered as a unique vertebrate model capable of high throughput drug screening and discovery, which cannot be done in other vertebrate models, and is particularly valuable for studying human sickness and associated therapies (Deveau *et al*., 2016; Liu *et al*., 2016; Williams and Hong, 2016).

It was George Streisinger and colleagues at the University of Oregon that pioneered zebra fish research in the 1960s. To embrace this new paradigm for laboratory environments, they had to provide the circumstances necessary to do so. They also had to develop new, basic methods for manipulating genetic material and imaging that were crucial to inspiring and educating a new generation of scientists. Zebrafish research efforts concentrated on understanding normal vertebrate embryonic development for the following almost 30 years (Tanguay *et al.*, 2018).

Glycolysis is the major oxidative breakdown process for glucose. In this breakdown, the route supplies substrates for energy generation via ATP synthesis as well as substrates for glycogenesis and lipogenesis storage pathways. These glycolytic metabolic activities, which are both catabolic and anabolic, change in various tissues based on the metabolic requirements of those tissues. Glycolysis is involved in glycogen synthesis in the liver and muscle, glycerol phosphate, lipogenesis in the adipose via the production of the triglyceride backbone, and carbon sparing in the skeletal muscle and heart, all of which rely on free fatty acid as fuel to various degrees (Wu *et al.*, 2005).

Hexokinase

Hexokinase an enzyme that is responsible for phosphorylating hexose, a six-carbon sugar, into hexose phosphate in the glycolytic pathway. Most tissues and organisms use glucose as the primary substrate for hexokinases, and glucose 6-phosphate as the primary product. Humans and other animals have also been shown to possess hexokinases. A basic ATP-binding site core is shared by all actin fold proteins, whereas varied sequences control substrate affinity and other characteristics. There can be several hexokinase isoforms in a single species. Hexokinase is present in all mammalian tissues and is regarded as a "housekeeping enzyme". When hexokinase binds to glucose, it experiences an induced-fit conformational shift, which eventually inhibits ATP hydrolysis. Physiological quantities of its immediate product, glucose-6-phosphate, also block it allosterically. This is a control mechanism for substrate entrance into the glycolytic pathway (Valerie *et al.*, 2015).
Hexokinase's active site residues include Asp205, Lys169, Asn204, Glu256, and Thr168 (Koshland *et al.*, 2010). There are two ligands that can be bound to this active site: glucose and glucose-6-phosphate. Hexokinase undergoes a conformational change when glucose is bound, which is known as a "induced fit". Inhibition of this conformational change by physiological concentrations of glucose-6-phosphate is allosterically mediated. There are two conformational states of hexokinase. Prior to glucose binding, the open state occurs. In the active site, ATP is bound to the large lobe, far from the glucose binding site, and in a different position (Kitto *et al.*, 2008).

Cytochrome p450

Cytochrome p450 is not a glycolysis enzyme. However, they are very important fish liver proteins. Cytochromes are proteins that belong to superfamilies that contain heme as a cofactor. As a result, they are known as hemoproteins, and they serve as substrates in enzymatic processes. The name "P450" refers to the spectrophotometric peak obtained at the enzyme's highest optic density (450 nm) when it is in its reduced state associated with carbon monoxide (Bezirtzoglou *et al*., 2012).

This gene family is one of the most diverse eukaryotic gene families, with an incredible amount of diversity within and between species (Cytochrome P450s, or CYPs). All of these processes rely on the CYP enzymes' utilization of molecular oxygen to change substrate structure. It is the largest and most diverse cytochrome P450 gene family in vertebrates. Organs with endoplasmic reticulum express these membrane-bound enzymes. A family/subfamily/individual enzyme nomenclature is used to classify CYP enzymes within and between species (Nelson *et al*., 2013).

The human immune system has evolved over time to combat harmful germs. The immune system's ability to develop immunological responses declines with age, contributing to an age-related immunodeficiency known as "immunosenescence," which increases susceptibility to infection in all living things (Gruver *et al*., 2007). Several factors, including genotype and lifestyle, appear to influence aging. Drug metabolism and pharmacokinetics may be affected by aging, immunodeficiency, and infectious illness conditions. The liver and kidneys are the principal organs reported for drug clearance. Among these, the liver is an important location for metabolic clearance (2). Infection and inflammation are linked to the hepatic and extrahepatic metabolism of cytochromes P450 (CYPs), which are enzymes (Bezirtzoglou *et al*., 2012).

Phosphofructokinase

Phosphofructokinase is an essential enzyme in glycolysis. Through allosteric inhibition, PFK can control glycolysis, allowing the cell to raise or decrease the rate of glycolysis in response to the cell's energy requirements. A high ATP-to-ADP ratio, for example, will impede PFK and glycolysis. Phosphofructokinase (PFK) is a tetrameric enzyme made up of three unique subunits: muscle, platelet and liver all of which are expressed differently in various tissues. The M isoform is found solely in mature human muscle, whereas erythrocytes express both the M and L subunits (Hellinga *et al*., 1987).

Tetrameric PFK-1 in mammals is made up of three subunits: muscle type (PFK-M), liver type (PFK-L), and platelet type (PFK-P) (PFK-P, previously called C-type). In humans, the genes encoding these subunits are allocated to separate chromosomes and exhibit tissue-specific expression. The PFK-1 isozyme in skeletal muscle is exclusively constituted of PFK-M, while in other tissues, all three subunits are expressed in different amounts (Dunaway *et al*., 1988).

2.6 PROBLEM STATEMENT

Rivers are complex ecosystem that are often severely contaminated due to over-exploitation and increasing population growth (Goff, 2017). Close relationship of rivers and human societies along with socio economic development have led to degradation and considerable changes in river ecosystems (Khorooshi et al., 2016). One such river, the Jukskei river is one of the largest rivers in Johannesburg. The river begins in Ellis Park and meanders in a northerly direction through Bedfordview and Edenvale before flowing through Alexandra township, ultimately joining the Crocodile River. The river is heavily polluted by urban runoff and a lack of infrastructure maintenance has allowed raw waste and industrial effluent flow into the river on a daily basis (Olukunle *et al.,* 2012). This often results in a reduction in dissolved oxygen levels in the river. Furthermore, the hazardous nature of heavy metals, a form of conservative toxin, has been well documented in many studies. Humans have continuously been exposed to these toxins either directly through the polluted water or indirectly through the consumption of contaminated fish products (Jordaan and Bezuidenhout, 2013).

In extreme environments, some microorganisms adapt and grow as they use pollutants as a source of energy, synthesis of cell components and for microbial activities. These microorganisms have been shown to have unique genes, able to express enzymes to assist them to survive and thrive in unfavourable conditions (Sogin *et al.,* 2006). The effects produced by environmental stressors on microbial diversity can be multifaceted, leading to significant changes in community composition and diversity, high spatiotemporal variability, and alteration of community functionality (Chakraborty and Bhaduty, 2015)

Green solutions are needed for a variety of biotechnology applications such as the use of microbial enzymes which may play a potential role in bioremediation. The findings of this research will reveal the diversity of novel microorganisms that exist in this tributary and their functional potential to spatio-temporary express proteins/enzymes. This study will be able to construct and supply details of enzymes that may be of novelty and their metabolic pathways associated with microorganisms in an urban lotic environment on how they are able to survive such a severe environment. In addition, a better understanding of the structure, function and evolution of protein catalysis would be possible by identifying pathways

CHAPTER 3: PHYSICOCHEMICAL, METAL AND SECONDARY METABOLITE ANALYSIS OF THE WATER SAMPLES

3.1 INTRODUCTION

Total dissolved solids (TDS), pH and electrical conductivity (EC) are the most routinely measured physical parameters in water quality studies across the world, including South Africa (Venkatesharaju *et al*., 2010). The pH values in water research have been used to study the acidity or alkalinity of water samples. Low pH values can cause gastrointestinal problems such as hyperacidity and ulcers in humans (Buridi & Gedala, 2014). In water sources, it corrodes natural rocks which results in the occurrence of more chemical substances such as metals in water. Therefore, increasing contaminants in water (Ayesha, 2012).

Electrical Conductivity and total dissolved solids (TDS) are widely employed as water quality indicators, particularly in coastal areas. These two metrics are salinity level indicators, making them highly helpful as one method of analysing seawater incursion. Electrical conductivity and TDS values have been correlated in most water quality studies (Daniel *et al*., 2016). The correlation of these parameters can be estimated by the following equation:

$$\text{TDS}\ \left(\frac{mg}{L}\right) = k * \text{EC}\ \left(\frac{\mu S}{cm}\right)$$

As the concentration of ions in water increases, so will the value of k. The relationship between EC and TDS, on the other hand, is not directly linear. The activity of individual dissolved ions, the average activity of all ions in the liquid, and ionic strength all have an effect on it (Siosemarde *et al*., 2010).

3.2 STUDY AREA:

The criteria used for selecting sampling points were based on a variety of factors such as industrial, agricultural areas and areas with high population densities.

Jukskei River

The Jukskei River is a watershed of the Limpopo River, which flows into the Indian Ocean. The river is located upstream of Bruma Lake. The river runs through a number of neighbourhoods, including Alexandra. It runs northward until it meets the Crocodile River, which drains into the Hartbeespoort Dam. The Klein Jukskei Spruit, Braamfontein Spruit, and Modderfontein Spruit are three important tributaries that join the Jukskei before it meets the Crocodile River. The Crocodile River is a contributor to the Jukskei River as it runs into the city of Johannesburg and passes through the informal settles of Alexandra Township as seen in Figure 2.

The Jukskei River was sampled at 4 different points, 2 upstream (26°06'09.8"S 28°06'22.8"E) and 2 downstream (26°06'38.9"S 28°06'44.1"E) in order to highlight different activities happening at those four points, ranging from cement spills (from ongoing house constructions), sewage spills, domestic wastes and other unknown liquid spills from pipelines thrown into the river.

Jukskei Upstream

Figure 2: The geographical location of the first sample site upstream of the Jukskei River in the Alexandra Township (Source: Google Maps). Sample site indicted with the red cross.

Figure 3: The sample site as seen in Figure 2 upstream of the Jukskei River. There is a sewage spill as indicated by the arrow.

Figure 4: The second sample site taken upstream of the Jukskei River.

As the Jukskei River passes through the township of Alexandra, the waste material is deposited along the banks upstream of the river, as well as continuously depositing waste downstream as seen in Figure 5

Jukskei Downstream

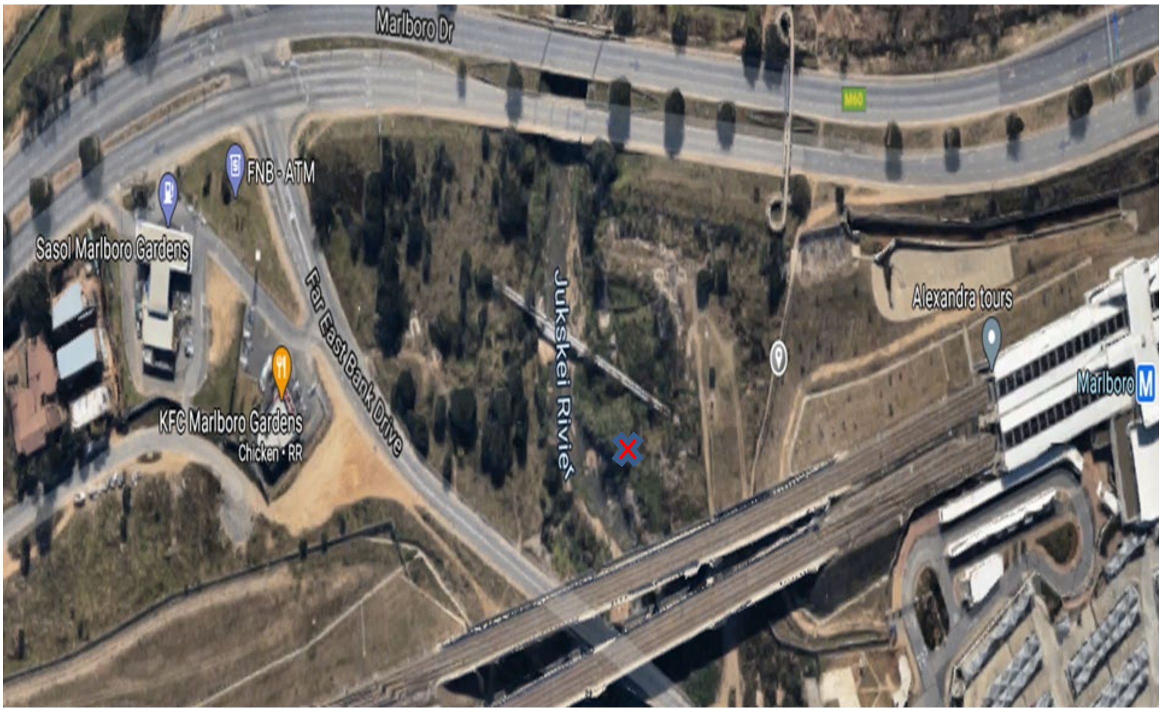

Figure 5: Sample site taken downstream on the Jukskei river (Source: Google maps)

Figure 6: Downstream of the Jukskei River.

3.3 SAMPLE COLLECTION

Thirty samples were collected in 1 L plastic bottles, 15 samples upstream and 15 samples downstream of the initial sampling location. Samples were taken at least 30 cm deep along the riverbank. All surface water samples were taken aseptically in a sterile plastic bottle with some aeration space. Within 3 hours of collection, all samples were analysed for physiochemical characteristics. Following that, more analytical processes were carried out.

3.4 PHYSICOCHEMICAL PARAMETERS

3.4.1 Electrical conductivity, pH and TDS

All physical parameters were determined on-site with the portable HI9813-5 Portable pH/EC/TDS Meter (Smithfield, USA). The probe was inserted into the running rivers to determine the different variables studied.

3.5 HEAVY METAL ANALYSIS

All sample preparations and heavy metal analysis was performed at the spectrau laboratory, at the University of Johannesburg, Auckland Park. Heavy metal concentrations in samples were determined as described in Elemental Analysis Manual (EAM) of the Food and Drug Association of America (FDA) compiled by Dolan *et al*. (2007). This technique is used to detect metals and non-metals at low concentrations as low as 10-15, parts per quadrillion.

3.5.1 Sample preparation for ICP-MS

All 90 contribute tubes (15 ml) were soaked in 2% nitric acid solution overnight and then rinsed thoroughly with deionized water. One ml of water sample was filtered through a 0.2 µm membrane, directly into contribute tube using a filtration syringe. A 10X dilution was made by adding 9 ml of distilled water to the water sample. One-hundred µl of concentrated nitric acid (55%) was added to the diluted sample to stabilize them. All samples were later run on the ICP-MS.

3.5.2 Calibration standards

In order to be able to read and compare varying concentrations of analytes, calibration standards were prepared by first making up intermediates of two different solutions. ICP-MS was set to read concentrations of up to 10-9. The first solution (Solution A) was the ICP-MS calibration standard for given trace metals. The second solution (solution B) was a 1000 ppb of Scandium (Sc). Ten µl of Solution A was pipetted into a 15 ml contribute tube and then made up to 10 ml with 1% nitric acid. One-hundred µl of sample was pipetted into a contribute tube and made up to 10 ml with 1% nitric acid.

Four concentrations of calibration standards were prepared, i.e. 0, 0.1, 1 and 10 ppb. For the calibration standard of concentration 0 ppb, 50 µl of the Sc intermediate solution was pipetted out into a contribute tube and made up to 10 ml with 1% nitric acid solution. The calibration standard with a concentration of 0.1 ppb was prepared by pipetting 10 µl of intermediate A and 50 µl of Sc intermediate into a contribute tube and filled to a 10 ml mark with 1% nitric acid solution. Intermediate with the concentration of 1 ppb were prepared by pipetting 100 µl of solution A intermediate and 50 µl of Sc intermediate solution and made up to 10 ml with 1% nitric acid solution. Lastly, intermediate with 10 ppb concentration were made by pipetting 1 ml of intermediate A and 50 µl of Sc intermediate into a contribute tube and volume adjusted to 10 ml with 1% nitric acid.

3.5.3 Quality control

In order to validate and evaluate the results, the extracted sample concentrations had to be correlated with the CRM data values. A Standard Reference Material (SRM), 1640a was used for quality control purposes. The SRM 1640a consisted of acidified spring water with mass fractions and mass concentrations assigned for 29 elements, 22 of which were gravimetrically added. The solution also contained 2% nitric acid.

3.5.4 Running of the instrument.

Helium and Argon gases that were connected to the ICP-MS were turned on. The chiller was then switched on to keep the instrument warm. The ICP-MS machine was turned on and allowed to warm up for 10 minutes. Samples were placed in the auto-sampler with deionized water and 2% nitric acid for rinsing. The Heavy metals were analysed with the ICP-MS machine (Perkin & Elmer, Norwalk, CT, USA) with the instrument software, Syngistx™, as specified by the manufacturer. The analysis was run on Kinetic Energy Discrimination (KED) mode so to avoid polyatomic interferences. Isobaric interferences were programmed and the instrumental and data acquisition parameters were set before running the software. As the software was then run, the probe analysed each sample on the auto-sampler automatically and data was generated and analysed. The qualitative analysis method is shown in Table 3 below.

Table 3: ICP-MS Quantitative analysis method

	Time (sec)	Speed (± rpm)
Sample flush	35	-24.0
Read delay	15	-20.0
Analysis		-20.0
Wash	50	-24.0

3.6 STATISTICAL ANALYSIS

The IBM SPSS statistical software (version 20) was used to examine physiochemical and microbiological data. The physicochemical analysis results and mean physiochemical counts of the examined water samples were compared to the specified standards (WHO drinking water quality guidelines) and evaluated as acceptable or unsatisfactory. The computed coefficient of variation was used to evaluate the significance of variations between samples (%CV). One-way ANOVA was used to determine the mean separation between sample categories. Using Pearson's correlation, the different physiochemical and microbial data was correlated against one another to establish their connection. In all situations, the 95% confidence interval was used to determine significance.

3.7 MOLECULAR DOCKING

Molegro Virtual Docker (v7.0.0) has a graphical user interface that allows users to perform all tasks related to docking simulations from its window. The step-by-step process of molecular docking is shown in Figure 7.

3.7.1 Protein acquisition and preparation

All 3D structural proteins were downloaded from the RCS Protein Data Bank and saved as *pdb* files. During the uploading of files, water molecules were removed from the protein, so to perform rigid docking. Thereafter, the flowchart method is Figure 7 was followed.

3.7.2 Ligand acquisition and preparation

All ligands were downloaded in 2D formats from PubChem.ncbi.nlm.nih.gov. All files were saved in *sdf* format and the flow chart workflow in Figure 7 was followed.

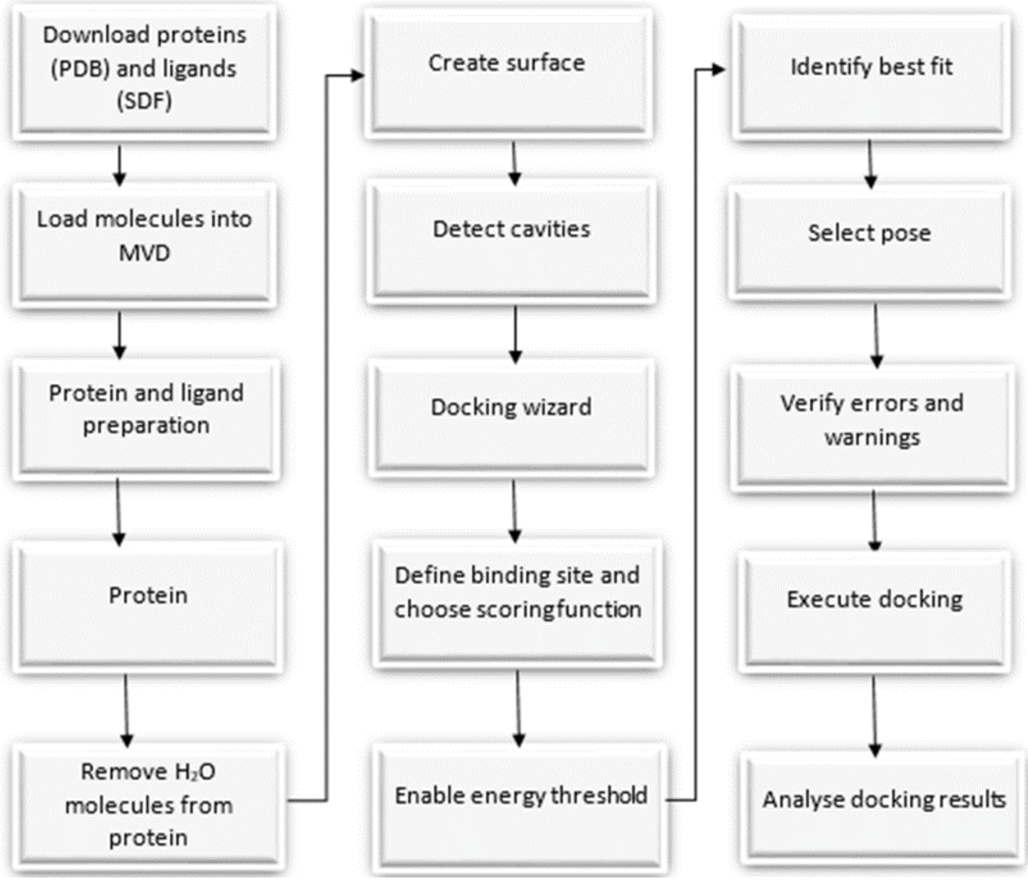

Figure 7: Flow chart illustrating the steps taken for the molecular docking of different ligands (heavy metals and phenols).

The top 1 docking results for each protein-ligand were used for analyses. Molecular scores (Molscores) and Hydrogen bond scores were recorded, as see in chapter 4. The docking procedure consisted of two fundamental steps: predicting the ligand structure as well as its position and orientation inside these sites (often referred to as pose) and determining the binding affinity. These two phases concern sample methods and scoring algorithms, respectively.

3.7.3 Inhibitory potential of metals and metabolites

Metal and phenol induced inhibition of Phosphofructokinase, Hexokinase and Cytochrome P450 will be performed according to the methods of Malina *et al*. (2014), Olmo *et al*. (2002) and Letelier *et al*. (2009) respectively at various metal and phenol concentrations.

3.8 RESULTS

3.8.1 TDS, pH, EC and metal analysis

Table 4: Mean Physicochemical parameters at Jukskei River (n= 90).

Parameters	WHO limit	JU	JD	Maximum
TDS (ppm)	500	210	240	349
pH	7.0-8.5	8.24	7.68	8.51
EC (µs/cm)	600	419	479	688

The overall means for each parameter were calculated. The overall mean TDS levels were 225.125±47.99575 ppm. The overall mean pH was 7.9829±0.35. The mean EC levels found in the Jukskei River were 449.58±93.81 µs/cm. Most pH levels in the Jukskei river were mostly within the WHO water standard guidelines, with the exception of one reading at 8.51 which was found upstream of the Jukskei River. The Jukskei River's mean conductivity values (µs/cm) varied from 352 to 688 µs/cm, with a mean average of 418 and 479 s/cm upstream and downstream, respectively. The highest value was observed downstream of the river, at 688 s/cm. Other than the studied physicochemical qualities found along the river, other characteristics such as odour and colour were observed. The river had bad odour both upstream, but more extremely downstream of the river due to sewage spills along the river. The bad odour may have been due to oxidative reactions of the sludge by microorganisms. There was also a considerable difference in the water colour. Upstream and downstream of the river, it had a grey colour and black sludge.

Comparative heavy metal concentrations to the WHO standards and the South African water quality guidelines for aquatic ecosystems are shown in table 5.

Table 5: Mean average concentrations of Heavy metals (µg/L) vs WHO and South African water quality guidelines for aquatic ecosystems.

Heavy metals	WHO	SA Target Water Quality Range (TWQR)	JU	JD
Al	0.9	≤ 10	843.56	645.38
As	0.01	≤ 10	21.94	10.928
Cd	0.003	≤ 0.15	0.646	0.71125
Cr	0.05	≤ 7	36.55	47.846
Cu	2	≤ 1.2	264.82	343.475
Mg	0.1	-	152531.25	81380.75
Mn	-	≤ 180	5201.28	3194.12
Ni	0.07	-	370.77	208.36
Pd	0.01	≤ 1.0	29.586	29.06
Se (82)	0.04	≤ 2	21.32	18.1986
Zn	-	≤ 2	870.80	983.886

The samples taken upstream of the Jukskei, as well as downstream of the Jukskei were taken within the Alexandra Township. The samples collected upstream were within a radius of 3 km of a community clinic, community building sites, as well as other informal businesses. The River as previously reported has been used as a dumping site for most residents, as the municipal council PickitUP service is not accessible to most residents in the Alexandra Township. The high population density in the Alexandra Township has led to high pressure in the river, as most waste is thrown in it, as witnessed when we were sampling. All heavy metal concentrations were well above the WHO water quality standards and the South African water quality guidelines for aquatic ecosystems. This came as no surprise, given the high population density of the informal township, as well as the poor infrastructure. The samples taken downstream had higher levels of heavy metal concentration. This may have been due to the additional pollution activities occurring in that area. Two sewage pipes were seen protruding from the settlement, to be dumping waste into the river, as well as construction workers at the time of sampling. According to Titilawo *et al.* (2018), sewage discharge into rivers is a major source of heavy metals entering aquatic ecosystems.

3.8.2 Secondary metabolite analysis

Phenolic compounds were identified using the UV wavelengths and retention times of the pure compounds, which were as follows:

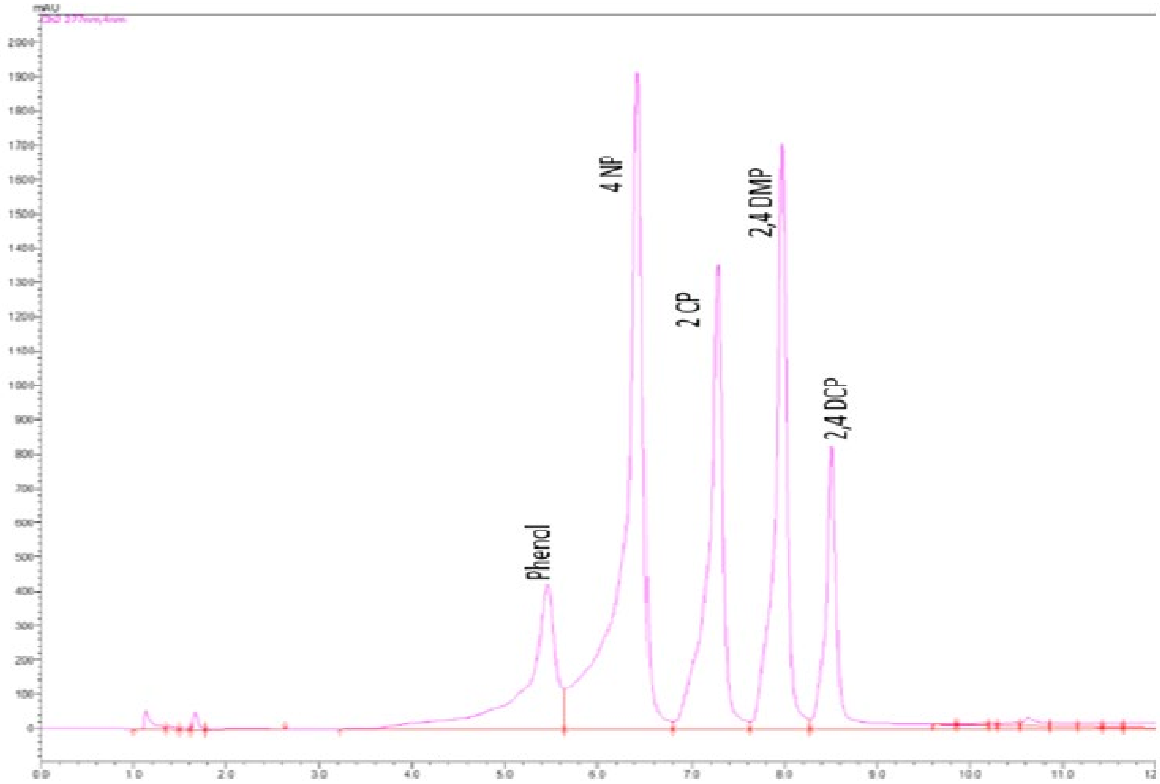

Figure 8: Chromatogram of phenolic standard with methanol/water separation of (1) Phenol (2) 2,4-dimethylphenol, (3) 4-nitrophenol (4) 2,4-dichlorophenol, (5)2-chlorophenol.

Table 6: Standard phenolic compound identification data.

Compound	Retention time (minutes)	MAU (milli-Absorbance Units)
Phenol	5.447	450
4-Nitrophenol	6.448	1920
2-Chlorophenol	7.281	1380
2,4-Dimethylphenol	7.974	1700
2,4-Dichlorophenol	8.507	820

After running phenolic compounds standards against water samples, raw data was used to calculate the amount of analyte in each of the samples. The following equation below was used:

Sample concentration= (sample area)/ (standard sample area) ×standard solution concentration × dilution factor.

2-Chlorophenol

JD

Sample concentration= (177509÷14551005) 111.407× 0.03 = 0.041 mg/L

JU

Sample concentration= (118846 ÷65159524) 498.882× 0.03= 0.027 mg/L

2,4-dichlorophenol

Sample concentration= (444663÷20687032) 249.604× 0.03= 1.6884 mg/L

Table 7: Phenolic compound concentration (ppm) of pooled samples.

Phenolic Compound	Correlation coefficient (R^2) of standards	EPA Standards for consumption	JU	JD
Phenol	0.998	10	0.00	0.00
2-chlorophenol	0.999	0.081	0.027	0.041
4-nitrophenol	0.999	-		
2,4-dichlorophenol	0.999	0.077	0.00	1.609
2,4-dmethylphenol	0.998	0.38	0.00	0.00

As seen in the table above, 2-chloropheol was found in all water samples. Phenol and 2,4-dimethylphenol were undetected. 2,4-dichlorophenol was the only pollutant phenol that was found to be above the limits stated by EPA, more than two times over. No quality guideline was provided by EPA (2009) with regards to the permissible limits allowed for 4-nitrophenol. Therefore, not much can be said about their concentration levels.

3.8.3 DOCKING RESULTS OF METALS AND PHENOLIC COMPOUNDS

The top 1 docking results for each protein-ligand were used for analyses. Molecular scores (Molscores) and Hydrogen bond scores were recorded, as seen in table 9. The docking procedure consisted of two fundamental steps: predicting the ligand structure as well as its position and orientation inside these sites (often referred to as pose) and determining the binding affinity. These two phases concern sample methods and scoring algorithms, respectively.

Rigid docking (without water proteins) was done for all docking procedures due to hydrogen's affinity to water. Therefore, water molecules were removed in order to obtain a better understating of purely protein-ligand interactions. Eleven heavy metals (Aluminium (Al), Arsenic (As), Cadmium (Cd), Chromium (Cr), Copper (Cu), Magnesium (Mg), Manganese (Mn), Nickel (Ni), Lead (Pb), Selenium (Se), and Zinc (Zn) were docked against all proteins. Top 3 heavy metals with the docked proteins were recorded in Table 9 below. Five identified phenols (2-chlorophenol, 4-nitrophenol, 2,4-dichlorophenol, 2,4-dimethyphenol and phenol) using the UHPLC were docked against the three different proteins and the top 3 complexes with the highest affinities were recorded (Table 10).

Table 8: Top 3 molecular docking scores for proteins against heavy metals.

Protein	PubChem ligand code	Ligand	Hydrogen bond ΔH kJ mol^{-1}	Molscore kcal/mol
Cytochrome P450	23973	Cd	0	-22.8949
Cytochrome P450	23978	Cu	0	-21.2207
Cytochrome P450	23976	Cr	0	-22.8181
Hexokinase	5359596	Al	0	-21.2523
Hexokinase	935	Ni	0	-21.2528
Hexokinase	23973	Cd		-21.2524
Phosphofructokinase	23978	Cu	0	-48.9695
Phosphofructokinase	935	Ni	0	-48.9701

The more negative a Molscore score is, the higher the affinity. Therefore, give the data above, amongst all the docked metals against cytochrome p450, Cd had the highest affinity to the protein with Molscore -22.8949. Hexokinase however had the strongest affinity of Ni with a Molscore of -21.2528. Like Hexokinase, PFK had the highest affinity to Ni, with a Molscore -48.9701. Amongst the docked metals, Cu, Cd and Ni showed the highest affinities with all proteins studied. No Hydrogen interactions were observed for either protein-ligand conformation.

Table 9: Top 3 molecular docking scores for the studied proteins against phenols

Protein	Phenol	Ligand code	Hydrogen bond ΔH	Molscore kcal/mol	Amino acid-Hydrogen bond Interactions
Cytochrome P450	4-nitrophenol	980	-2.5	-68.967	Gln 215
Cytochrome P450	2,4-dimethylphenol	7771	-2.2264	-63.102	Gln 218
Cytochrome P450	2-chlorophenol	7245	-3.922	-63.102	Gln 218
Phosphofructo kinase	4-nitrophenol	980	-2.5	-75.928	Thr 136
Phosphofructo kinase	2,4-dimethylphenol	7771	-2.5	-69.841	Asp 260, Thr 136
Phosphofructo kinase	2,4-dichlorophenol	8449	-2.5	-68.193	Asp 260
Hexokinase	4-nitrophenol	980	-2.27389	-44.717	Asn 56, Arg 794
Hexokinase	2,4-dimethylphenol	7771	-2.5	-44.350	Arg 794
Hexokinase	2-chlorophenol	7245	-2.5	-41.91	Arg 794

Docked phenols against cytochrome p450 showed the highest affinity to 4-nitrophenol with Molscore -68.967. However, cytochrome p450 had the highest hydrogen bond with pentachlorophenol (-3.88099). The highest affinity with PFK was observed with 4-nitrophenol with Molscore -75.9286. Lastly, the highest affinity in hexokinase docking was with pentachlorophenol with Molscore -47.1745.

As mentioned previously, the presumption is that the more negative a molscore is, the higher the affinity due to interaction between the ligand and protein. It is evident that a more negative Molscore is not indicative of protein-ligand/steric interaction and potentially show inhibitory potential towards an enzyme. For this reason, in vitro assays were needed to be performed to authenticate a molscore (Table 10 and 11).

The results obtained in vitro demonstrate that there appears to be link between molescore and the inhibition activity of an inhibitor on hexokinase and phosphofructokinase. Heavy metals which are generally defined as metals with relatively high densities, atomic weights, or atomic numbers appear to exert a greater inhibition potential than “normal” metals (table 10). In addition, the phenolic compounds exert an even greater inhibitory action and at lower concentrations compared to the metals and heavy metals. This effect may be attributed to the chemical structure of phenolic compounds and their interaction with either the enzyme and/or the substrates. However, cytochrome P450 appear to still maintain a relatively higher % remaining activity. This may be due to the ability of this class of enzymes to detoxify xenobiotics. They oxidize substances using iron and are able to metabolize a large variety of xenobiotic substances.

Table 10: Inhibitory potential of metals against proteins.

Protein	Metal	Molscore kcal/mol	Metal conc	%Remaining activity
Hexokinase	Al	-21.2523	1μM	56
			10μM	20
			50μM	0
	Ni	-21.2528	1μM	44
			10μM	16
			50μM	0
	Cd	-21.2524		
	Cu		1μM	100
			10μM	100
			50μM	83
	Zn		1μM	100
			10μM	91
			50μM	80
	Mg		1μM	100
			10μM	100
			50μM	83
	Pb		1μM	58
			10μM	21
			50μM	0

	Cr		1µM	51
			10µM	38
			50µM	0
Phosphofructokinase	Cu	-48.9695	1µM	100
			10µM	100
			50µM	89
	Al		1µM	77
			10µM	43
			50µM	7
	Ni	-48.9701	1µM	61
			10µM	28
			50µM	0
	Zn		1µM	100
			10µM	96
			50µM	83
	Mg		1µM	100
			10µM	100
			50µM	73
	Pb		1µM	42
			10µM	6
			50µM	0
	Cr		1µM	56
			10µM	22
			50µM	0

Table 11: Inhibitory potential of phenols against proteins.

Protein	Phenol	Molscore kcal/mol	Conc of Inhibitor	% Remaining activity
Cytochrome P450	4-nitrophenol	-68.967	1μM	89
			5μM	73
			10μM	70
Cytochrome P450	2,4-dimethylphenol	-63.102	1μM	90
			5μM	64
			10μM	58
Cytochrome P450	2-chlorophenol	-63.102	1μM	81
			5μM	80
			10μM	62
Phosphofructokinase	4-nitrophenol	-75.928	1μM	0
			5μM	0
			10μM	0
Phosphofructokinase	2,4-dimethylphenol	-69.841	1μM	53
			5μM	0
			10μM	0
Phosphofructokinase	2,4-dichlorophenol	-68.193	1μM	22
			5μM	2
			10μM	0
Hexokinase	4-nitrophenol	-44.717	1μM	27
			5μM	2
			10μM	0

Protein	Phenol	Molscore kcal/mol	Conc of Inhibitor	% Remaining activity
Hexokinase	2,4-dimethylphenol	-44.350	1μM	45
			5μM	0
			10μM	0
Hexokinase	2,4-dichlorophenol	-41.91	1μM	44
			5μM	0
			10μM	0

CHAPTER 4: MICROBIAL COMMUNITY PROFILING AND PREDICTIVE FUNCTIONAL POTENTIAL ANALYSIS

4.1 INTRODUCTION

Microorganisms are diverse in the environment and play an important role in the breakdown of organic matter and other contaminants, such as pesticides and some xenobiotic chemicals, as well as in nutrient recycling. They can, however, have a detrimental influence on ecosystems and public health by generating poisonous compounds that endanger the health of different species, including people (Frączek *et al*., 2011).
Studies in the Alexandra Township in particular have been of importance in South Africa due to the constant potential pollution, and the constant domestic wastes thrown into the Jukskei River. Microbial community profiles of the different water systems are therefore significant. Waterborne diseases are mostly caused by bacteria linked with faecal pollution in water systems. This is especially concerning given the enormous effect of water-borne illnesses in South Africa (DWAF, 2010). Environmental disturbances have the greatest impact on aquatic microorganisms. Changes in their community structures can thus be utilized as possible bio-indicators of environmental degradation. Bacterial communities can be sensitive markers for pollutant stress, particularly metal contamination (Sun *et al*., 2012).

Microbial community profiling was done using NGS. Next Generation Sequencing allows massive parallel sequencing of environmental DNA. The Illumina ®MiSeq-2000 system produces a large number of reads (van Dijk *et al*., 2014). Due to its huge data output and relatively low cost per base pair, this technology was chosen as a solution for gene sequencing. Another factor in selecting the Illumina ®MiSeq-2000 system was that it is one of the most frequently utilized platforms for environmental metagenomics.

4.2 MICROBIAL COMMUNITY PROFILING

4.2.1 CTAB DNA Extraction Method

The Genomic DNA isolation: Genomic DNA was isolated by the method as described by Aboul-Maaty *et al.* (2019) with modifications to suite water sample DNA extraction. The CTAB extraction buffer was prepared as follows:

- 2% CTAB (hexadecyltrimethylammonium bromide)

- 100 Mm Tris-HCl (pH=8)
- 20 mM EDTA
- 1.4 M NaCl
- 0.2% of β-mercaptoethanol
- 0.1 mg/ml proteinase K

Added to samples just before the incubation step

The extraction buffer was then autoclaved and was ready for use. 900 mL of water sample was centrifuged at intervals in 50 ml centrifuge tubes at 10 000 rpm at 4°C for 10 minutes, in order to obtain a pellet. After which, 0.8 mL of pre-warmed (60°C) CTAB buffer was added to the tubes and incubated at 60°C for an hour. After 1 hour, 0.8 mL solution of chloroform/isoamylalcohol (24:1) was added to all the tubes in a fume hood. Samples were mixed gently for 2minutes by inverting and were then centrifuged at 10 000 rpm at 4°C for 10 minutes. The aqueous phase was then transferred to clean micro tubes. One micro litre of RNAse A was then added to the tubes and incubated at 37°C for 30 minutes. Isopropanol (0.6 mL) was then added to the tubes and incubated overnight at room temperature. After the overnight incubation, the tubes were then spun at 10 000 rpm, at 4°C for 15 minutes to pellet the DNA. The supernatant was carefully removed and washed with 70% ethanol. The tubes were the centrifuged again at 10 000 rpm, at 4°C for 15 minutes. The ethanol supernatant was removed, and pellets were dried and re-suspended in 50 µL of sterile water and quantified. They were later stored at -20°C.

Quantification of DNA: Concentration, purity (A260/A280 ratio) and absorbance ratio at 260 nm to 280 nm (A260/A230 ratio) were measured with the Implen Nano-photometer N60 (Westlake Village, California). Samples were later taken to Inqaba Biotech Inqaba Biotech (Pretoria, South Africa) for PCR amplification and thereafter, 16S rDNA amplicon gene sequencing. At Inqaba Biotech, PCR amplification of the 16S rRNA V1 to V3 regions was performed with oligonucleotide primers 27F (16S-50-AGAGTTTGATCMTGGCT-CAG-‘3) and 518R (16S-50-ATTACCGCGGCTGCTGG). The PCR amplicons were processed using the illumina® reaction kits according to the manufacturer's instructions and sequenced using the illumina® MiSeq-2000 with the MiSeq V3 kit. The acquired data was processed using the bioinformatics QIIME program.

4.3 RESULTS

All DNA samples were sent to Inqaba Biotech in Pretoria, South Africa for high-throughput sequencing. Polymerase Chain Reaction procedures were performed by the Inqaba Biotech team and thereafter, 16S rDNA sequencing was performed using their Illumina ®MiSeq-2000 system.

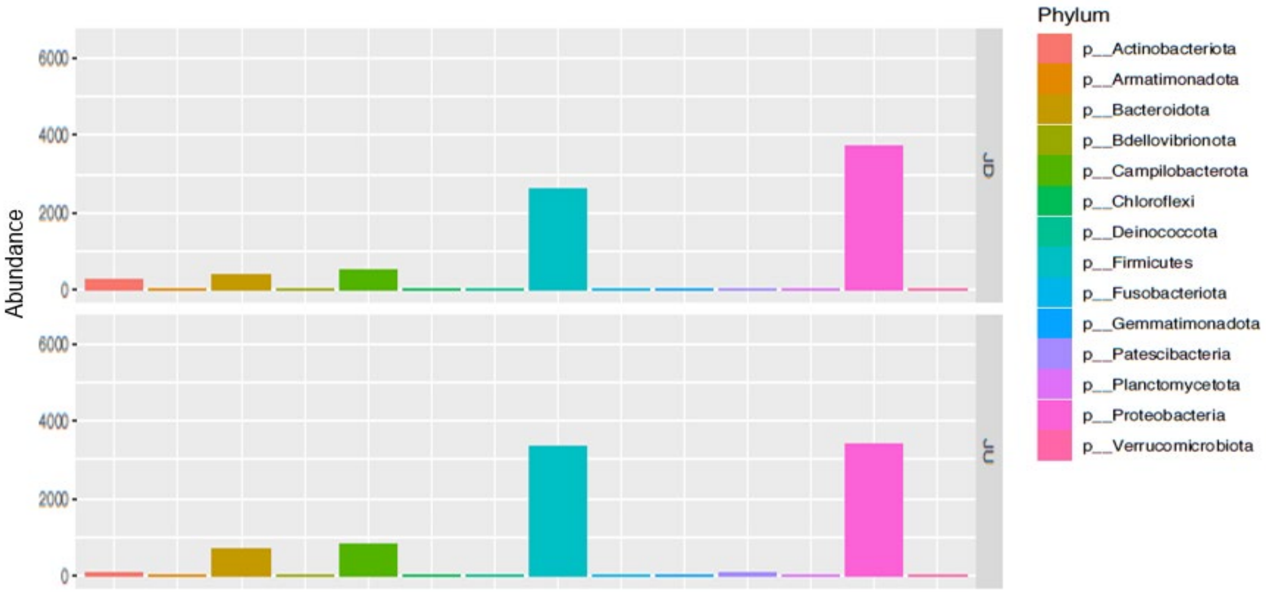

Figure 9: Relative abundance of bacterial phylum in samples of the Jukskei River.

The most abundant phylum observed were *Proteobacteria* and *Firmicutes*, as seen in Figure 8 *Firmicutes*, mostly gram-positive bacteria producing endospores that can survive in extreme conditions. Taxonomic analysis of the data revealed that *Proteobacteria and Firmicutes* were present and the two most dominant phylum. Given the activities in and around the Jukskei River, faecal related bacteria were expected. Bacterial community composition upstream of the Jukskei River were most dominant in the genus *Pseudomonas, Flavobacterium* and *Prevotella.* Downstream of the Jukskei, Pseudomonas was also seen to be the most abundant genus whilst, *Bacillus* and *Massilla* were second and third most abundant genus.

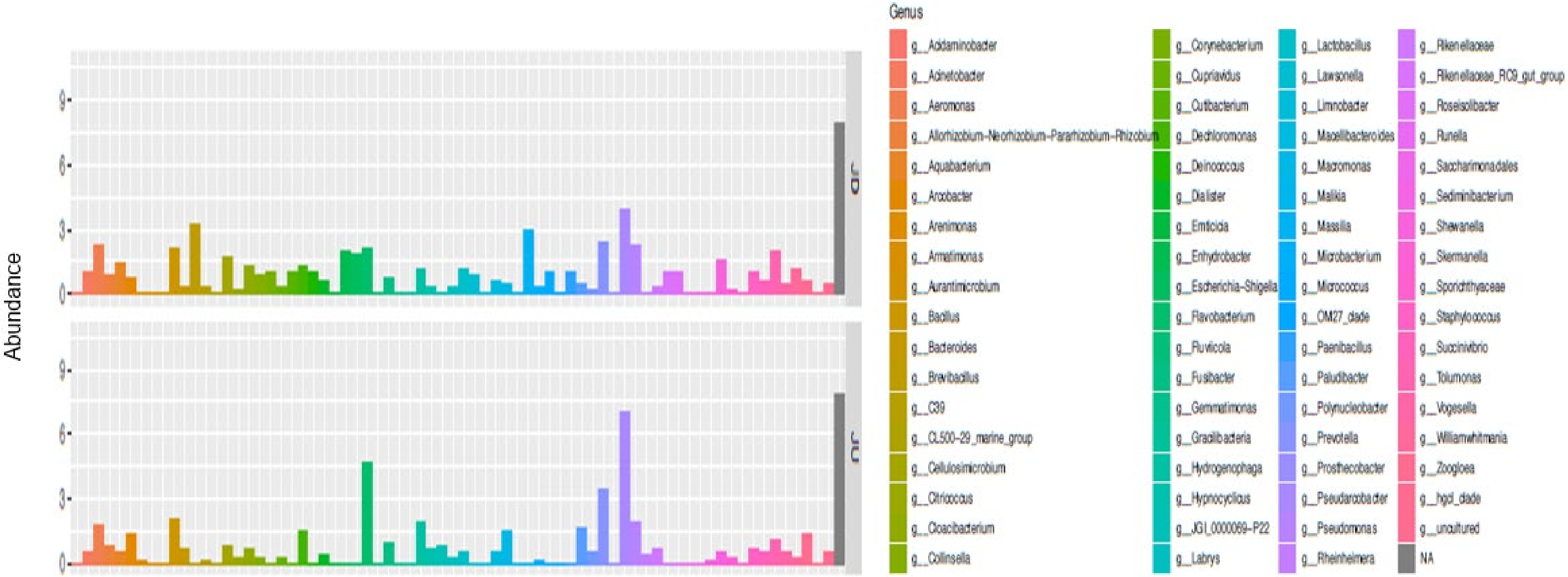

Figure 10: Relative abundance of the bacterial genus found.

The most abundant bacterial genus is *Staphylococcus*, *Pseudomonas*, and *Proteobacteria*, *Flavobacterium*, *Bacillus* and *Allorhizobium Neorhizobium Pararhizobium Rhizobium.*

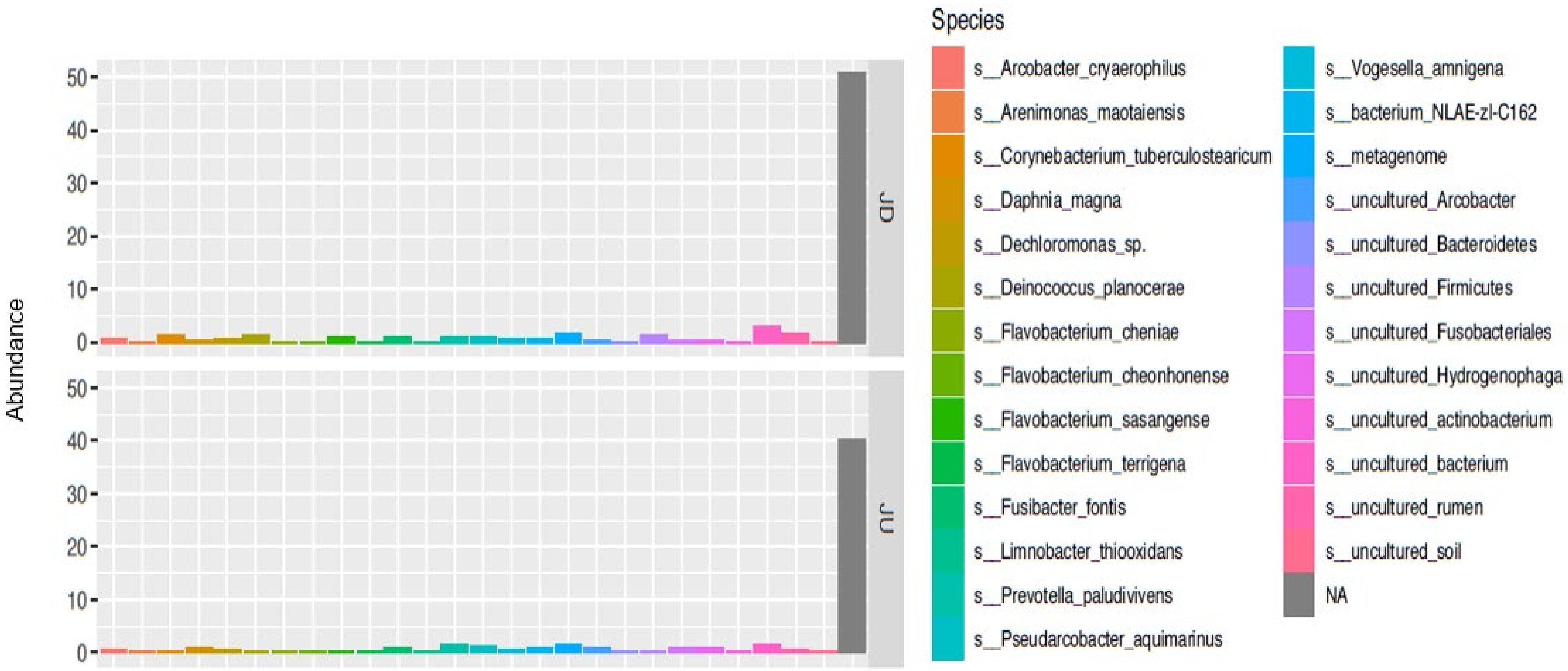

Figure 11: Relative abundance of bacterial species found in the Jukskei River.

Uncultured *Bacteriodetes* were mostly abundant upstream in the Jukskei river. Identified species *Flavobacterium terrigena* and *Flavobacterium cheonhonense* were also only found downstream of the river and not upstream of the river. One species of concern is the *Corynebacterium tuberculostearicum* which was found to be most abundant downstream of the Jukskei River. Given the diverse activities occurring in the Jukskei, the origins of this bacterium cannot be identified.

In addition to colonizing the skin and mucous membranes of people, *Corynebacteria* are widely dispersed in the environment. Clinical specimens are commonly found to have them and has been in the news lately. *Tuberculostearicum* was confirmed to be the causative agent in 7/18 cases with clinical infections. Patients with empyema or rib osteomyelitis were more likely to have the bacterium (Hinic *et al.*, 2012).

CHAPTER 5: PREDICTIVE FUNCTIONAL PROFILING OF MICROBIAL COMMUNITIES IN THE JUKSKEI RIVER USING 16S RRNA MARKER GENE SEQUENCES.

5.1 INTRODUCTION

Microorganisms colonize extreme environments like rivers because they can thrive in a varietyof harsh conditions like acidic (pH 4) and alkaline (pH >9) pH values, high salinity, high and low temperatures, high metal concentrations, and toxic secondary metabolites. The molecularstrategies used to survive in such environments are still being investigated. However, it is known that under extreme conditions, these microbes have biomolecules that are catalytically active. As a result, functional-based metagenomic data screening can detect both novel and previously known gene classes related to a variety of cellular functions (Lewin *et al*., 2013; Elleuche *et al*., 2014). PICRUSt 2.0 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States), a bioinformatics software tool that uses 16S rRNA genesurveys to estimate metagenome functional content, is one such program that can be used to survey genomic data for functional potential (Langille *et al*., 2013).

Rivers present a store of bacteria that have got diversified functional properties (Mittal *et al*., 2019). However, the technology for discovery of these functional properties through traditional techniques such as culturing has only led to the discovery of less than 1% of the total microbial community available (Awaswthu *et al*., 2020). The metabolic pathways of bacteria andexpressed genes are now being understood through the use of high throughput technologies (Lorens-Marès, *et al*., 2015). These technologies have resulted in discovery of potential biomarkers, novel enzymes and genes (Mallick *et al*., 2019). This is equally important considering that polluted river environment also demonstrate the level of tolerance to toxicity and potential of such microorganisms to become novel bioremediates (Mallick *et al*., 2019). Inorder to study the diversity of a particular function and to demonstrate changes in the composition of prokaryote communities contributing to the function, in this investigation, functional potential studies were used.

5.2 METHOD AND MATERIALS

5.2.1 Analysis of the bacterial community metabolic potential

Langille *et al*. (2013) states that microbiome profiling is an investigation that seeks to reconstruct the phylogenetic relationship of unobserved states. One of the most used tools to do so is PICRUSt which uses the 16s rRNA gene marker to search for metabolic function within the Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathways database (Awaswthu *et al*., 2020). With the assistance of Qiime, the clustered-OUT file positioned at 79%, a phylogenetic tree is constructed using the Greengenes database, whereby the contents are given nodes (Deval *et al*., 2020). Langille *et al*. (2013) adds that PICRUSt makes use of algorithms that align with the ancestral state when the sequenced genomes are not there. Withineach sample gene counts are given and inferred within the KEGG database using a classifier in PICRUSt (Deval *et al*., 2020). STAMP version 2 was used to calculate and visualize relative abundance and profile of microbiome community in terms of available pathways at the third level for some of the most used pathway.

5.3 RESULTS AND DISCUSSION

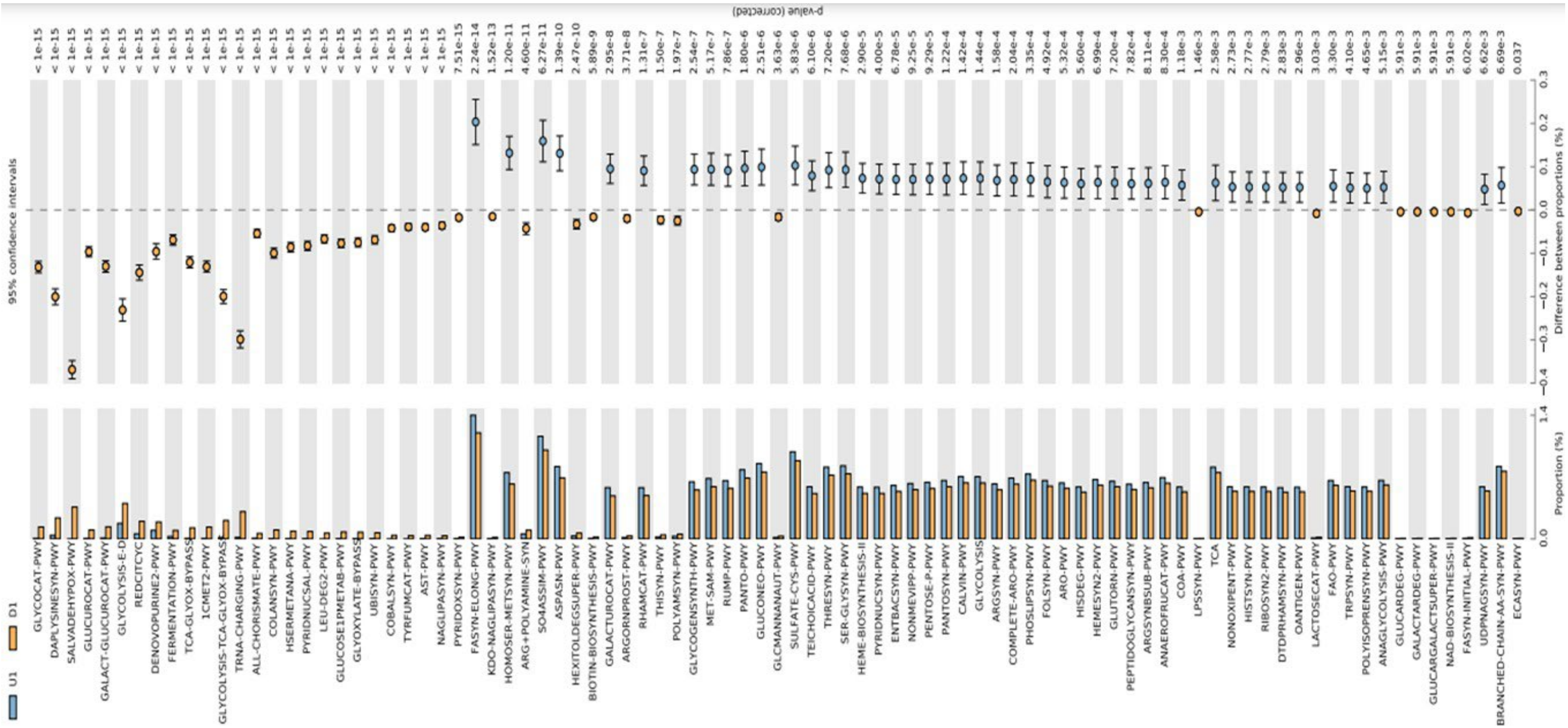

Figure 12:A presentation of comparison of the metabolic and functional potential of the Jukskei river microbial community.

The inferred functional analysis of the bacterial communities revealed several differences in gene abundance. The KEGG pathway predictions revealed a high abundance of functional potential. With regards to hydrocarbons biodegradation, two pathways have been identified which include both aerobic and anaerobic processes. The aerobic processes involve the use ofoxidation of hydrocarbons to alkanols which requires NADH, whilst that of alkenes are oxidized with fatty acids acting as intermediaries which are made from alkanols using an aldehyde derivative (Chen *et al*., 2015). These fatty acids are only released after they had been degraded through carboxylic degradation and released into the environment(Chen *et al*., 2015).

With regards to biotin, which is a co-factor of carboxylation reactions and is synthesized by several marine bacteria, it is not well understood how it is produced for certain bacteria but for those that were identified in the study such as some *Bacillus spp*, it is synthesized from fatty acids after cleavage with cytochrome p450. In *Bacillussubtilis* it is synthesized from ACP-bound fatty acids involving a C-C bond cleavage by a cytochrome P450 containing enzyme. The reaction starts with condensation which results in atransaminase reaction which leads to eight isomers of biotin. The binding of biotin is catalyzed by several enzymes which represses the release of biosynthetic enzymes when biotin becomes a repressor. In particular biotin is degraded by *Pseudomonas spp* species which happens after oxidation which makes the side chain short andattacks the imidazolidone ring producing urea (Michal and Schomburg, 2012).

The citrate cycle and the glycolysis cycles are major key pathways for bacteria and essential for a major role in energy metabolism respectively. Within thecontext of marine bacteria in the form of green sulfur bacteria, they utilize a reductive cycle and produces acetyl coA under anaerobic conditions which utilizes ferrodoxin and reduces carbon dioxide. It is an essential process for amino acids and porphyrin production (Michal and Schomburg, 2012).

In terms of upstream processes, related pathways identified were more concerned with lipid biosynthesis, lipid oxidation, pentose phosphate and glucogenesis production. Firstly, anaerobic marine bacteria are produced through the fatty acid synthase system. In particular, the 3-hydroxydecanoyl-ACP-dehydratase produces the cis-3,4 unsaturated acid which is then converted to cis-vaccenate 3-oxoacyl-ACP synthase,a major fatty acid of bacteria. Equally marine bacteria produce trans-unsaturated fatty acid which is an isomerization process which is helpful in controlling membrane fluidity (Kumar *et al.,* 2016).

Fatty acids themselves as previously articulated as products of alkene degradation which is facilitated by β-oxidation and has been reported in several species which include *Pseudomonas*and *Acetobacter spp*. This is done through oxidation to becomea methyl group which is then converted to alcohol through catalytic action of oxgenases whichfurther convert it to aldehyde and carboxylic acids (Selvam and Thatheyus, 2018). However, with *Rhodococcus spp*. the alkenes are converted to alcohols which are then oxidized by monooxygenases to become aldehydes. These hydrocarbons produced depend on the length of the chain, become surfactants which are also important for removal of organic pollutants from the surface of river sources. These fatty acids are taken up together with acetyl-CoA in the glyoxylate pathway which is performed in the glyoxisome which then release succinate in the mitochondrion and released in the cytosol as oxaloacetate (Waikhom *et al*., 2020).

The pentose phosphate pathway is a recognized pathway that is essential for the glucose turnover and is found in most bacterial species. Its main function is to release pentoses and NADPH which can be used for other biosynthetic reactions. Inparticular bacteria are then able to break xylulose to produce lactate and ethanol (Michal and Schomburg, 2012).

As part of the major nutrients in the diet, amino acids not only support the growth and survivalof bacteria, but also regulate energy and protein homeostasis in organisms. Amino acid such as methionine with the major role such as that it can be used to produce other important molecules. It is involved in the production of cysteine, the other sulfur-containing amino acid used to build proteins. Another amino acid is Histidine and these amino plays particularly important roles in the active site of enzymes, such as serine proteases (e.g. trypsin) where it isa member of the catalytic triad. Excess histidine may be converted to trans-urocanate by histidine ammonia lyase (histidase).

CHAPTER 6: RESULTS SYNTHESIS, CONCLUSION AND RECOMMENDATIONS

The current study assessed the physicochemical and bacterial diversity in water samples of a heavily utilized South African river system in order to determine how diverse land usage along the river stretch could adversely impact the river. The Jukskei River is at high risk of direct pollution from domestic, industrial and agricultural activities in surrounding areas. The research carried out on the Jukskei River aimed to assess physicochemical, HMs, pollutant phenols and bacterial pollution of water. The pH levels in all river samples were compliant with the WHO standards. However, other physical qualities such as EC and TDS were of concern. Conductivity in streams and rivers is impacted by a variety of elements, including soil type, bedrock, the presence of inorganic dissolved particles, sewage, and wastewater. Water's electrical conductivity is related to its dissolved mineral matter concentration (Singh and Katiyar, 2020). Higher electrical conductivity values in the downstream might be attributed to substantial deposition of metal irons from urban and industrial regions into the river via surface runoffs, channels, and sewage effluents (Medeiros *et al.,* 2014). The high concentration of heavy metals present in the river might be the results of higher electrical conductivity values in the downstream.

Several conventional wastewater treatment methods are available such as batch sequence, vertical treatment, photocatalysis and oxidation ditches. The wastewater treatment with an external energy supply however is not suitable on a large scale. Hence in future studies, a modern approach to treat wastewater along with energy generation must therefore be explored (Fadzli *et al*., 2021). During the rainy season, turbidity in South African rivers typically rises. However, the months of June through August are chilly and dry. Higher microbial load and human activities such as road and bridge building can cause increased turbidity and TDS levels (Zhao *et al.,* 2019). High turbidity can reduce the effectiveness of disinfection against microbes and allow bacteria to proliferate. The highest turbidity levels downstream may be attributed to the emission of coloured particles and metallic elements into the streams, particularly those created by various industries. Suspended particles can have detrimental impacts on aquatic life by causing abrasive injuries, blanketing the stream bottom, disrupting spawning grounds, and filtering out light required for aquatic plants' photosynthetic activities (Wu *et al.,* 2019).

The presence of phenolic compounds in water can occasionally be attributed to the decay of dead plants and animals, as well as runoff from various land uses into water bodies. Phenolic compounds are widely distributed in the environment, including wastewater and natural environmental waters, and are among the most dangerous chemicals because of their long-term persistence in the environment and harmful effects (Mainali, 2020). Phenolic compounds are refractory organic compounds having chemical structures that include an aromatic ring and one or more hydroxyl functional groups (Chen *et al.,* 2021). In aquatic environments, certain bacteria are known to breakdown non-ionic surfactants into alkyl phenolic chemicals, which are often more toxic or damaging than the original compounds. For example, bacterial degradation of nonylphenol polyethoxylate surfactant results in the formation of nonylphenol (Anku *et al*., 2017).

According to Zhou *et al.* (2017) Phenol, 2-nitrophenol (2-NP), 2,4-dichlorophenol (2,4-DCP), 2,4,6-trichlorophenol (2,4,6-TCP), and pentachlorophenol (PCP) are common phenolic compounds that can be released into the environment through industrial sewage drainage, agricultural irrigation, pesticide application, and pharmaceutical drugs that are very likely to enter the human body through the food chain.

The composition of bacterial communities in a given environment is determined by the interaction of various factors such as anthropogenic activities (Wu *et al*., 2012). The physico-chemical parameters in this research upstream and downstream of the Jukskei River were similar. Coupled with the microbial community profile, it is evident that the EC levels had the most significant correlation with the top bacterial species. Given the high EC levels in the Jukskei River and its positive correlation to *Actinobacteria, Proteobacteria,* and *Firmicutes*, this study further supports studies by Fagorzi *et al.* (2019) on their ability to survive extreme conditions. A study conducted by Zhu *et al.* (2013) was largely consistent with the results obtained in this research. Both heavy metal concentrations, as well as microbial community profiles were similar. In their study, sediments were abundant in *Proteobacteria* and *Firmicutes*. It was also noted that, *Proteobacteria* abundance increased with heavy metal concentrations.

Additionally, a study performed by Abia *et al.* (2018) on the metagenomic analysis and functional profiles of bacterial communities in water and sediments of the Apies River, corroborates our study done on the Jukskei River with regards to the impact of land use on bacterial communities. Just like the Jukskei River, informal settlement samples had the highest bacterial abundance and diversity when compared to rural and peri-urban settlement samples, highlighting the influence of informal settlements on the poor microbiological quality of water bodies, particularly in developing nations. Furthermore, their findings also indicated that the bacteria exhibited a variety of functional profiles

.

According to literature, the bacterial phyla *Proteobacteria, Bacteroidetes,* and *Firmicutes*, which harboured a larger suite of metal-resistance genes, were found to be the core functional phyla in the water samples. The results in this study is also consistent with that done by Chen *et al.* (2018). Their study revealed the primary phyla in the polluted sediments had metal-resistance genes *Proteobacteria* and *Bacteroidetes* and were the most common phyla in the water samples.

Recommendations and future research/perspectives

Due to the high population density in the Alexandra Township, it would be difficult to implement sewage sanitation services. The shacks are built so close together that there is little room for the construction of services such as sewer systems. Additionally, the main sewer lines are approximately 1.5 km away from the informal settlements. The Alexandra township has very little town planning, as shacks are built haphazardly. As these structures are mostly informal houses, constructing pipelines and sewer systems to connect these would be impossible unless the entire township undergoes town planning. This would be an expensive project for the municipality to take on.

To comprehend and utilize unculturable microorganisms, metagenomics provides access to the enormous variety of the microbial world and has significantly advanced both academic communities and industrial settings. A compelling method for the identification of new enzymes and bioactives derived from uncultured bacteria is functional metagenomics. Though, functional metagenomics is still facing obstacles because it is a relatively new technique, the potential of a method that has already demonstrated success in its infancy shows that there may be large benefits if suitable solutions and additional optimization are implemented. The development of new screening and selection techniques along with faster and cheaper sequencing technologies will allow for the expansion of a very promising field in microbiology, genetics and the food and pharmaceutical industries. This work discussed the potential of various aspects of physicochemical analysis, microbiome profiling and functional metagenomics to facilitate the development of novel industrial products sourced from as yet uncultured microorganisms. However, difficulties arise in the creation of a consumer-friendly and commercially viable product that can be produced in industrially significant amounts and maintains its activity when scaled up after the discovery of beneficial proteins and bioactives (for example when present in high amounts in a large industrial reaction vessel), can be purified and formulated appropriately into a finished product and maintains its stability during shipping and storage. The product must work effectively under the suggested/outlined circumstances to do the task it was purchased to undertake. It must also be relatively simple to use and adaptable to contemporary industrial expectations. A respectable reaction accomplished in a lab setting could be challenging to replicate on a large scale. To find any variables or flaws in the reaction that were not obvious during the reaction's development in the laboratory, pilot plant studies must be conducted first. These investigations serve as a bridge between the identification of the intriguing active ingredient and its formulation into a finished, marketable product. Further research is then required to qualify the agent at an industrial level and ensure the production of a robust product that is effective and faithful to its intended purpose once inadequacies and other issues in the pilot plant phase have been resolved. The acceptability of the novel enzyme or bioactive and its source microorganism to the relevant regulatory authorities must also be considered.

REFERENCES

Abbas, M. and Cheema, K.J., 2015. Arsenic levels in drinking water and associated health risk in district Sheikhupura, Pakistan. *Journal of Animal and Plant Sciences*, *25*(3), pp.719-724.

Abraham, M.R. and Susan, T.B., 2017. Water contamination with heavy metals and trace elements from Kilembe copper mine and tailing sites in Western Uganda; implications for domestic water quality. *Chemosphere*, *169*, pp.281-287.

Adam, E.A., Collier, S.A., Fullerton, K.E., Gargano, J.W. and Beach, M.J., 2017. Prevalence and direct costs of emergency department visits and hospitalizations for selected diseases that can be transmitted by water, United States. *Journal of water and health*, *15*(5), pp.673-683.

Ahmed, S. and Ismail, S., 2018. Water pollution and its sources, effects & management: a case study of Delhi. *Shahid Ahmed and Saba Ismail (2018)'Water Pollution and its Sources, Effects & Management: A Case Study of Delhi', International Journal of Current Advanced Research*, *7*(2), pp.10436-10442.

Alavanja, M.C., 2009. Introduction: Pesticides use and exposure, extensive worldwide. *Reviews on environmental health*, *24*(4), pp.303-310.

Amin, M.T., Alazba, A.A. and Manzoor, U., 2014. A review of removal of pollutants from water/wastewater using different types of nanomaterials. *Advances in Materials Science and Engineering*, *2014*.

Anbu, P., Gopinath, S.C., Chaulagain, B.P. and Lakshmipriya, T., 2017, Microbial enzymes and their applications in industries and medicine.

Anirban, C., Chanchal, K.G., Punyasloke, B., 2014, Application of Molecular Techniques for the Assessment of Microbial Communities in Contaminated Sites. *Microbial Biodegradation and Bioremediation,* Elsevier. pp. 85-113.

Barbosa, A., Solano, M.D.L. and Umbuzeiro, G.D.A., 2015. Pesticides in drinking water – the Brazilian monitoring program. *Frontiers in public health*, *3*, p.246.

Barnett, J.B. and Brundage, K.M., 2010. Immunotoxicology of Pesticides and Chemotherapies.

BBC News 2015 'Telephone farmers' reaping the benefits of agri-tech. Available at: https://www.bbc.com/news/business-33610593 (accessed 14 November 2020)

Brahmachari, G., Demain. A.L. and Adrio, J.,L 2017, Biotechnology of microbial enzymes 11, pp 267-98.

Brown, C.M., Staley, C., Wang, P., Dalzell, B., Chun, C.L. and Sadowsky, M.J., 2017. A high-throughput DNA-sequencing approach for determining sources of fecal bacteria in a Lake Superior estuary. *Environmental science & technology*, *51*(15), pp.8263-8271.

Chakraborty, A. and Bhadury, P., 2015, Effect of pollution on aquatic microbial diversity. In: Environmental Microbial Biotechnology (pp. 53-75). Springer, Cham.

Chen, Q.Y., DesMarais, T. and Costa, M., 2019. Metals and mechanisms of carcinogenesis. *Annual review of pharmacology and toxicology*, *59*, pp.537-554.

Chow, C., Saint, C., Zappia, L., Henderson, R., Roeszler, G., Dexter, R., Nguyen, T., Stuetz, R., Byrne, A., Trolio, R. and Lucas, J., 2014. Online water quality monitoring: The voice of experience. *Water: Journal of the Australian Water Association*, *41*(2).

Collier, S.A., Stockman, L.J., Hicks, L.A., Garrison, L.E., Zhou, F.J. and Beach, M.J., 2012. Direct healthcare costs of selected diseases primarily or partially transmitted by water. *Epidemiology & Infection*, *140*(11), pp.2003-2013.

Daughton, C.G., 2020. Wastewater surveillance for population-wide Covid-19: the present and future. *Science of the Total Environment*, *736*, p.139631.

De Vocht, F., Sobala, W., Wilczynska, U., Kromhout, H., Szeszenia-Dabrowska, N. and Peplonska, B., 2009. Cancer mortality and occupational exposure to aromatic amines and inhalable aerosols in rubber tire manufacturing in Poland. *Cancer epidemiology*, *33*(2), pp.94-102.

De Vries, W., Kros, J., Kroeze, C. and Seitzinger, S.P., 2013. Assessing planetary and regional nitrogen boundaries related to food security and adverse environmental impacts. *Current Opinion in Environmental Sustainability*, *5*(3-4), pp.392-402.

FAO [Food and Agriculture Organization of the United Nations]. Joint FAO/WHO Meeting on Pesticide Residues. Rome, Italy, 2019, ISBN 978-92-5-132086-0.

Folayan, M.O., El Tantawi, M., Schroth, R.J., Kemoli, A.M., Gaffar, B., Amalia, R. and Feldens, C.A., 2020. Association between environmental health, ecosystem vitality, and early childhood caries. *Frontiers in pediatrics*, *8*, p.196.

Gamble, A., 2019. Ullmann's encyclopedia of industrial chemistry. *The Charleston Advisor*, *20*(4), pp.46-50.

Ghassabian, A., Bell, E.M., Ma, W.L., Sundaram, R., Kannan, K., Louis, G.M.B. and Yeung, E., 2018. Concentrations of perfluoroalkyl substances and bisphenol A in newborn dried blood spots and the association with child behavior. *Environmental Pollution*, *243*, pp.1629-1636.

Goff, C., 2017. BDS conference – tour 2 – the gateway to the lakes. Dams and Reservoirs, 27(2), pp.54-55.

Grossman, M.R., 2007. Agriculture and the polluter pays principle (keynote speech). In *XVII Congress of the International Academy of Comparative Law, Utrecht, 16-22 July 2007* (pp. 409-479).

Gurung, N., Ray, S., Bose, S. and Rai, V., 2013. A broader view: microbial enzymes and their relevance in industries, medicine, and beyond. BioMed research international, 2013.

Hamdi, H. and Hellal, A., 2019. Optimization of phenol biodegradation by immobilized Bacillus subtilis isolated from hydrocarbons-contaminated water using the factorial design methodology. *Journal of the Serbian Chemical Society*, *84*(7), pp.679-688.

Han, D., Currell, M.J. and Cao, G., 2016. Deep challenges for China's war on water pollution. *Environmental Pollution*, *218*, pp.1222-1233.

Haward, M., 2018. Plastic pollution of the world's seas and oceans as a contemporary challenge in ocean governance. *Nature communications*, *9*(1), pp.1-3.

Heikkinen, S., Pitkäniemi, J., Sarkeala, T., Malila, N. and Koskenvuo, M., 2015. Does hair dye use increase the risk of breast cancer? A population-based case-control study of Finnish women. *PloS one*, *10*(8), p.e0135190.

Hemme, C.L., Deng, Y., Gentry, T.J., Fields, M.W., Wu, L., Barua, S., Barry, K., Tringe, S.G., Watson, D.B., He, Z. and Hazen, T.C., 2010. Metagenomic insights into evolution of a heavy metal-contaminated groundwater microbial community. *The ISME journal*, *4*(5), pp.660-672.

Henderson, C. Xylem technology used to study major river systems. Available on https://www.xylem.com/en-in/making-waves/industrial-news/xylem-technology-used-to-study-major-river-systems/ (Accessed 13/07/2021).

Huang, K., Chen, C., Shen, Q., Rosen, B.P. and Zhao, F.J., 2015. Genetically engineering Bacillus subtilis with a heat-resistant arsenite methyltransferase for bioremediation of arsenic-contaminated organic waste. *Applied and environmental microbiology*, *81*(19), pp.6718-6724.

Hunter-Cevera, J.C., 1998. The value of microbial diversity. Current Opinion in Microbiology, 1(3), pp.278-285.

Inyinbor Adejumoke, A., Adebesin Babatunde, O., Oluyori Abimbola, P., Adelani Akande Tabitha, A., Dada Adewumi, O. and Oreofe Toyin, A., 2018. Water pollution: effects, prevention, and climatic impact. *Water Challenges of an Urbanizing World*, *33*, pp.33-47.

Izah, S.C., Chakrabarty, N. and Srivastav, A.L., 2016. A review on heavy metal concentration in potable water sources in Nigeria: Human health effects and mitigating measures. *Exposure and Health*, *8*(2), pp.285-304.

Jordaan, K. and Bezuidenhout, C.C., 2013. The impact of physico-chemical water quality parameters on bacterial diversity in the Vaal River, South Africa. Water Sa, 39(3), pp.385-396.

Jurkowski, A., Reid, A.H. and Labov, J.B., 2007. Metagenomics: a call for bringing a new science into the classroom (while it's still new). *CBE—Life Sciences Education*, *6*(4), pp.260-265.

Khan, M.R., Saha, M.L. and Afroz, H., 2001. Microorganisms associated with gemstones. Bangladesh Journal of Botany, 30(2), pp.93-96.

Khorooshi, S., Mostafazadeh, Raoof, Esmali-Ouri, Abazar, and Raoof, Majid, 2016, River health, importance and application. Extension and Development of Watershed Management, 4(13).

Kokkinaki, A., Kokkinakis, M., Kavvalakis, M.P., Tzatzarakis, M.N., Alegakis, A.K., Maravgakis, G., Babatsikou, F., Fragkiadakis, G.A. and Tsatsakis, A.M., 2014. Biomonitoring of dialkylphosphate metabolites (DAPs) in urine and hair samples of sprayers and rural residents of Crete, Greece. *Environmental research*, *134*, pp.181-187.

Kolya, H., Hashitsume, K. and Kang, C.W., 2021. Recent Advances in Colorimetric Detection of Arsenic Using Metal-Based Nanoparticles. *Toxics*, *9*(6), p.143.

Krishnaveni, M., Asha, S., Vini, S.S. and Punitha, S.M.J., 2018. Metagenomics of Marine Invertebrate-Microbial Consortium. In Metagenomics (pp. 255-272). Academic Press.

Kshetri, N. and Voas, J.M., 2021. Major Computing Technologies of the Past 75 Years. *Computer*, *54*(5), pp.15-21.

Leketa, K., Abiye, T., Zondi, S. and Butler, M., 2019. Assessing groundwater recharge in crystalline and karstic aquifers of the Upper Crocodile River Basin, Johannesburg, South Africa. Groundwater for Sustainable Development, 8, pp.31-40.

Mansour, S.A., 2004. Pesticide exposure — Egyptian scene. *Toxicology*, *198*(1-3), pp.91-115.

Manzano-Salgado, C.B., Granum, B., Lopez-Espinosa, M.J., Ballester, F., Iñiguez, C., Gascón, M., Martínez, D., Guxens, M., Basterretxea, M., Zabaleta, C. and Schettgen, T., 2019. Prenatal exposure to perfluoroalkyl substances, immune-related outcomes, and lung function in children from a Spanish birth cohort study. *International journal of hygiene and environmental health*, *222*(6), pp.945-954.

Matowanyika, W., 2010. Impact of Alexandra Township on the water quality of the Jukskei River (Doctoral dissertation, University of the Witwatersrand).

Mohamed, N.N., 2021. Optimum Economic Uses of Precious Costly Ground Water in Marginal and Desert Lands; Case Study in Egypt. In *Groundwater in Egypt's Deserts* (pp. 373-393). Springer, Cham.

Msadala, V.C. and Basson, G.R., 2017. Revised regional sediment yield prediction methodology for ungauged catchments in South Africa. Journal of the South African Institution of Civil Engineering, 59(2), pp.28-36.

Nawy, T., 2013. Microbial planet. *Nature methods*, *10*(9), pp.826-826.

Nema, V., 2019. The Role and Future Possibilities of Next-Generation Sequencing in Studying Microbial Diversity. In Microbial Diversity in the Genomic Era (pp. 611-630). Academic Press.

Ngara, T.R. and Zhang, H., 2018. Recent advances in function-based metagenomic screening. Genomics, proteomics & bioinformatics, 16(6), pp.405-415.

NX Filtration, 2020 NX Filtration's hollow fiber nanofiltration attacks summer droughts in the Netherland. Available at: https://nxfiltration.com/knowledge-base/publications/nx-filtrations-hollow-fiber-nanofiltration-attacks-summer-droughts-in-the-netherlands/ (accessed 23 November 2020)

Ockenden, M.C., Deasy, C., Quinton, J.N., Bailey, A.P., Surridge, B. and Stoate, C., 2012. Evaluation of field wetlands for mitigation of diffuse pollution from agriculture: Sediment retention, cost and effectiveness. *Environmental Science & Policy*, *24*, pp.110-119.

Olukunle, O., Okonkwo, J., Kefeni, K. and Lupankwa, M., 2012. Concentrations of polybrominated diphenyl ethers in sediments from Jukskei River, Gauteng, South Africa. Bulletin of environmental contamination and toxicology, 88(3), pp.461-466.

Parmar, S. and Singh, V., 2015. Phytoremediation approaches for heavy metal pollution: a review. *J Plant Sci Res*, *2*(2), p.135.

Pepper, I.L., 2019. Biotic characteristics of the environment. In *Environmental and Pollution Science* (pp. 61-87). Academic Press.

Pinheiro, J.C., dos Santos, L.T., Henriques, I., Silva, A. and Baraúna, R.A., 2019. Linking the Environmental Microbial Diversity and Antibiotic Resistance. In Microbial Diversity in the Genomic Era (pp. 451-457). Academic Press.

Qiao, F., Huang, J., Zhang, L. and Rozelle, S., 2012. Pesticide use and farmers' health in China's rice production. *China Agricultural Economic Review*.

Qureshi, S.; Qamar, F.N., 2020, Miscellaneous Bacterial Enteritides. 10th edition. *Hunter's Tropical Medicine and Emerging Infectious Diseases*, CT, USA, pp. 512-517.

Ray, S.G. and Ghangrekar, M.M., 2019. Comprehensive review on treatment of high-strength distillery wastewater in advanced physico-chemical and biological degradation pathways. *International Journal of Environmental Science and Technology*, *16*(1), pp.527-546.

Rezania, S., Park, J., Rupani, P.F., Darajeh, N., Xu, X. and Shahrokhishahraki, R., 2019. Phytoremediation potential and control of Phragmites australis as a green phytomass: an overview. *Environmental Science and Pollution Research*, *26*(8), pp.7428-7441.

Richey, A.S., Thomas, B.F., Lo, M.H., Reager, J.T., Famiglietti, J.S., Voss, K., Swenson, S. and Rodell, M., 2015. Quantifying renewable groundwater stress with GRACE. *Water resources research*, *51*(7), pp.5217-5238.

Rojas-Downing, M.M., Nejadhashemi, A.P., Harrigan, T. and Woznicki, S.A., 2017. Climate change and livestock: Impacts, adaptation, and mitigation. *Climate Risk Management*, *16*, pp.145-163.

Sanchez, S. and Demain, A., 2012. Special issue on the production of recombinant proteins. Biotechnology Advances, 5(30), pp.1100-1101.

Santhosh, C., Velmurugan, V., Jacob, G., Jeong, S.K., Grace, A.N. and Bhatnagar, A., 2016. Role of nanomaterials in water treatment applications: a review. *Chemical Engineering Journal*, *306*, pp.1116-1137.

Sharma, A., Kumar, V., Shahzad, B., Tanveer, M., Sidhu, G.P.S., Handa, N., Kohli, S.K., Yadav, P., Bali, A.S., Parihar, R.D. and Dar, O.I., 2019. Worldwide pesticide usage and its impacts on ecosystem. *SN Applied Sciences*, *1*(11), pp.1-16.

She, Y., Liu, Y., Deng, Y. and Jiang, L., 2020. Can China's Government-Oriented Environmental Regulation Reduce Water Pollution? Evidence from Water Pollution Intensive Firms. *Sustainability*, *12*(19), p.7841.

Shrivas, K., Shankar, R. and Dewangan, K., 2015. Gold nanoparticles as a localized surface plasmon resonance based chemical sensor for on-site colorimetric detection of arsenic in water samples. *Sensors and Actuators B: Chemical*, *220*, pp.1376-1383.

Singh, J., Saharan, V., Kumar, S., Gulati, P. and Kapoor, R.K., 2018. Laccase grafted membranes for advanced water filtration systems: a green approach to water purification technology. *Critical reviews in biotechnology*, *38*(6), pp.883-901.

Smith, E., Smith, J., Smith, L., Biswas, T., Correll, R. and Naidu, R., 2003. Arsenic in Australian environment: an overview. *Journal of Environmental Science and Health, Part A*, *38*(1), pp.223-239.

Sogin, M.L., Morrison, H.G., Huber, J.A., Welch, D.M., Huse, S.M., Neal, P.R., Arrieta, J.M. and Herndl, G.J., 2006. Microbial diversity in the deep sea and the underexplored "rare biosphere". Proceedings of the National Academy of Sciences, 103(32), pp.12115-12120.

Sposito, J.C., Montagner, C.C., Casado, M., Navarro-Martín, L., Solórzano, J.C.J., Piña, B. and Grisolia, A.B., 2018. Emerging contaminants in Brazilian rivers: occurrence and effects on gene expression in zebrafish (Danio rerio) embryos. *Chemosphere*, *209*, pp.696-704.

Stehle, S., Bline, A., Bub, S., Petschick, L.L., Wolfram, J. and Schulz, R., 2019. Aquatic pesticide exposure in the US as a result of non-agricultural uses. *Environment international*, *133*, p.105234.

Subramaniam, M., 2018. Controlling Water Resources from "Above": Global Water Forums. In *Contesting Water Rights* (pp. 95-152). Palgrave Macmillan, Cham.

Sultana, S. and Satyanarayana, A.N.V., 2021. Impact of Urbanization on Surface Energy Balance Components Over Metropolitan Cities of India During 2000-2018 Winter Seasons.

Sun, H.J., Rathinasabapathi, B., Wu, B., Luo, J., Pu, L.P. and Ma, L.Q., 2014. Arsenic and selenium toxicity and their interactive effects in humans. *Environment international*, *69*, pp.148-158.

Sweileh, W.M., Sa'ed, H.Z., Al-Jabi, S.W., Sawalha, A.F. and Shraim, N.Y., 2016. Drinking and recreational water-related diseases: a bibliometric analysis (1980-2015). *Annals of occupational and environmental medicine*, *28*(1), pp.1-11.

Tamabayeva, D., Duffy, L., Loring, P.A. and Barnes, D., 2013. Mitigation history of the industrial Hg contamination in the Nura River watershed of the Republic of Kazakhstan: Evolution of an Adaptive Management Approach. *Environmental Management and Sustainable Development*, *2*(1), p.183.

Tarrass, F. and Benjelloun, M., 2012. The effects of water shortages on health and human development. *Perspectives in public health*, *132*(5), pp.240-244.

Tchounwou, P.B., Yedjou, C.G., Patlolla, A.K. and Sutton, D.J., 2012. Heavy metal toxicity and the environment. *Molecular, clinical and environmental toxicology*, pp.133-164.

Thirunavukkarasu, A., Nithya, R. and Sivashankar, R., 2020. A review on the role of nanomaterials in the removal of organic pollutants from wastewater. *Reviews in Environmental Science and Bio/Technology*, pp.1-28.

UNEP, A., 2016. A snapshot of the world's water quality: Towards a global assessment. *Nairobi, United Nations Environment Programme*.

Varkey, T.J., 2016, Design and Development of Novel antibacterial nanofiber tea bags for clean drinking water. *J. Sci. Eng. Res.* 7, 699.

Vikesland, P.J. and Wigginton, K.R., 2010. Nanomaterial enabled biosensors for pathogen monitoring-a review. *Environmental science & technology*, *44*(10), pp.3656-3669.

Wang, L., Liu, X., Zhang, Q., Zhang, C., Liu, Y., Tu, K. and Tu, J., 2012. Selection of DNA aptamers that bind to four organophosphorus pesticides. *Biotechnology letters*, *34*(5), pp.869-874.

Water, U.N., 2020. World Water Development Report: Water and Climate Change.

Wells, H., 2018, How Melbourne, Australia Uses AI to Cut Water Treatment Costs. Available at https://news.itu.int/melbourne-cut-down-water-costs-using-ai/ (accessed 14 November 2020)

Wen, Y., Schoups, G. and Van De Giesen, N., 2017. Organic pollution of rivers: Combined threats of urbanization, livestock farming and global climate change. *Scientific reports*, *7*(1), pp.1-9.

Woodlief, T., Vance, S., Hu, Q. and DeWitt, J., 2021. Immunotoxicity of Per-and Polyfluoroalkyl Substances: Insights into Short-Chain PFAS Exposure. *Toxics*, *9*(5), p.100.

World Health Organization, 1993. *Guidelines for drinking-water quality*. World Health Organization.

Zeng, X., Chen, Q., Zhang, X., Li, H., Liu, Q., Li, C., Ma, M., Zhang, J., Zhang, W., Zhang, J. and Huang, L., 2019. Association between prenatal exposure to perfluoroalkyl substances and asthma-related diseases in preschool children. *Environmental Science and Pollution Research*, *26*(29), pp.29639-29648.