THE EFFECT OF TOXICANT POLLUTION ON THE GROWTH OF BACTERIA IN THE JUKSKEI RIVER

Mini dissertation submitted for the partial fulfilment of the Honors degree

Submitted by

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To

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DECLARATION

I hereby declare that the project work entitled "**The effect of toxicant pollution on the growth of bacteria in the Jukskei river**" submitted to the University of South Africa, Life and consumer science is a record of an original work done by me under the guidance of Dr Tracy Masebe Senior Lecturer (Agriculture and Life Sciences) Life and Consumer Sciences, College of Agriculture and Environmental Sciences , and this project work is submitted in the partial fulfilment of the requirements for the award of the degree of Honors in Life Sciences (Microbiology stream). The results embodied in this project have not been submitted to any other University or Institute for the award of any degree or diploma.

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DEDICATION

I dedicate this research project to my family and friends. I am grateful to my parents, Christopher and Omone Odiley who encouraged, supported and believed in me through the whole process. My best friend, Tumi Makofane never left my side and supported me through every step of the project and I am truly grateful for that. Finally, I dedicate this project to God Almighty. I believe that everything happened through Divine inspiration, wisdom and understanding and I have nothing but appreciation in my heart.

ABSTRACT

Toxicants are chemical substances that have negative effects on the health of an organism. Different toxicants are released into the environment especially rivers through anthropogenic activities such as mining, industrial activities and agriculture. These chemicals affect the health of the organisms that inhabit the river including microorganisms. The purpose of this study was to investigate the effect of some prominent toxicants on the growth of naturally occurring bacteria in the Jukskei river. The toxicants of choice were Naphthalene and Atrazine which have previously been found in the Jukskei river. Six bacterial species were isolated from two sampling sites (Marlboro and Bruma) in the Jukskei river through culturing methods like serial dilutions, spread and streak plating. The effect of Naphthalene and Atrazine was tested by exposing the bacterial isolates to the toxicants using 96-well microplates. The absorbance of the isolates was measured in a microplate reader at 600nm at 24 hour intervals over a duration of 144 hours. The same process was followed for *Escherichia coli* since it is used as an indicator organism for faecal contamination in rivers. Growth curves were plotted from the optical density in relation to time at the different concentrations. PCR was performed to confirm that the isolates were bacterial isolates and also to confirm that they are not *E.coli.* Atrazine and Naphthalene had negative effects on the growth of the bacteria but the bacteria did not die. The bacteria grew slower than the control (bacteria without toxicant). The isolates were indeed bacterial isolates. Toxicants have negative effects on the growth of naturally occurring bacteria in the Jukskei river. The consequences of these effects can disrupt the ecosystem.

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LIST OF ABBREVIATIONS

BLAST – Basic Local Alignment Search Tool

CFU/ML – Colony Forming Units per Millilitre

DEA – Department of Environmental Affairs

DMSO - Dimethylsulfoxide

DNA – Deoxyribonucleic Acid

DNTP – Deoxyribonucleotide Triphosphate

DO – Dissolved Oxygen

DWAF – Department of Water Affairs and Forestry

DWS – Department of Water and Sanitation

EC – Electrical Conductivity

MGCL2 – Magnesium Chloride

NCBI – National Center for Biotechnology Information

NMMP – National Microbial Monitoring Programme

NTMP – National Toxicity Monitoring Programme

NWA – National Water Act

PCR – Polymerase Chain Reaction

POPs – Persistent Organic Pollutants

RNA – Ribonucleic Acid

SOP – Standard Operating Procedure

TAE – Tris-Acetate-Ethylenediaminetetraacetic acid

TFTC – Too Few To Count

TNTC – Too Numerous To Count

1. INTRODUCTION

Microorganisms are important inhabitants of the aquatic environment. They play major roles in the niches they inhabit such as nutrient cycling and degradation of organic materials. Water pollution is a worldwide problem due to constant discharge of industrial and agricultural effluents into rivers. Chemicals which are harmful to living organisms, humans and the environment end up in rivers through direct discharge or run-off. Examples of these chemicals include pesticides such as Naphthalene, herbicides such as Atrazine and Simazine. These harmful chemicals have a negative impact on the living organisms that inhabit the rivers including microorganisms. Toxicity data involving microorganisms are limited. Coliform bacteria are used for monitoring faecal contamination in water systems and research regarding toxicants and bacteria has mostly been focused on the degradation of toxicants by aquatic bacteria. Information on the effect of toxicants on the naturally occurring microorganisms in rivers is limited (DeLorenzo *et.al*, 2001).

In this study, the effect of the toxicants Naphthalene and Atrazine on bacteria isolated from the Jukskei river was investigated. Bacteria isolated from the river were exposed to different concentrations of each toxicant and the effect on their growth was monitored. The goal of the study was to present the potential for the use of bacteria for assessing the level of chemical toxicity in the Jukskei river and other water systems.

1.1. RATIONALE OF THE STUDY

The Jukskei River receives effluent from industrial activities and run-off from waste dumps and agricultural activities (Sibali *et.al*, 2008). In addition, litter and rubble are deposited in the river and along the river banks. There are informal human settlements that are in close proximity to the river (Moropa, 2015). Taking into consideration the level of pollution in the Jukskei River, the use of the water poses a health hazard especially to those who live in the informal settlements.

The South African Department of Water and Sanitation (DWS) is the custodian of the country's water resources and they are responsible for monitoring and providing adequate water quality information according to the National Water Act (NWA) of South Africa (Act 36 of 1998). The National Toxicity Monitoring Programme (NTMP) and the National Microbial Monitoring Programme (NMMP) are examples of the ways in which the DWS are

carrying out the mandate of the NWA (DWAF, 2002 and 2005). The NTMP reports on the status and trends observed in surface water regarding the toxicants found in the water and the toxic effects of the water on living organisms. The NMMP reports on status and trends observed in surface waters with regards to faecal contamination and the health risks to humans using the water. Monitoring sites for both monitoring programmes are chosen based on the anthropogenic activities that occur at those areas and likelihood of water contamination from these activities e.g. industrial, agricultural, mining and domestic activities (DWAF, 2002 and 2005).

Currently, microbial water quality monitoring involves the use of indicator organisms for faecal contamination of water. The main concern with microbial water quality monitoring is the release of waste water from municipal sewers. Rivers contaminated by such effluent usually contain pathogenic bacteria and are a health hazard especially if the river is a direct source of drinking water. *Escherichia coli* and total coliforms are the main focus of this type of water quality monitoring (DWAF, 2002). The use of indicator organisms for faecal contamination does not provide the overall pollution status of the rivers. However, microbial monitoring of all microbial species present in the water would be too expensive and not all microbes can be cultured in the laboratory environment. The release of toxic chemicals (toxicants) into the environment has a negative effect on the aquatic flora and fauna. Investigation of the effects of chemical pollution on the microbial population in the water resources has been previously recorded especially for trace and heavy metals (Staley *et.al*, 2014). However, this has not been investigated in the context of the Jukskei River. It is therefore an area worthy of exploration.

Investigating the relationship between chemical pollution and the growth of bacteria in the Jukskei river may lead to the discovery of novel indicator organisms specifically for chemical pollution. Information gathered from this research may provide the possibility to assess the level of chemical toxicity in the water resources using the microbiota of that water body. The creation of a profile of the types of bacteria that are more prominent at different sampling sites and the level of toxicity such a profile represents is also a possibility. This could provide a range of new ways to monitor toxicity in the National Toxicity Monitoring Programme (NTMP). The potential for this type of research is vast however; the immediate focus of the research is to observe the effect of chemical pollutants on the bacteria in the Jukskei River.

1.2. HYPOTHESIS

Toxicant pollution has a negative effect on the growth of naturally occurring bacteria in the Jukskei river.

1.3. RESEARCH QUESTIONS

A. What effect does toxicant pollution have on the growth of the naturally occurring bacteria in the Jukskei river?
B. What are the prevalent bacterial strains in highly chemically contaminated rivers such as the Jukskei river?

1.4. AIM

To investigate the effect of organic pollutants on the growth of naturally occurring bacteria in the Jukskei river

1.5. SPECIFIC OBJECTIVES

- Isolate naturally occurring bacteria from water samples obtained from the Jukskei river
- Investigate the effect of the selected toxicants on the bacterial isolates
- Identify the bacteria isolated from the river

2. LITERATURE REVIEW

A toxicant is defined as a chemical substance that can have negative health effects on an organism. The effect can be to the organism as a whole, to particular tissues or organs or on a cellular level (DWAF, 2005). Toxicants have negative effect on the environment. In rivers, these effects can be observed as fish deaths and sometimes, the disruption of normal hormonal activities in some animals i.e. endocrine disruption (DWAF, 2005). Toxicants such as plastic and their degraded products have been documented to cause the deaths of animals such as birds, fish and turtles by suffocation or blocking of the digestive tract causing death by starvation. The toxicants that pollute water have also been observed to have negative effects on the growth, survival and reproductive ability of aquatic organisms such as frogs, crabs, plants, many invertebrates and fish (Lithner, 2011). In a study done by Manikkam et al. (2012), rats were exposed to various toxicants i.e. the pesticide permethrin, a mixture of phthalates, dioxin and a hydrocarbon mixture. Upon investigation of the effect of the

toxicants on sexual reproduction and puberty, it was discovered that the toxicants caused early puberty in the rats and a reduction in their reproductive abilities, and these effects persisted for three generations (Mannikam et al., 2012). The effects of pollutants like Persistent Organic Pollutants (POPs) which are organic compounds that are resistant to environmental degradation ranges from cancer, reproductive disorders, nervous system damage and endocrine disruption (DEA, 2011).

Microorganisms are important members of an ecosystem and they perform activities crucial to the ecosystem such as biogeochemical cycling of elements like Carbon, Nitrogen and Sulphur. They form symbiotic relationships with plants and fungi which is important for the provision of oxygen. They are the primary decomposers in an ecosystem and facilitate the process of returning nutrients back into the soil and the atmosphere. Due to the significance of microorganisms in the environment, it is important to investigate the effect of toxicants on the natural microbial communities that occur within rivers. It has been found that the microbiota in rivers is affected by environmental pollutants present in the rivers. Barnhart and Vestal (1983) investigated the effect of toxicants on the metabolic activity of natural microbial communities. They determined that Mercury, Cadmium and heavy metals were toxic to the microorganisms that are naturally occurring in waters and sediments of the Ohio river. These metals inhibited the microbial activity of the microbiota found in the river and sediments (Barnhart and Vestal, 1983). A study performed by Brosche (2010) displayed the toxic effects of the release of pharmaceutical chemicals into rivers. The pharmaceuticals exhibited antibiotic effects against the natural microbiota that occur within surface waters (Brosche, 2010). Many other studies have been carried out that demonstrate the effect of chemical pollution on microorganisms. For instance, *Thiobacillus sp* has been recorded to be an indicator for the presence of Mercury in the marine environment. These bacteria were observed to oxidize toxic mercury and release mercury ions into the food chain which are stored in fat tissue, thereby causing harm to other species of microbes, plants and animals in the environment (Sumampouw and Risjani, 2014). Yao and colleagues performed a study that showed that the composition of the bacterial community in the Jiaozhou Bay is strongly influenced by heavy metals (Yao et.al, 2017).

Toxicants such as Naphthalene and Atrazine have been observed to be toxic towards microorganisms. They have been shown to interfere with photosynthesis, respiration and many biosynthetic reactions as well as cell growth and cell division. Herbicides such as

Atrazine tend to be more toxic towards photosynthetic bacteria by inhibiting chlorophyll formation. Many studies regarding the degradation of toxicants by soil bacteria have been performed well (DeLorenzo *et.al*, 2001). However, research concerning the effect of toxicants on the naturally occurring aquatic microorganisms is limited. Most of the studies that have been conducted are regarding the effect of herbicides especially Atrazine on algae. The concentrations used in these studies were far greater than the concentration used in this present study. Concentrations ranged from 0.04µg/L to 5000µg/L of Atrazine in most of the studies performed on algae. Studies on the effect of pesticides such as Naphthalene on aquatic bacteria are limited as well (DeLorenzo *et.al*, 2001).

Hudak and Fuhrman (1988) observed that certain planktonic bacteria can adapt to growing in high concentrations of Naphthalene (Hudak and Fuhrman, 1988). Muturi and colleagues observed that Atrazine and a mixture of other toxicants (malathion, carbaryl, permethrin and glyphosate) can disrupt aquatic microbial communities which has a negative effect on the invertebrates that feed on the bacteria (Muturi *et.al*, 2017).

This research project will focus on the bacterial microbiota of the Jukskei river and their interaction with the toxicants present in the water. The aim is to improve on current knowledge regarding the effect of toxicants on the microbial communities that occur naturally within surface waters.

3. MATERIALS AND METHODS

3.1. SAMPLE COLLECTION

Permission to collect water samples from the Jukskei River was obtained from the Department of Water and Sanitation. Samples were collected from two of the Department's toxicity monitoring programmes sampling sites. The sites were the Marlboro and the Bruma monitoring sites on the Jukskei River. Sample bottles were obtained from Resource Quality Information Services' (RQIS) laboratories. Samples were obtained by using the subsurface grab method whereby the bottle was dipped into the water and a forward scooping motion was performed until the bottle is full. Two 500mL sample bottles sterilized with Sodium thiosulphate were used for obtaining the water samples (DWAF, 2002). The bottles were labelled according to the names of the monitoring sites. Samples were stored in a cooler box with ice packs and transported to the RQIS laboratories (DWAF, 2005). The physicochemical

data of the river at both sites were recorded using the YSI 556 instrument. The parameters monitored were temperature, electrical conductivity, dissolved oxygen and pH.

3.2. ISOLATION OF BACTERIA FROM WATER SAMPLES

A serial dilution was set up using twelve test tubes, with six test tubes for each of the two water samples. The test tubes were filled with 9mL sterile distilled water and labelled from 10^{-1} to 10^{-6}. About 100mL of the Marlboro water sample was transferred into a 250mL Erlenmeyer flask. Working aseptically in a laminar flow hood, 1mL of the Marlboro sample was transferred into the first test tube (10^{-1}) using a micropipette and the test tube was vortexed. From this test tube, 1mL was transferred to the 10^{-2} tube and the tube was vortexed. This was repeated until the 10^{-6} tube. About 1mL of the sample in the 10^{-2} to 10^{-5} tubes were plated on four nutrient agar plates using the spread plate technique (Wise, 2006). These dilution factors were chosen because it was expected that this dilution range will yield distinct colonies that can be counted. This procedure was repeated for the Bruma water sample. The eight plates were labelled according to the dilution factor and sampling site and incubated at 30°C for 24 hours. The incubation temperature was 30°C which is the optimum temperature for isolating mesophilic bacteria which thrive in temperatures between 20-40°C and include mostly pathogenic and common environmental bacteria (Graw, 2018). The plates were not incubated at 37°C in order to minimize the risk of isolating human pathogens because pathogens tend to grow faster at 37°C than common environmental bacteria (Microbiology Society, 2016).

After incubating the plates for 24 hours, the colonies on the plates were counted and pure isolates were isolated from plates containing bacteria from the two sampling sites, Marlboro and Bruma. Three colonies were picked from the 10^{-3} plates of the two sampling sites using an inoculation loop working close to a flame. The colonies were streaked onto fresh nutrient agar plates using the streak plate method (Katz, 2008). The isolates from the Marlboro monitoring site were labelled isolates 1-3 and those from the Bruma monitoring site were labelled isolates 4-6.

3.3. PREPARATION OF TOXICANTS

3.3.1. Selection of toxicants

The toxicants were selected based on data obtained from previous National Toxicity Monitoring Programme (NTMP) reports. In the 2015/2016 and 2016/2017 hydrological years, Naphthalene and Atrazine were detected at the Marlboro monitoring site at levels lower than 0.1µg/L (DWS, 2016) and 0.4µg/L (DWS, 2017) respectively. Based on these findings, the two toxicants were selected toxicants to use for response testing on the six bacterial isolates.

3.3.2. Preparation of toxicant concentrations for the bacterial exposure

Stock solutions were provided of Atrazine and Naphthalene by Mr Cornelius Rimayi from RQIS. The stock solutions were 1L each and had a concentration of 50mg/L. The toxicants were dissolved in distilled water. Naphthalene was first dissolved in 7mL of Dimethylsulfoxide before filling up the volumetric flask to the 1L mark with distilled water. This was done because Naphthalene does not dissolve in water. Preparation of these standards was done according to the Standard Operating Procedure (SOP) of the RQIS organic chemistry laboratory (Rimayi and Ngwandula, 2015).

Preparation of the toxicants was done by making 0.5 dilutions. These diluted concentrations were used for exposing the bacteria to the toxicants i.e. Atrazine dilutions were 0.4, 0.2 & 0.1µg/L and Naphthalene dilutions were 0.1, 0.05 & 0.025µg/L. One more concentration was added to increase the range of concentrations for both toxicants i.e. 1µg/L. The concentrations used for the exposure of the isolates were as follows:

Atrazine: 1µg/L, **0.4µg/L**, 0.2µg/L and 0.1µg/L

Naphthalene: 1µg/L, **0.1µg/L**, 0.05µg/L and 0.025µg/L

The 1L stock solutions were diluted multiple times in order to achieve the desired concentrations for the exposure of the bacterial isolates. Firstly, the 1L stock solution was diluted into a 100ml volumetric flask by adding 1ml of the stock solution into the flask and filling it up to the 100ml mark with distilled water. This was done for both Atrazine and Naphthalene. The concentration of both toxicants in 100ml distilled water was 500µg/L respectively.

The different concentrations were then diluted in 500mL volumetric flasks. In order to prepare the 500ml equivalent of the 0.4µg/L concentration, 4ml of the Atrazine and 1ml of the Naphthalene from the 100ml solution was pipetted into a 500ml volumetric flask and filling it with distilled water. The flasks were labelled according to the toxicant name and concentration. The concentration of Atrazine in 500ml of water was 4µg/L. Half of this (250ml) was transferred into another flask and filling it up to the 500ml mark to make 2µg/L. This was repeated to make a 1µg/L solution of Atrazine. The same was done for Naphthalene and the following concentrations were achieved: 1µg/L, 0.5µg/L and 0.25µg/L. the final concentration was achieved by pipetting 10ml of the toxicants from the 100ml solution into a 500ml flask and filling it with distilled water to the 500ml mark. The concentration achieved from this was 10µg/L for both atrazine and naphthalene respectively.

The 96-well microplate was used to determine the effect of the toxicants on the bacterial isolates. The toxicant concentrations needed to be diluted further to suit the small volumes of the 96-well microplate. The wells in the microplate have a total capacity of 400µL. For the exposure of the isolates, the total volume for the tests was 300µL per well comprising the following volumes: a total of 200µL of Nutrient broth, 90µL of toxicant and 10µL of the bacterial suspension.

Table 3.3a abd 3-3b show the concentration (C1) of the toxicants that were prepared to achieve the desired toxicant concentrations (C2) in the 300µL mixture for the microplates taking into consideration the concentration prepared in 20mL flasks.

Table 3.3a: Concentrations (C1) of Atrazine and Naphthalene that need to be prepared in order to achieve the desired concentrations (C2) when 90µL of the toxicant is added in a total volume of 300µL in the microplates.

ATRAZINE				NAPHTALENE			
C1 (µg/L)	**V1 (mL)**	**C2 (µg/L)**	**V2 (mL)**	**C1 (µg/L)**	**V1 (mL)**	**C2 (µg/L)**	**V2 (mL)**
3.33	90	1	300	3.33	90	1	300
1.33	90	0.4	300	0.33	90	0.1	300
0.67	90	0.2	300	0.17	90	0.05	300
0.33	90	0.1	300	0.083	90	0.025	300

Table 3.3b shows the volume (V1) of the toxicants that were transferred from the 500mL volumetric flasks to the 20mL bottles to achieve the desired toxicant concentrations (C2) mixture in the 96-well microplates

Table 3.3b: Volumes (V1) of Atrazine and Naphthalene that need to be prepared in 20mL bottles from the 500mL stock solutions so as to achieve the desired concentrations (C2).

ATRAZINE				NAPHTALENE			
C1 (µg/L)	**V1 (mL)**	**C2 (µg/L)**	**V2 (mL)**	**C1 (µg/L)**	**V1 (mL)**	**C2 (µg/L)**	**V2 (mL)**
10	6.7	1	20	10	6.7	1	20
4	6.7	0.4	20	1	6.6	0.1	20
2	6.7	0.2	20	0.5	6.7	0.05	20
1	6.6	0.1	20	0.25	6.7	0.025	20

Therefore, approximately 7mL of each toxicant concentration was pipetted from the 500mL volumetric flasks into a 20mL bottle respectively. The bottle was filled up with distilled water up to the 20mL mark. The 20mL solution of each toxicant concentration of Naphthalene and Atrazine is what was used to carry out the exposure of the bacterial isolates to the toxicants.

3.3.3. Preparation of solvent for bacterial exposure

Dimethytsulfoxide (DMSO) was used to dissolve Naphthalene before mixing it with distilled water in the stock solution. During exposure of the bacterial isolates to the toxicants, a solvent control was added to the experiment to test whether the solvent (i.e. DMSO) has an effect on the growth of the bacterial isolates. During the preparation of the naphthalene stock solution, 7mL of DMSO was used to dissolve the Naphthalene. For the exposure tests, 0.7% (v/v) of DMSO was prepared by pipetting 700µL of DMSO into a 100mL volumetric flask and filling it to the 100mL mark with distilled water. In order to adjust this volume to suit the 300µL volume of the microplates, 6mL of the prepared DMSO solution was transferred to a 20mL bottle and filled to the 20mL mark with distilled water. This is the volume that was used to perform the exposure tests on the bacterial isolates.

3.4. EXPOSURE OF ISOLATES TO TOXICANTS

3.4.1. Determination of cell density

Isolates 1-6 were previously stored in a freezer in 1mL eppendorf tubes until they were ready to use. The isolates were allowed to thaw and 500µL of each isolate was transferred into test tubes containing 10mL of fresh nutrient broth respectively. The test tubes were labelled according to isolate number and incubated at 30°C for 18 hours. This same procedure was repeated for an *Escherichia coli* culture used as a negative control which was incubated at 37°C (optimum growth temperature for *E.coli*) for 18 hours. After 18 hours of growth, the bacteria reached the log phase of growth and were ready to be used for the exposure to the toxicants. However, the concentration of cell used needed to be determined. Saline solution (0.9%v/v) was previously prepared by adding 1L of distilled water to 9g of Sodium Chloride. About 1mL of each bacterial isolate and *E.coli* was transferred into 9mL saline solution respectively. Thereafter, 4mL of the saline solution and bacteria mixture were transferred into the appropriately labelled cuvettes. A spectrophotometer was used to measure the turbidity of the saline solution and bacteria mixture at 600nm.

A comparison to the McFarland turbidity standard was made using the absorbance of the saline solution to determine the concentration of cells in the saline solution. All the isolates including *E.coli* corresponded with the absorbance for McFarland standard 0.5 which ranges from 0.08 to 0.1 at 600nm. Therefore, the cell density in the saline solution for all the isolates and *E.coli* was approximately $1.5\,X\,10^8$ CFU/mL. The McFarland standards and their corresponding absorbances and cell density are shown in Figure 3.4a. Table 3.4 shows the absorbance values of each bacterial isolate.

McFarland Standard No.	0.5	1	2	3	4
1.0% barium chloride (ml)	0.05	0.1	0.2	0.3	0.4
1.0% sulfuric acid (ml)	9.95	9.9	9.8	9.7	9.6
Approx. cell density (1X10^8 CFU/mL)	1.5	3.0	6.0	9.0	12.0
% transmittance*	74.3	55.6	35.6	26.4	21.5
Absorbance*	0.08 to 0.1	0.257	0.451	0.582	0.669

*at wavelength of 600 nm

Figure 3.4a: Absorbance and cell density that corresponds with the McFarland standards obtained from https://openwetware.org/wiki/BISC209/F13:_Lab7

Table 3.4: The absorbance (600nm) values of the bacterial isolates in saline solution that corresponds with MacFarland standard 0.5.

Isolate	Absorbance at 600nm
1	0.084
2	0.182
3	0.079
4	0.097
5	0.087
6	0.084
E.coli	0.115

3.4.2. Inoculation of 96-well microplates

The 96-well microplates were inoculated according to the structure presented in the figures 3.4b and 3.4c below. The activity of Naphthalene and Atrazine were determined in separate plates. The microplates were inoculated with two isolates to determine the sensitivity of the isolates to the toxicants. Isolate 1 and 2 were exposed to the toxicants in a plate with Atrazine and one with Naphthalene.

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLANK											
B	SOLVENT CONTROL FOR ISOLATE 1											
C	SOLVENT CONTROL FOR ISOLATE 2											
D	CONTROL FOR ISOLATE 1											
E	1µg/L			0.1µg/L			0.05µg/L			0.025µg/L		
F	STERILE WATER											
G	CONTROL FOR ISOLATE 2											
H	1µg/L			0.1µg/L			0.05µg/L			0.025µg/L		

Figure 3.4b: illustrating the layout of the Naphthalene plates for all the isolates

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLANK											
B	STERILE WATER											
C	CONTROL FOR ISOLATE 1											
D	1µg/L			0.4µg/L			0.2µg/L			0.1µg/L		
E	STERILE WATER											
F	CONTROL FOR ISOLATE 2											
G	1µg/L			0.4µg/L			0.2µg/L			0.1µg/L		
H	STERILE WATER											

Figure 3.4c: illustrating the layout of the Atrazine plates for all the isolates

For the Atrazine plates:

About 300µL of nutrient broth was transferred into Row A using a multi-channel pipette. This row contains the blank which is the nutrient broth without toxicant or bacteria. Row B was filled with 300µL of sterile water, this is to prevent evaporation. Row C was filled with 290µL of nutrient broth and inoculated with 10µL of isolate 1 from the saline solution. This was used as the control for isolate 1. Row D was filled with 200µL of nutrient broth, 90µL of the 4 different concentrations of Atrazine were added to 3 wells each (triplicate) and 10µL of isolate 1 was added to all the wells in Row D. Row E was filled with 300µL of sterile water in order to prevent evaporation. Row F was filled with 290µL of nutrient broth and inoculated with 10µL of isolate 2 from the saline solution. This was used as the control for isolate 2. Row G was filled with 200µL of nutrient broth, 90µL of the 4 different concentrations of Atrazine were added to 3 wells each (triplicate) and 10µL of isolate 2 was added to all the wells in Row G. Row H was filled with 300µL of sterile water in order to prevent evaporation. The whole procedure was repeated for isolates 3 and 4, 5 and 6 and *E.coli.*

For the Naphthalene plates:

About 300µL of nutrient broth was transferred into Row A using a multi-channel pipette. This row contains the blank which is the nutrient broth without toxicant or bacteria. Row B was filled with 200µL of nutrient broth, 90µL of the 0.7% DMSO was added and 10µL of isolate 1 was added to all the wells in Row B. This was used as the solvent control for isolate 1. Row C was filled with 200µL of nutrient broth, 90µL of the 0.7% DMSO was added and 10µL of isolate 2 was added to all the wells in Row C. This was used as the solvent control for isolate 2. Row D was filled with 290µL of nutrient broth and inoculated with 10µL of isolate 1 from the saline solution. This was used as the control for isolate 1. Row E was filled with 200µL of nutrient broth, 90µL of the 4 different concentrations of Naphthalene were added to 3 wells each (triplicate) and 10µL of isolate 1 was added to all the wells in Row E. Row F was filled with 300µL of sterile water in order to prevent evaporation. Row G was filled with 290µL of nutrient broth and inoculated with 10µL of isolate 2 from the saline solution. This was used as the control for isolate 2. Row H was filled with 200µL of nutrient broth, 90µL of the 4 different concentrations of Naphthalene were added to 3 wells each

(triplicate) and 10µL of isolate 2 was added to all the wells in Row H. The whole procedure was repeated for isolates 3 and 4, 5 and 6 and *E.coli*.

The plates were placed on a shaker for 2 minutes at 600rpm before reading the absorbance for t0 at 600nm using a Biotek Powerwave XS microplate reader. The absorbance at 600nm reading was taken every 24 hours at 24hours, 48 hours, 72 hours and the final reading was taken at 144 hours. Before taking any reading on the microplate reader, the plates were shaken for 2 minutes at 600rpm. The absorbance values were measured and growth curves were plotted using the mean absorbance values of the isolates.

3.5. AMPLIFICATION OF 16SrRNA AND UIDA GENE REGIONS

3.5.1. DNA EXTRACTION

The 16S rRNA is a region that is highly conserved in different types of bacteria. Amplification of this region was performed in order to confirm that the isolates are indeed bacterial isolates. The Polymerase Chain Reaction (PCR) was performed on the extracted DNA from the isolates and *E.coli* in order to identify the bacterial isolates through DNA sequencing. Universal primers for the 16SrRNA region were used for the PCR process i.e. 27F as the forward primer and 518R as the reverse primer (Jacobs, 2016). The uidA primers specific for *E.coli* were included to determine whether some of the isolates are *E.coli* (Molina et.al, 2015).

Isolates 1-6 and *E.coli* were stored in a freezer in 1mL eppendorf tubes until they were ready to use. The isolates were allowed to thaw and 1mL of each isolate was transferred into test tubes containing 10mL of fresh nutrient broth respectively. The test tubes were labelled according to isolate number and incubated at 37°C for 18 hours.

DNA extraction was performed using the ZymoResearch Quick DNA^{TM} Miniprep Plus Extraction kit. Seven centrifuge tubes were labelled according to isolate name and the bacteria in the test tubes were then transferred into centrifuge tubes and centrifuged for 2 minutes at 4,400 x g. The supernatant was discarded with only a small portion left in order to enable resuspension of the bacterial cells. The resuspended cells were transferred into appropriately labelled microcentrifuge tubes and 200µL of Biofluid and cell buffer was added into each tube. About 20µL of Proteinase K was added to each tube and the suspension was vortexed. The tubes were incubated at 56°C for 10 minutes. After incubation, 500µL of Genomic DNA binding buffer was added to the tubes and mixed thoroughly. The mixture

was transferred to the Zymo-Spin™ IIC-XL Column in a Collection tube and centrifuged at 12000 x *g* for 1 minute and the collection tube was discarded with the flow through. Thereafter, 400µL of DNA pre-wash buffer was added to the Zymo-Spin™ IIC Column in a new collection tube and centrifuged at 12000 x *g* for 1 minute. About 700 µL of g-DNA wash buffer was added to the tubes and centrifuged at 12000 x *g* for 1 minute. G-DNA wash buffer was then added again but at 200 µL and the tubes were centrifuged at 12000 x *g* for 1 minute. The collection tubes were discarded and the columns were transferred into clean microcentrifuge tubes and 35µL of DNA Elution buffer was added to elute the DNA. The tubes were incubated at room temperature for 5 minutes and centrifuged at 12000 x *g* for 1 minute.

The BioDrop instrument was used to measure the concentration of the extracted bacterial DNA. The instrument was blanked using the DNA Elution buffer. Thereafter, 1µL of DNA from each tube was transferred onto the BioDrop instrument and the absorbance was measured for each drop of DNA from the different isolates.

3.5.2. Gel electrophoresis to confirm DNA extraction

The gel was prepared by weighing 1.25g of agarose gel powder and adding 100mL of 1xTAE buffer. The mixture was microwaved for 2 minutes and 2µL of ethidium bromide was added. The mixture was poured into a tray, a comb was placed into the gel and it was allowed to set. The gel was then placed in the electrophoresis chamber which was filled with 1xTAE buffer and the comb was removed from the gel. The DNA ladder was added to the first column of the gel. About 2µL of the DNA samples were mixed with 2µL of loading dye and the samples were loaded on the gel. The chamber was covered and plugged to the power supply which was set to run for 60 minutes at a voltage of 90V. After running the gel for 60 minutes, the BioRad Gel Doc was used to visualize the gel under UV light using the ImageLab software (Jacobs, 2016).

3.5.3. Polymerase Chain Reaction (PCR)

Fourteen PCR tubes were labelled according to the isolate number and primer used. Seven tubes were used for the 16SrRNA primers and the other seven tubes contained the uidA gene primers. Two master mixes were prepared by pipetting the volumes as shown in the table 4 below. Thereafter, 9µL of both master mixes containing the two primer sets was transferred into the 7 PCR tubes for each master mix and 1µL of the extracted DNA was added to the

tubes containing the master mix. A control PCR tube containing only master mix and water was included for both primer sets. Therefore each primer set includes 8 PCR tubes. The procedure was carried out in a PCR workstation to prevent contamination and all the reagents were kept on ice. Table 3.5b shows the volumes of each component of both of the mastermixes. The 16 PCR tubes were placed into the thermal cycler and the reaction was performed according to the temperatures shown in table 3.5c.

Table 3.5a: The volumes of each component used for the master mixes

PCR REAGENT	VOLUME (µL)
Buffer	9
MgCl2	5.4
dNTP	0.9
Forward primer – 27F or Forward primer for uidA gene	1.8
Reverse primer – 518R or Reverse primer for uidA gene	1.8
Taq polymerase	1.8
Nuclease free water	60.3
Total	**81**

Table 3.5b: The conditions in the thermal cycler for the PCR

STAGE	TEMPERATURE (°C)	TIME	
Pre-denaturation	95°C	60s	
Denaturation	95°C	30s	x 32 cycles
Annealing	55°C	30s	
Elongation	72°C	90s	
Final Elongation	72°C	7 minutes	
	Cool down to 4°C		

The PCR products were used to perform gel electrophoresis as described in section 3.5.2. The gel was run for 45 minutes at a voltage of 80V. The BioRad Gel Doc was used to visualize the gel under UV light using the ImageLab software.

3.6. Statistical analysis

The exposure test for each isolate in each toxicant concentration was done in triplicate. The average (mean) of the absorbance values were calculated using Microsoft Excel and growth curves were plotted of the average absorbance values in relation to time at different toxicant concentrations. The standard deviations of the triplicate absorbance values were also calculated. The standard deviation tables can be viewed in the Appendix 1.

4. RESULTS

4.1. SAMPLE COLLECTION

The sites which the water samples were collected from are the Marlboro and the Bruma monitoring sites on the Jukskei river. The Marlboro monitoring site is downstream of both the Alexandra Township and a major industrial complex. The other site is located in the suburb of Bruma which is known for having a small lake (DWAF, 2002). Table 4.1a provides more information about the two monitoring sites. Figures 4.1a and 4.1b show the current conditions at the sampling sites.

Table 4.1a: Description of the two sampling sites

Site name	Site I.D.	Coordinates	Date sampled	Time	No. of samples
Marlboro	188571	S 26.084 E 28.109	26-09- 2018	12:45	1
Bruma	193282	S 26.178 E 28.107	26-09-2018	13:22	1

Figure 4.1a: The Marlboro monitoring site

Figure 4.1b: The Bruma monitoring site

The physicochemical data of the river at both sites were recorded using the YSI 556 instrument. The parameters monitored were temperature, electrical conductivity, dissolved oxygen and pH. Table 4.1b shows the recorded physicochemical parameters at the sampling sites.

Table 4.1b: showing the physicochemical data of the river at the two sampling sites

Marlboro		Bruma	
Physicochemical properties	**Unit**	**Physicochemical properties**	**Unit**
Temperature	21.84	**Temperature**	23.72
Electrical Conductivity (µS/cm)	757	**Electrical Conductivity (µS/cm)**	552
pH	7.32	**pH**	7.15
Dissolved Oxygen (mg/L)	4.85	**Dissolved Oxygen (mg/L)**	3.24

4.2. Isolation of bacterial isolates from water samples

A serial dilution was performed to determine the colony forming units per mL of the samples. The results of the plate count are shown in Table 4.2a:

Table 4.2a: showing the plate counts and colony forming units per mL of the bacteria isolated from the water samples from the Marboro and Bruma monitoring sites

MARLBORO			BRUMA		
DILUTION FACTOR	**PLATE COUNT**	**CFU/ML**	**DILUTION FACTOR**	**PLATE COUNT**	**CFU/ML**
10^{-2}	TNTC	TNTC	10^{-2}	TNTC	TNTC
10^{-3}	283	$\mathbf{2.38 \times 10^{5}}$	10^{-3}	92	$\mathbf{9.2 \times 10^{4}}$
10^{-4}	124	$\mathbf{1.24 \times 10^{6}}$	10^{-4}	12	TFTC
10^{-5}	8	TFTC	10^{-5}	0	0

TFTC = Too few to count, TNTC = Too Numerous To Count, CFU/ML = Colony Forming Units per Milliliter

The bacterial isolates were picked randomly and differentiated according to colony morphology. The colony morphology of each isolate is shown in the Table 4.2b below:

Table 4.2b: Colony morphology of the selected isolates

	ISOLATES					
Colony Property	**Isolate 1**	**Isolate 2**	**Isolate 3**	**Isolate 4**	**Isolate 5**	**Isolate 6**
Form	Spindle	Circular	Rhizoid	Circular	Filamentous	Spindle
Elevation	Raised	Convex	Raised	Flat	Flat	Convex
Margin	Undulate	Entire	Lobate	Entire	Lobate	Curled
Appearance	Shiny	Shiny	Dull	Shiny	Dull	Shiny
Optical properties	Opaque	Opaque	Translucent	Opaque	Translucent	Opaque
Pigmentation	Cream	Cream	Cream	Cream	Cream	Cream
Texture	Smooth	Smooth	Rough	Smooth	Rough	Smooth

4.3. EXPOSURE OF ISOLATES TO TOXICANTS

4.3.1. Inoculation of 96-well microplates

The exposure of the bacteria to different concentrations of the toxicants was done in triplicate and the absorbance at 600nm was measured at 24 hour intervals. The average of the triplicate absorbance values was calculated and these average values were used to plot the growth curves for each isolate at different toxicant concentrations over time as illustrated in the respective figures. The standard deviation of the triplicate absorbance values were also calculated and are also indicated in the respective tables in Appendix 1.

4.3.2. Exposure of the isolates to Atrazine

When the test isolates were exposed to Atrazine, the toxicant inhibited the growth of the bacterial isolates and *E.coli*. Five of the bacterial isolates showed a growth pattern that was slower than the control (bacteria grown without toxicant). Isolate 1 is the only isolate that showed little difference in growth rate from its control at all the different Atrazine concentrations. The growth patterns of the six isolates and *E.coli* in the presence of different Atrazine concentrations are illustrated in figures 4.3.2a-4.3.2g.

Isolate 1 displayed little difference in growth patterns from the control at most of the Atrazine concentrations. It only showed a difference from the control when grown in 0.2µg/L of Atrazine. Isolate 1 had higher absorbance values than the control at 0.2µg/L of Atrazine than any other concentration. At 0.2µg/L of Atrazine, Isolate 1's absorbance values were 0.062, 0.902, 1.287, 1.352 and 1.143 at the respective times while the control absorbance values were 0.069, 0.608, 0.998, 1.085 and 1.179 at the respective times. Figure 4.3.2a illustrates the growth rate of isolate 1 at the different Atrazine concentrations overtime.

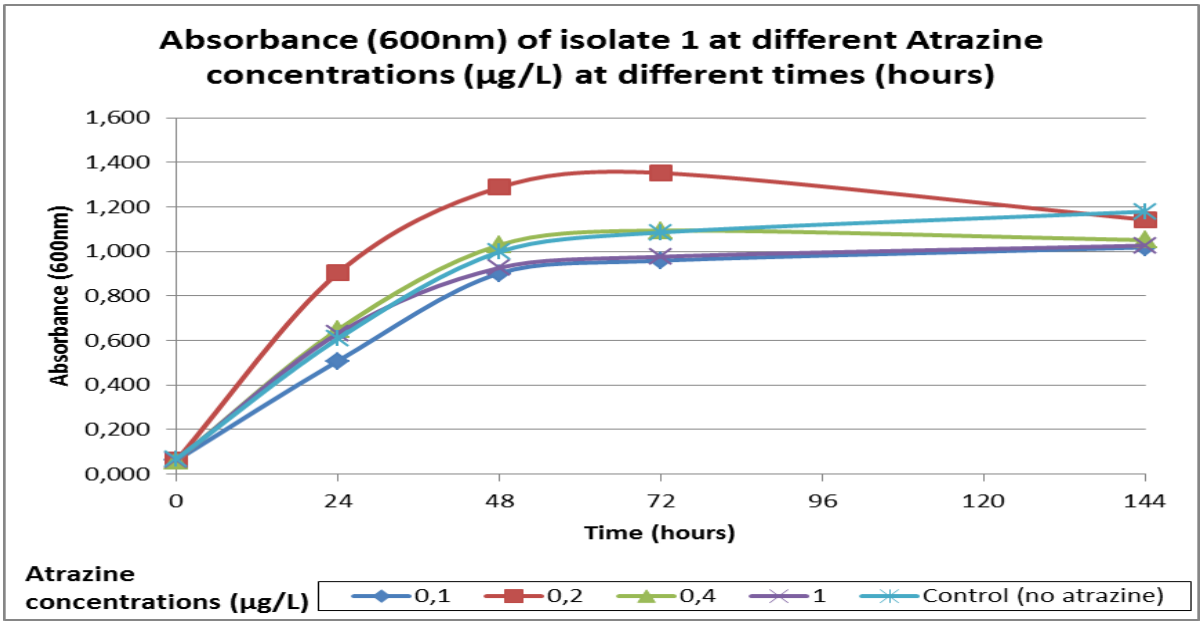

Figure 4.3.2a: showing the growth of Isolate 1 at absorbance (600nm) in different Atrazine concentrations over a period of time

Isolates 2-6 and *E.coli* all showed a slowed down growth rate compared to their controls (isolates grown without atrazine). They had lower absorbance values that the control at all the Atrazine concentrations. They grew at similar paces regardless of the Atrazine concentration. At the concentration of 0.2µg/L, the bacteria were growing a bit faster than their controls at 144 hours except isolate 4. This is the point where the bacteria have reached the death phase of their growth. The growth curves (Figures 4.3.2b – 4.3.2g) illustrate the mean absorbance values of the bacteria at different times and in different Atrazine concentrations.

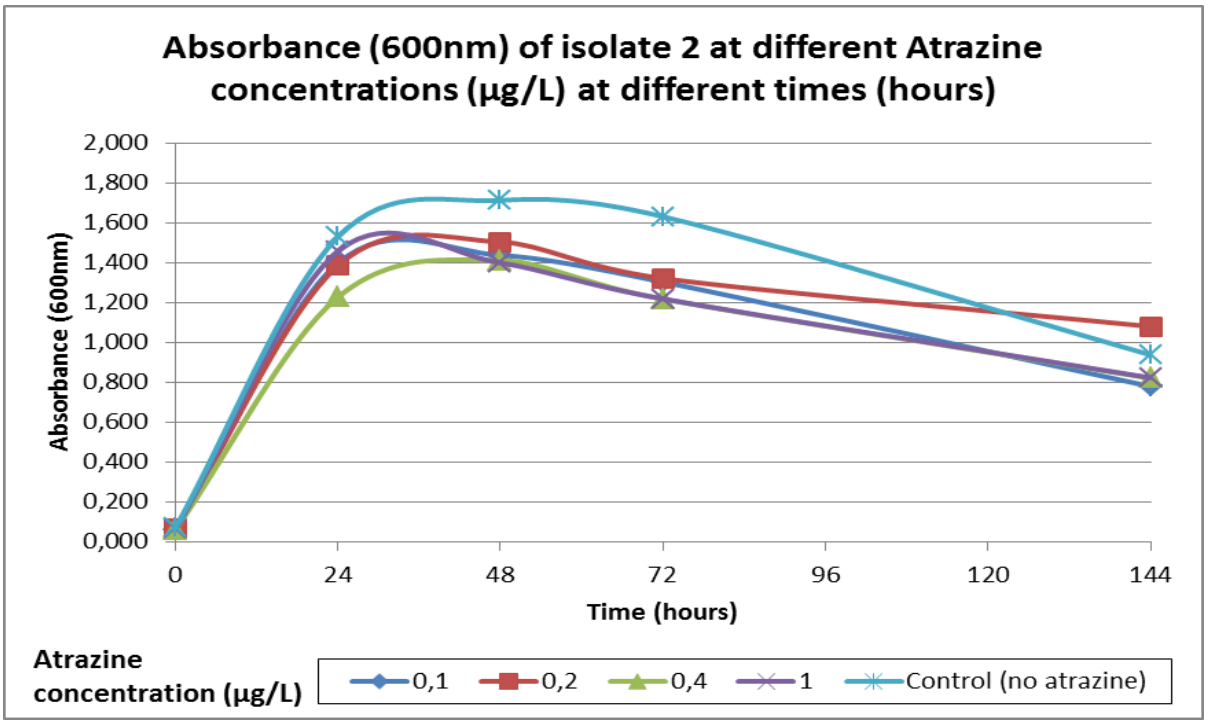

Figure 4.3.2b: showing the growth of Isolate 2 at absorbance (600nm) in different Atrazine concentrations over a period of time

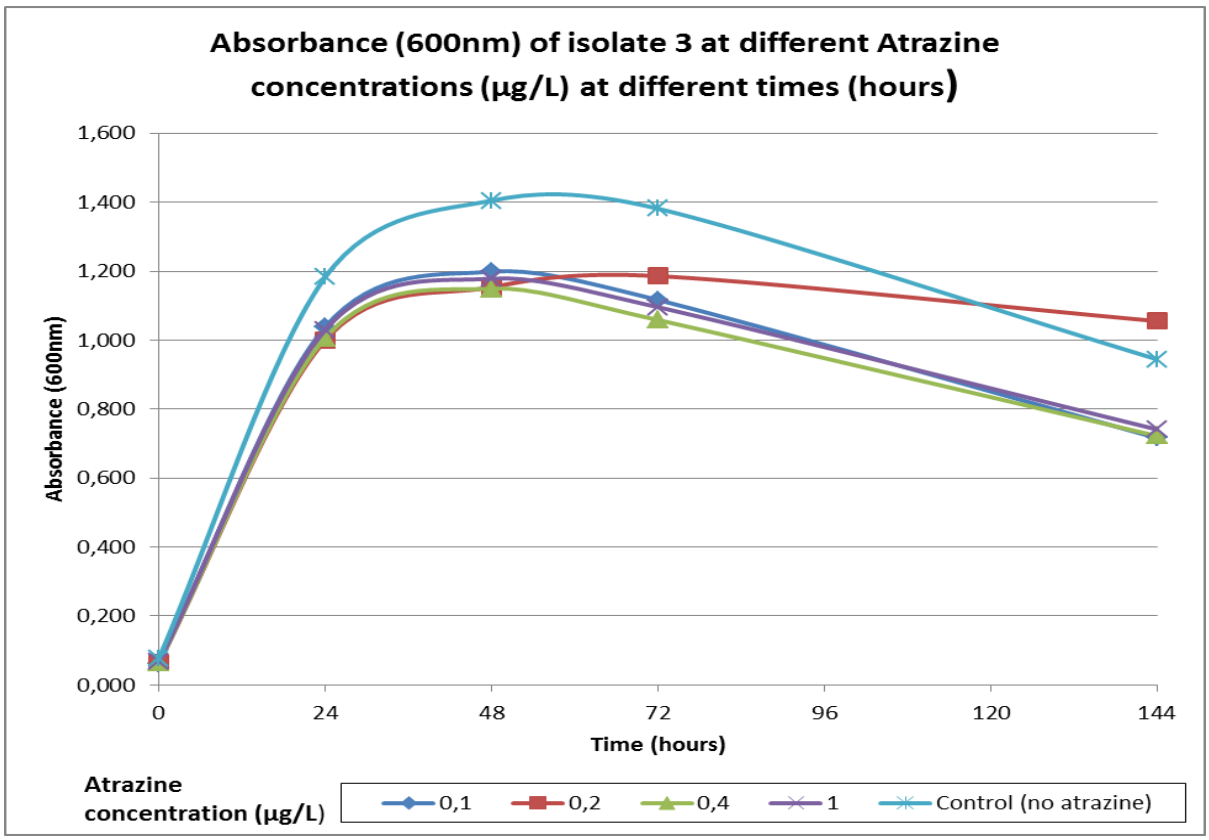

Figure 4.3.2c: showing the growth of Isolate 3 at absorbance (600nm) in different Atrazine concentrations over a period of time

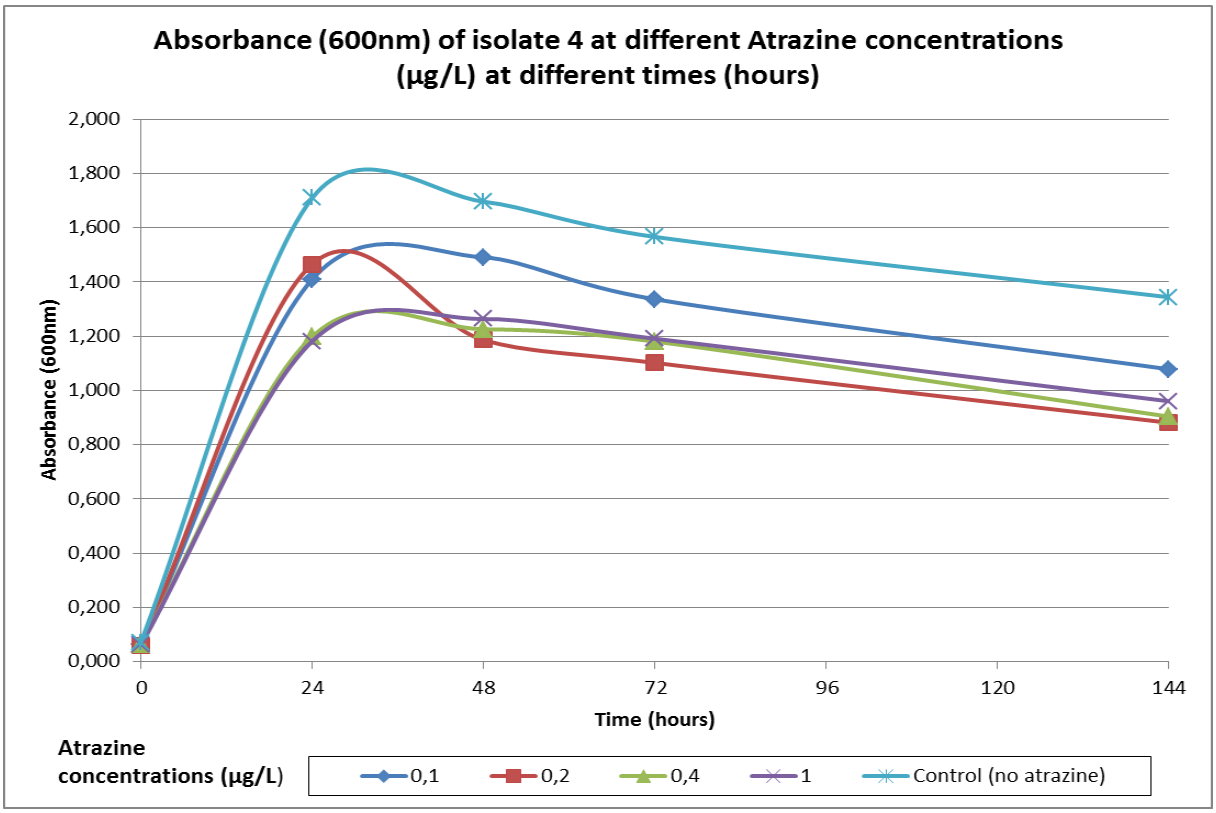

Figure 4.3.2d: showing the growth of Isolate 4 at absorbance (600nm) in different Atrazine concentrations over a period of time

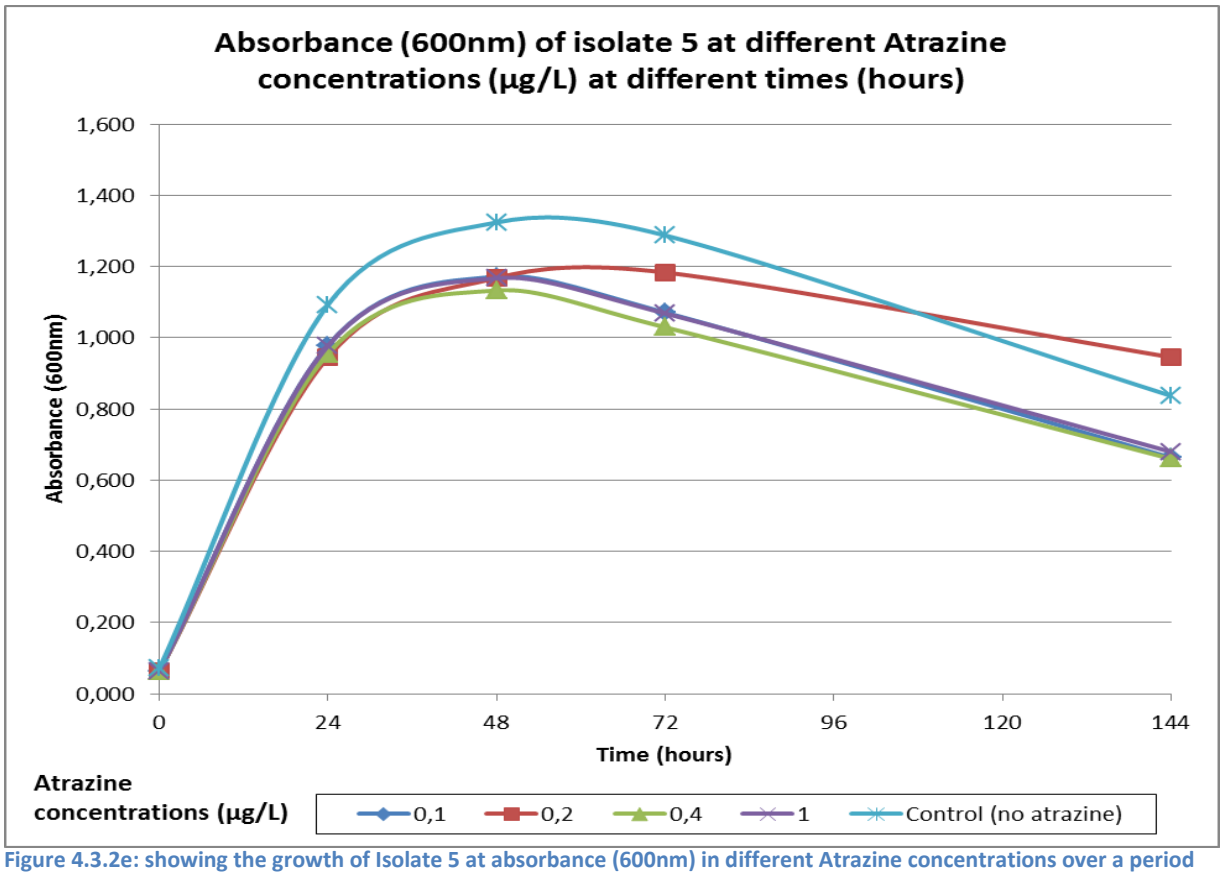

Figure 4.3.2e: showing the growth of Isolate 5 at absorbance (600nm) in different Atrazine concentrations over a period of time

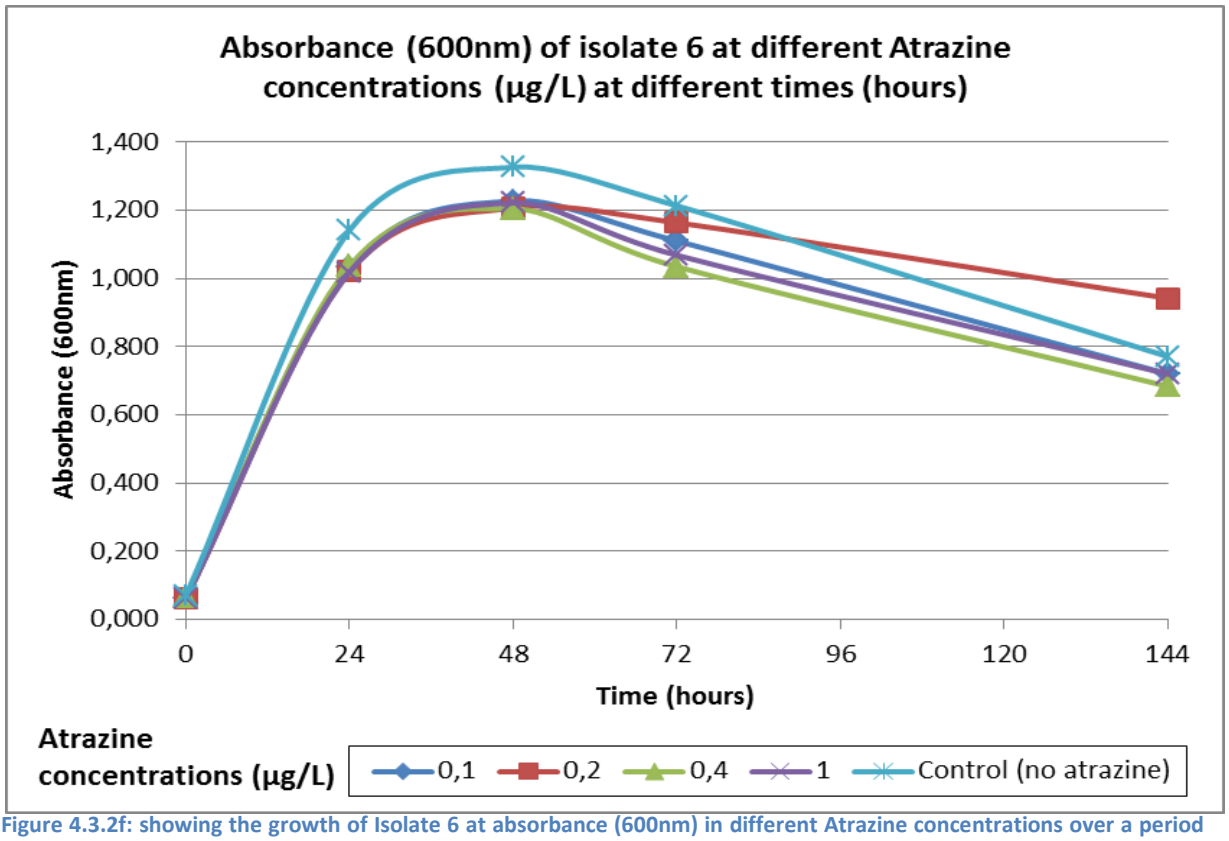

Figure 4.3.2f: showing the growth of Isolate 6 at absorbance (600nm) in different Atrazine concentrations over a period of time

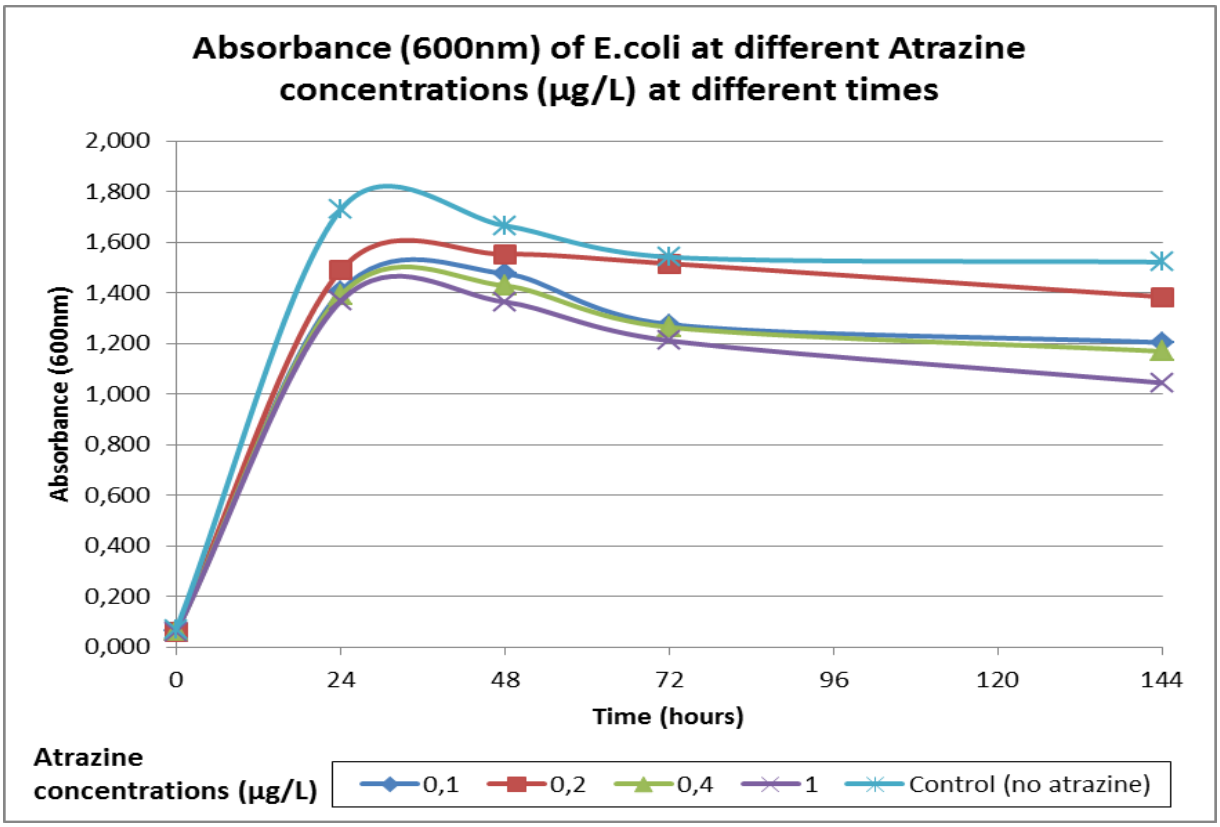

Figure 4.3.2g: showing the growth of *E.coli* at absorbance (600nm) in different Atrazine concentrations over a period of time

4.3.3. Exposure of the isolates to Naphthalene

The total effect of Naphthalene on the growth of the bacteria was calculated using the formula indicated in Equation 1. This was done in order to determine the true effect of Naphthalene alone on the bacterial isolates without the effect of the solvent (DMSO). The average absorbance values were calculated and the formula was applied to the average. Growth curves were plotted based on the final absorbance values of Naphthalene after applying the formula.

The growth curves (Figures 4.3.3a-4.3.3b) show that the growth of the isolates was slowed down by Naphthalene. For instance, Figure 4.3.3a shows the effect of Naphthalene on Isolate 1. The control had higher absorbance values than when the isolate was grown in different Naphthalene concentrations. A similar pattern was observed for *E.coli*. The bacteria did not die but they grew slower than their controls (Bacteria grown without Naphthalene).

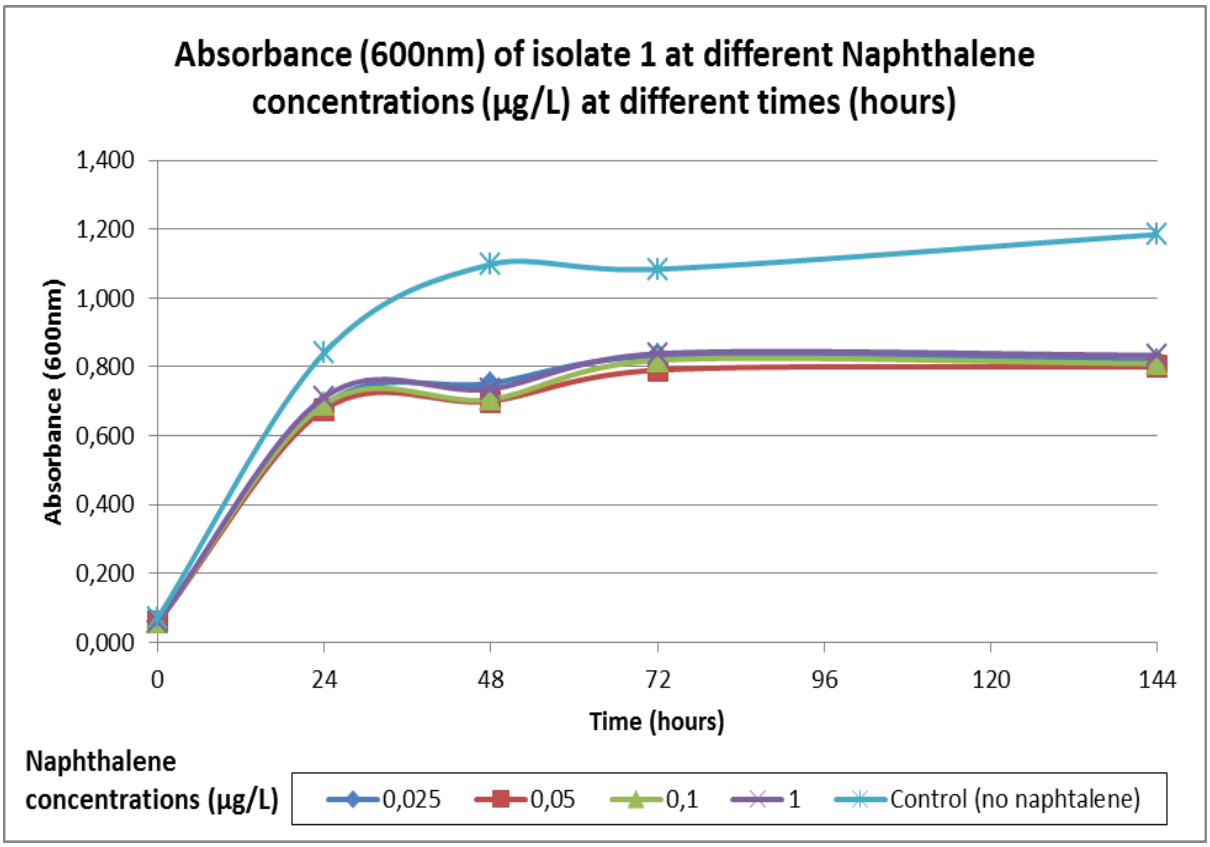

Figure 4.3.3a: showing the growth of Isolate 1 at absorbance (600nm) in different Naphthalene concentrations over a period of time

Equation 1: used for determining the true effect of Naphthalene on the isolates

Total Napthalene Absorbance =
Observed Naphthalene Absorbance − (Control Absorbance − Solvent control Absorbance)

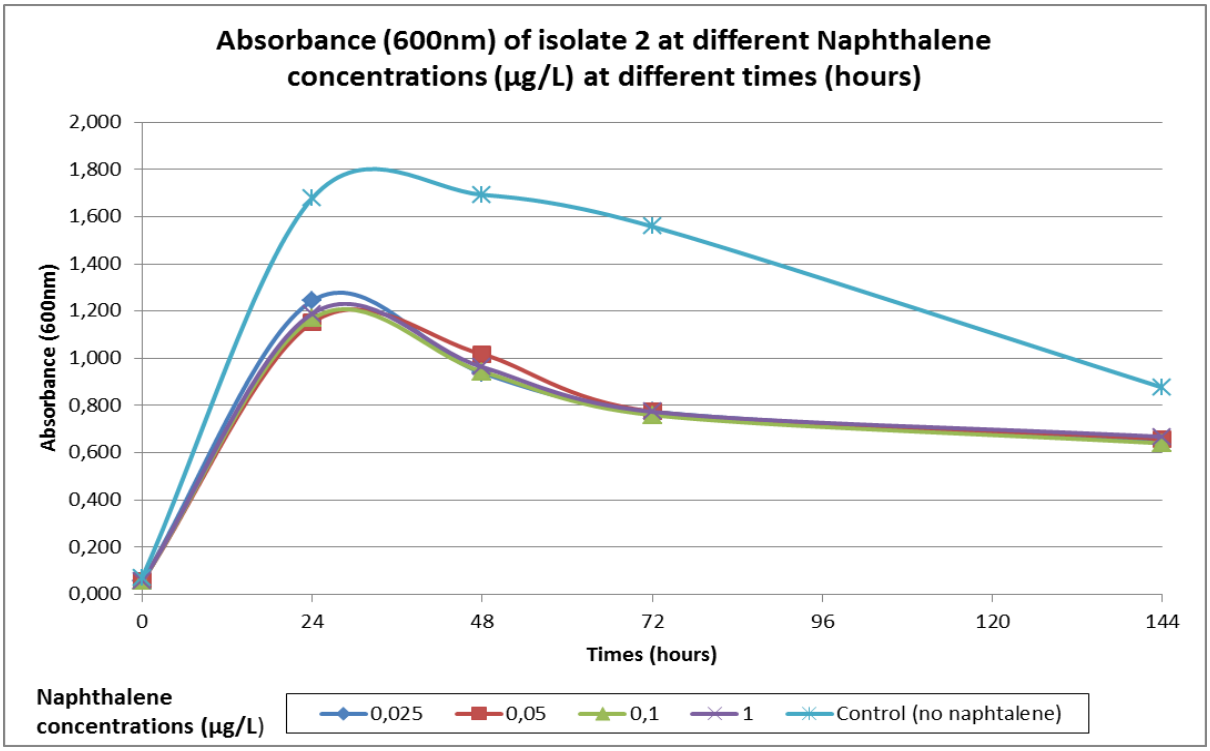

Figure 4.3.3b: showing the growth of Isolate 2 at absorbance (600nm) in different Naphthalene concentrations over a period of time

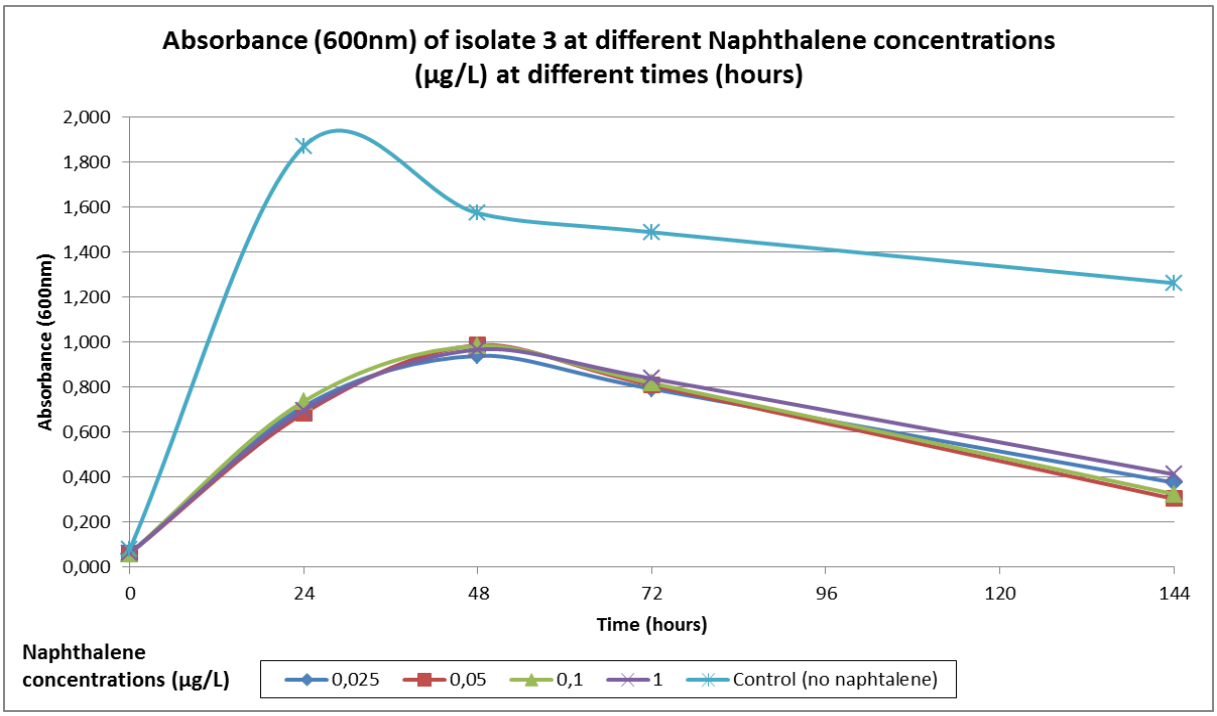

Figure 4.3.3c: showing the growth of Isolate 3 at absorbance (600nm) in different Naphthalene concentrations over a period of time

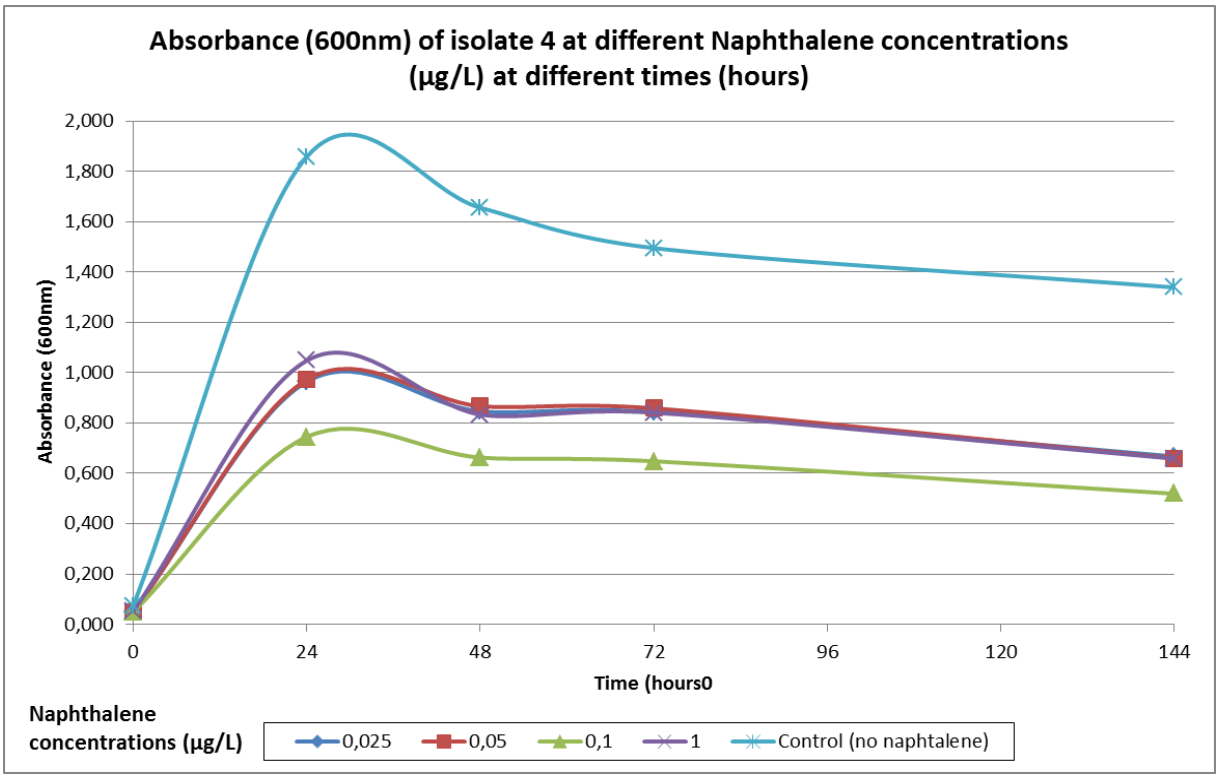

Figure 4.3.3d: showing the growth of Isolate 4 at absorbance (600nm) in different Naphthalene concentrations over a period of time

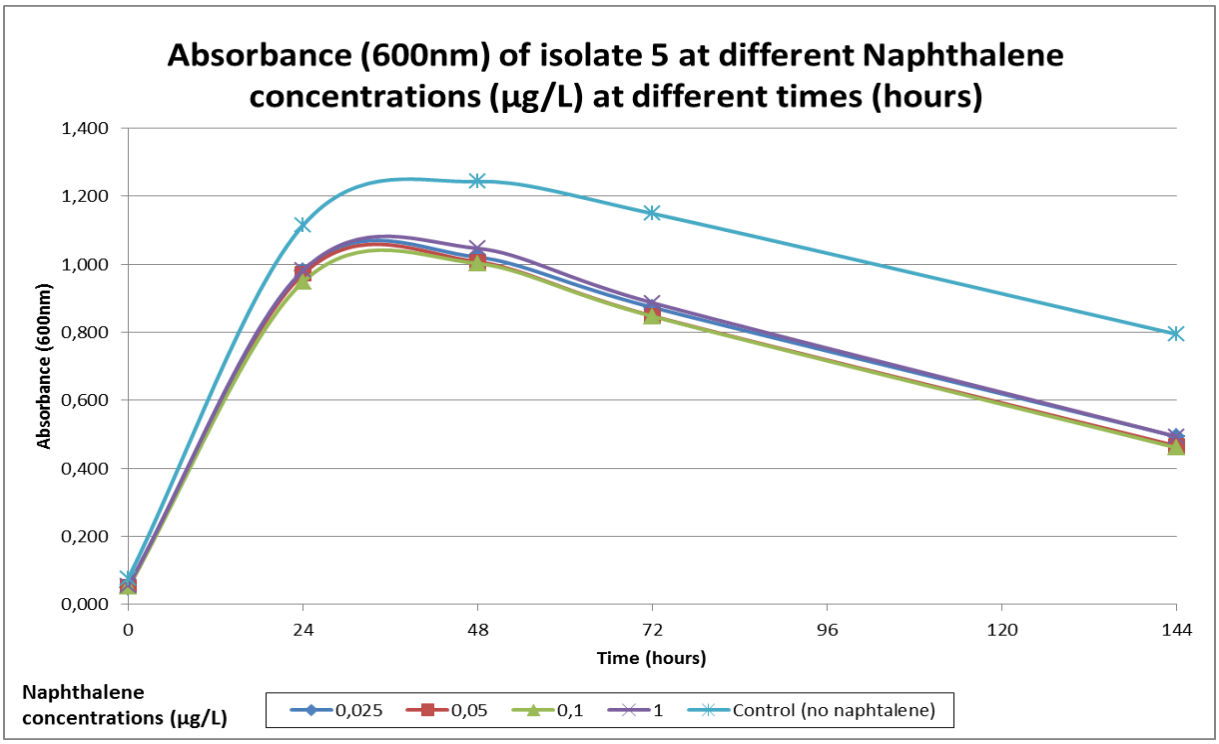

Figure 4.3.3e: showing the growth of Isolate 5 at absorbance (600nm) in different Naphthalene concentrations over a period of time

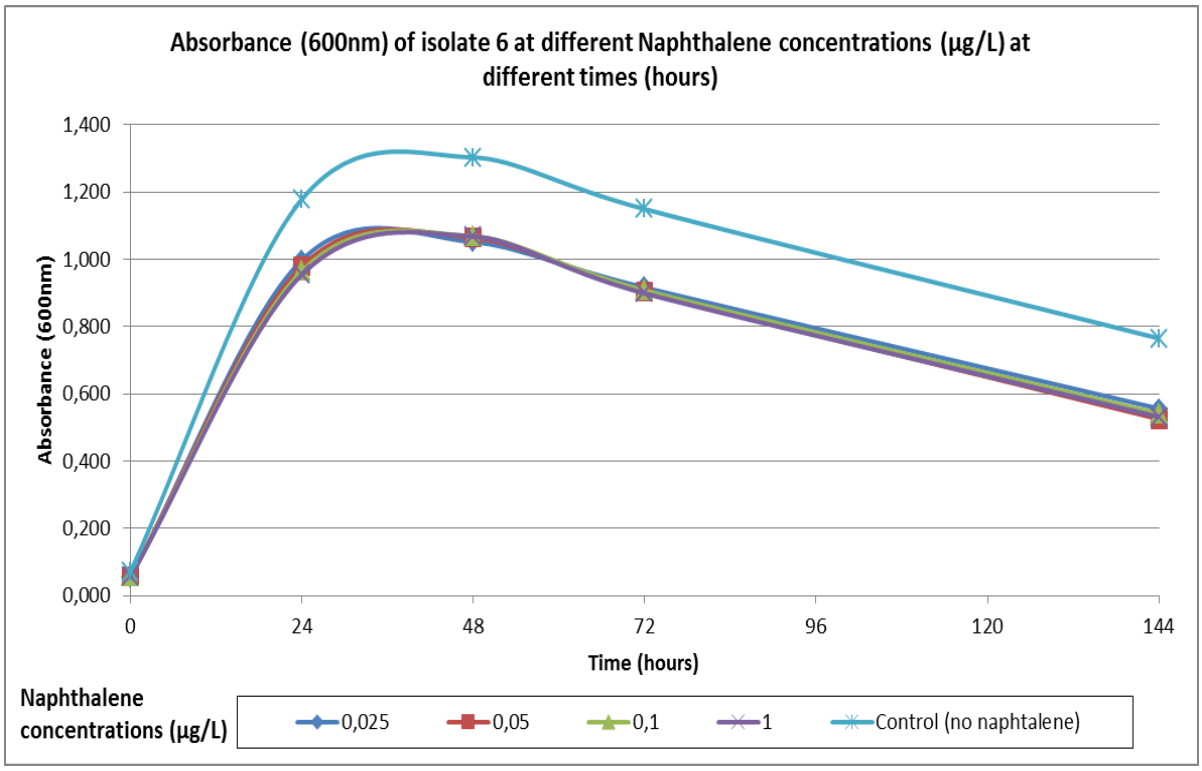

Figure 4.3.3f: showing the growth of Isolate 6 at absorbance (600nm) in different Naphthalene concentrations over a period of time

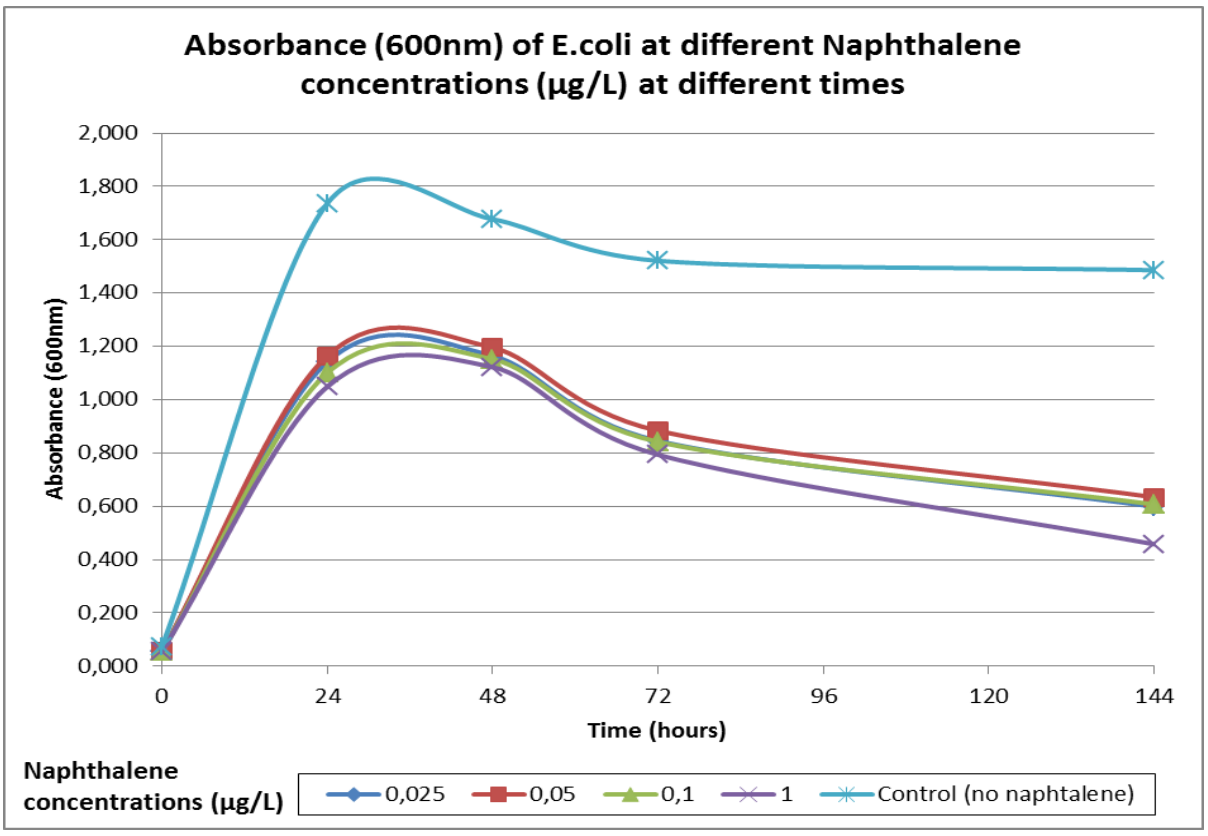

Figure 4.3.3g: showing the growth of Isolate *E.coli* at absorbance (600nm) in different Naphthalene concentrations over a period of time

4.4. AMPLIFICATION OF 16SrRNA and UIDA GENE REGIONS

4.4.1. DNA Extraction

The BioDrop instrument was used to measure the concentration of the extracted bacterial DNA by measuring the absorbance of the extracted DNA. Table 4.4.1 shows the absorbance ratios and corresponding DNA concentrations of the bacteria.

Table 4.4.1: Absorbance ratios and DNA concentration of the extracted DNA for each bacterial isolate

Isolate	Absorbance		DNA concentration (µg/mL)
	A260/A230	A260/A280	
1	1.568	2.295	10.90
2	0.157	1.737	1.650
3	0.427	2.000	5.100
4	0.679	2.000	1.800
5	1.070	1.872	5.350
6	0.031	2.667	0.400
E.coli	0.187	2.000	6.700

4.4.2. Gel electrophorersis to confirm DNA extraction

After DNA extraction, gel electrophoresis was performed to confirm that the DNA was extracted. All the bacterial isolates showed bands of DNA indicating adequate extraction of DNA except isolate 2. This indicates that there may have not been adequate DNA extracted for Isolate 2. Figure 4.4.2 shows the DNA bands from the gel electrophoresis.

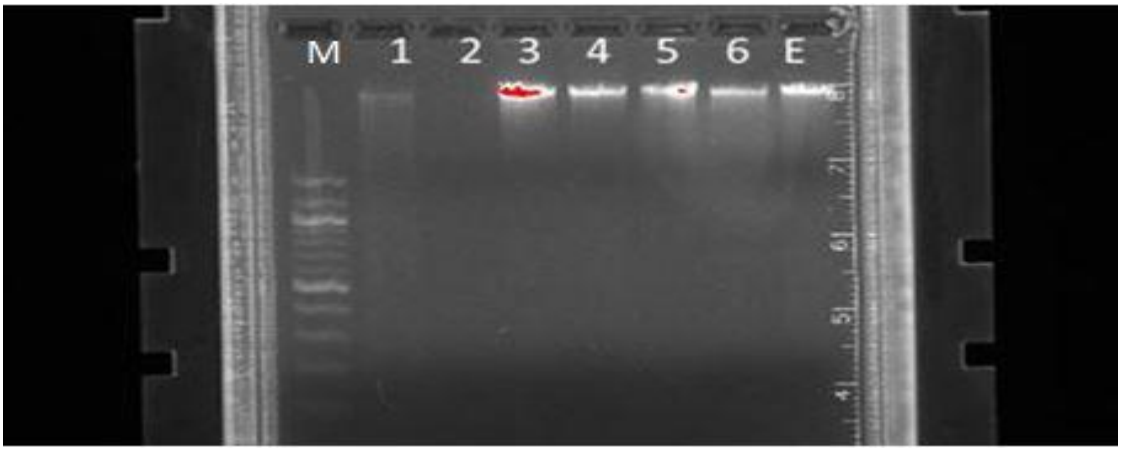

Figure 4.4.2: Gel electrophoresis showing bands of the extracted DNA from the isolates. The bands are labelled as follows: Lane 1: M-Molecular ladder, Lane 2-7: Isolate 1-6 and Lane 8: E- *E.coli.*

4.4.3. PCR

Two different PCR were set up using two different sets of primers. The first primer set was for the 16SrRNA region which is a gene that is conserved in all bacterial species and is used for the identification of bacteria. This reaction was carried out in order to confirm that the isolates were indeed bacterial isolates. The second primer set was for the uidA gene region which is used in the identification of *E.*coli. The figure below shows the results of the PCR for the 16SrRNA region and the uidA gene.

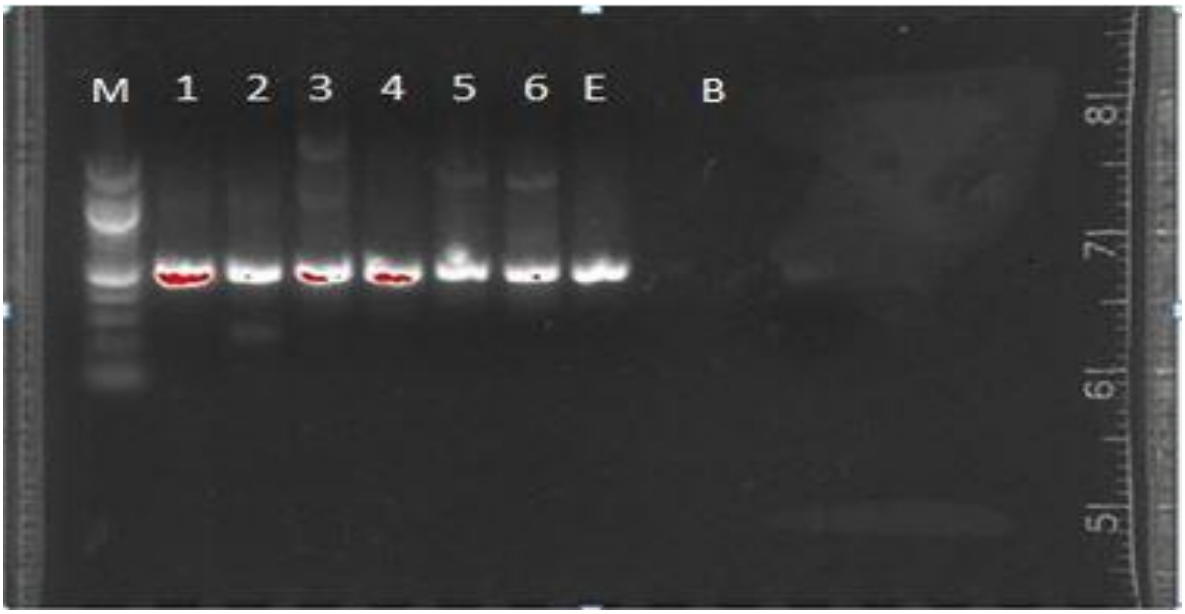

Figure 4.4.3a: PCR DNA bands for 16SrRNA region. The bands are labelled as follows: Lane 1: M-Molecular ladder, Lane 2-7: Isolate 1-6, Lane 8: E- *E.coli* and Lane10: B- Blank (Mastermix without DNA)

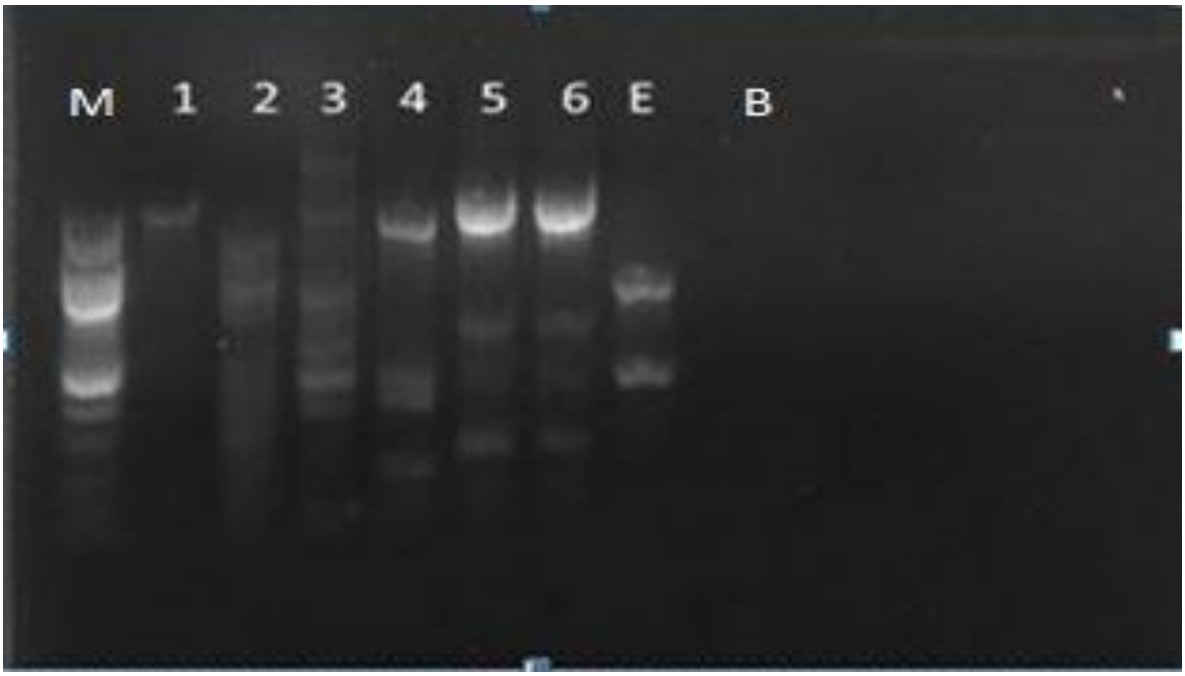

Figure 4.4.3b: PCR DNA bands for uidA gene region. The bands are labelled as follows: Lane 1: M-Molecular ladder, Lane 2-7: Isolate 1-6, Lane 8: E- *E.coli* and Lane10: B- Blank (Mastermix without DNA)

5. DISCUSSION

Microbial water quality monitoring involves the use of organisms such as *Escherichia coli* and total coliforms as indicators for faecal contamination of water (DWAF, 2002). However, this type of monitoring give little information with regard to the overall pollution status of the rivers. Consequently, the release of toxic chemicals such as trace and heavy metals into the environment has a negative effect on the aquatic flora and fauna (Staley *et.al*, 2014). A river such as the Jukskei, receives effluent from industrial activities and run-off from waste dumps and agricultural activities (Sibali *et.al*, 2008). Therefore, investigating the relationship between chemical pollution and the growth of bacteria in the Jukskei River may lead to the discovery of novel indicator organisms specifically for chemical pollution. This could provide a range of ways to monitor toxicity in the National Toxicity Monitoring Programme. Thus, this study sought to investigate the effect of organic pollutants on the growth of naturally occurring bacteria in the Jukskei River.

Water samples collected from the Juskei River were investigated for the presence of microbial pathogens and the pathogens were exposed to Naphthaline and Azatrine toxicants commonly found in water contaminated with chemical effluents. The physicochemical properties such as temperature, pH, dissolved oxygen and electrical conductivity collected at

point of water collection provides information on the types of bacteria that may be isolated from the river as well as the level of toxicity by the toxicants. High temperatures and pH may increase the toxicity of many toxicants as well as the type of bacteria that may be found in the water. The temperatures at the monitoring sites during sampling were 21.84°C for Marlboro and 23.72°C for Bruma (Table 4.1b). The pH at the monitoring sites during sampling was 7.32 for Marlboro and 7.15 for Bruma (Table 4.1b). The observed pH values fall within the normal range (6.5-8.5) according to the South African water quality guidelines. The influence of pH in the river results in many the increase of toxicity of many toxicants such as aluminium. It also influences microbial biodiversity in the river (DWAF, 1996).

Dissolved oxygen (DO) may influence the type of bacteria isolated in the sense that they may be aerobic or anaerobic bacteria. The DO at the monitoring sites during sampling was 4.85mg/L for Marlboro and 3.24mg/L for Bruma (Table 4.1b). The normal DO range in surface waters is usually 6-14 mg/L (DWS, 2016). The observed DO values at the sampling sites were lower than the guideline values. This implies that at the time of sampling, the conditions in the Jukskei river were anoxic. However, aerobic bacteria were still isolated. The Electrical Conductivity (EC) at the monitoring sites during sampling was 757µS/cm for Marlboro and 552µs/cm for Bruma (Table 4.1b). A high electrical conductivity may disrupt the salt and water (osmoregulatory) balance of the water sample. Table 4.1b shows the values for temperature, pH, dissolved oxygen and electrical conductivity of the two monitoring sites on the date and time of sample collection.

Atrazine is used as an herbicide to control weeds in crops such as maize, sugar-cane and pineapple. It is does not easily undergo biodegradation and can persist in the environment for a long time. Atrazine is a priority toxicant because it is widely used in the agricultural sector (Jain *et.al*, 2009). Naphthalene is a volatile organic compound that is used as a pesticide. It's most common usage is in moth balls to prevent moth larvae from damaging clothing. Naphthalene is known to be highly toxic to humans (Pajaro-Castro *et.al*, 2017). The widespread use of Atrazine and Naphthalene are evident in their prominent environmental presence. The South African guideline value for Naphthalene in water is 16µg/L and for Atrazine is 10µg/L (DWAF, 1996). The toxicant concentrations used in this study ranged from 0.025µg/L to 1µg/L for Naphthalene and 0.1µg/L to 1µg/L for Atrazine. These concentrations are lower than that of the guideline values yet they still had a significant effect on the bacterial isolates and *E.coli* even at such low concentrations.

Exposure of the bacteria to Atrazine and Naphthalene was performed on an acute (72 hours) and chronic basis (≥96 hours). Atrazine and Naphthalene had a negative effect on the growth of the six bacterial isolates and *E.coli*. The toxicants slowed down bacterial growth. There were no bactericidal effects. The concentrations of both toxicants did not seem to matter because the bacteria grew at the more or less the same rate no matter the concentration of both toxicants although this was lower when compared to that of the control isolate. Isolate 1 was the only outlier in the sense that it outgrew the control (isolate 1 grown without Atrazine) at the 0.2µg/L Atrazine concentration. This suggests that at the 0.2µg/L concentration, isolate 1 was able to break down Atrazine and possibly use it as an energy source for growth. This is illustrated in Figure 4.3.2a. The growth curves in figures 4.3.2a-4.3.2g illustrate the growth of the bacteria in different Atrazine concentrations.

Naphthalene had more of a negative effect on the growth of the bacteria than Atrazine. This can be seen in the growth curves above (Figures 4.3.3a-4.3.3g). The gap between the growth curve of the control and the growth curves of the bacteria is wider for all the isolates in Naphthalene than it was for Atrazine. The concentrations of Naphthalene used on the bacteria were also lower than the concentrations of Atrazine used on the bacteria. This suggests that at lower concentrations, Naphthalene is toxic to the bacterial isolates.

Toxicants such as Naphthalene and Atrazine have been observed to be toxic towards microorganisms. They have been shown to interfere with photosynthesis, respiration and many biosynthetic reactions as well as cell growth and cell division. Herbicides such as Atrazine tend to be more toxic towards photosynthetic bacteria by inhibiting chlorophyll formation. This suggests that there is a possibility that isolates 1-6 could be photosynthetic bacteria. Insecticides such as Naphthalene can also inhibit chlorophyll production as well as carbohydrate and protein synthesis in bacteria (DeLorenzo *et.al*, 2001).

Many studies regarding the degradation of toxicants by soil bacteria have been performed (DeLorenzo *et.al*, 2001). However, research concerning the effect of toxicants on the naturally occurring aquatic microorganisms is limited. Most of the studies that have been conducted are regarding the effect of herbicides especially Atrazine on algae. The concentrations used in these studies were far greater than the concentration used in this present study. Concentrations ranged from 0.04µg/L to 5000µg/L of Atrazine in most of the studies performed on algae. Studies on the effect of pesticides such as Naphthalene on aquatic bacteria are limited as well (DeLorenzo *et.al*, 2001).

Hudak and Fuhrman (1988) observed that certain planktonic bacteria can adapt to growing in high concentrations of Naphthalene (Hudak and Fuhrman, 1988). Muturi and colleagues observed that Atrazine and a mixture of other toxicants (malathion, carbaryl, permethrin and glyphosate) can disrupt aquatic microbial communities which has a negative effect on the invertebrates that feed on the bacteria (Muturi *et.al,* 2017). This may also be the case in the Jukskei river. Many aquatic organisms that feed on the bacteria may be at a disadvantage since Atrazine and Naphthalene are inhibiting the growth of the bacteria in the river. However, there may be an advantage to the inhibitory effect of Atrazine and Naphthalene on the bacteria. The sites sampled i.e. Marlboro and Bruma are both in proximity of residential areas. Marlboro is downstream an informal settlement which means there may be an increase in faecal contamination. *E.coli* is one of the indicator organisms used to detect faecal contamination and the growth of *E.coli* is inhibited by both toxicants. There is a possibility that other bacteria present during faecal contamination of the Jukskei river may be inhibited by both toxicants as well. This may be a good thing in the sense that both these toxicants are contributing to decreasing the effects of faecal contamination.

Two separate PCR reactions were performed for the 7 bacteria l isolates. The first one was performed using primers for the 16SrRNA region which is conserved in all bacterial species. This was done to confirm that the organisms isolated from the Jukskei river were indeed bacteria. The second reaction was carried out using primers for the uidA gene regionwhich is used for *E.coli* identification. This reaction was performed in order to confirm the presence of *E.coli* among the isolates. Figure 4.4a confirms that the isolates are indeed bacteria. This is shown by the single bands on the gel indicating the 16SrRNA region. Figure 4.4b shows the results of the PCR for the uidA gene region of *E.coli*. The results of this second reaction were inconclusive because multiple bands were derived from the DNA of the isolates. In order to confirm that the correct primers for the uidA gene were used, a BLAST search was performed on NCBI database. The search confirmed that the primers were indeed the correct primers for *E.coli*. The accession number for the primer on the NCBI database is AY447088.1. The resulting multiple bands for the second PCR may be a product of the primers binding to other regions of DNA other than the uidA gene or the annealing temperature of the primers was too low (Palumbi *et.al,* 2002).

6. LIMITATIONS

There were a few limitations encountered during this study. After PCR, the DNA of the isolated bacteria from the Jukskei river were supposed to be taken for DNA sequencing. Sequencing of the DNA would have enabled identification of the bacterial strains isolated from the water collected from the Jukskei river. Biochemical testing would have provided information in terms of whether the strains were gram positive or negative. Conducting the exposure tests using a mixture of both toxicants would provide more insight on the effects of the toxicants since they do not occur individually in the environment. Studying the mechanisms or mode of action of the toxicants effects on the bacteria would also be useful.

7. CONCLUSIONS

The hypothesis of this study has been confirmed by the results of the study. That is, the toxicants have a negative effect on bacterial growth. However, this effect was not bactericidal. Therefore, the toxicants slow down the growth of the naturally occurring bacteria in the Jukskei river. However, there is a possibility that isolate 1 may be able to degrade Atrazine but only at low concentrations. More research may be conducted to confirm isolate's 1 potential for its use in bioremediation or as an indicator organism for chemical pollution of rivers. It can be concluded that toxicant pollution negatively affects bacterial growth in the Jukskei river.

8. RECOMMENDATIONS

In order to improve this study in the future, it would be advisable to sequence the DNA of the isolates to identify them. This would give an idea of some of the bacteria that naturally inhabit the Jukskei river. It would also be good to conduct the exposure tests using a mixture of both toxicants. This would produce more insightful results because the toxicants do not occur individually in the environment. There is usually a mixture of many toxicants together in the river. Hence, doing the exposure tests with a mixture of the toxicant will present a more realistic representation of the environmental conditions. Studying the mechanisms or mode of action of the toxicants effects on the bacteria will also be useful. Most of the studies regarding the effect of toxicants on bacteria focus on bacterial degradation of these toxicants. More research needs to be conducted on the effect of chemical pollution on the naturally occurring bacteria in rivers and the consequences these effects have on the ecosystem. This

kind of research has the potential for the discovery of indicator organisms for chemical pollution in rivers.

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APPENDIX 1

Results of exposure tests for each bacterial isolate to the toxicants and standard deviation tables for the results

1. Isolate 1

Table 1.1: Absorbance (600nm) of Isolate 1 in the different Atrazine concentrations at the different times in triplicate

Isolate 1 ATRAZINE (TRIPLICATE)																
	Time	t0			t24			t48			t72			t144		
Toxicant Concentration(µg/L)	0.1	0.061	0.063	0.062	0.444	0.525	0.555	0.863	0.925	0.917	0.96	1.008	0.908	0.966	1.064	1.024
	0.2	0.061	0.062	0.062	0.887	0.951	0.869	1.262	1.317	1.283	1.316	1.382	1.359	1.107	1.184	1.137
	0.4	0.062	0.061	0.06	0.491	0.579	0.875	0.901	0.892	1.293	0.98	0.953	1.348	0.997	0.976	1.18
	1	0.061	0.063	0.062	0.689	0.629	0.575	0.948	0.928	0.903	0.98	0.998	0.951	1.085	1.014	0.98
	Control	0.07	0.069	0.067	0.679	0.555	0.589	1.029	1.006	0.958	1.092	1.083	1.081	1.166	1.171	1.2

Table 1.2: Average absorbance (600nm) of Isolate 1 in the different Atrazine concentrations at the different times

Isolate 1 ATRAZINE AVERAGE						
Toxicant Concentration (µg/L)	Time	t0	t24	t48	t72	t144
	0.1	0.062	0.508	0.902	0.959	1.018
	0.2	0.062	0.902	1.287	1.352	1.143
	0.4	0.061	0.648	1.029	1.094	1.051
	1	0.062	0.631	0.926	0.976	1.026
	Control	0.069	0.608	0.998	1.085	1.179

Table 1.3: The Standard Deviation of the absorbance values of Isolate 1 in the different Atrazine concentrations at the different times

Isolate 1 Atrazine Standard Deviation						
Toxicant Concentration(µg/L)	Time	t0	t24	t48	t72	t144
	0.1	0.001	0.057	0.034	0.050	0.049
	0.2	0.001	0.043	0.028	0.034	0.039
	0.4	0.001	0.201	0.229	0.221	0.112
	1	0.001	0.057	0.023	0.024	0.054
	Control	0.002	0.064	0.036	0.006	0.018

Table 1.4: Absorbance (600nm) of Isolate 1 in the different Naphthalene concentrations at the different times in triplicate

Isolate 1 NAPHTHALENE (TRIPLICATE)																
Toxicant Concentration (µg/L)	Time	t0			t24			t48			t72			t144		
	0.025	0.065	0.065	0.061	0.745	0.749	0.791	0.941	0.955	0.983	0.953	0.964	0.980	0.985	1.019	1.042
	0.05	0.064	0.063	0.063	0.755	0.75	0.739	0.874	0.943	0.908	0.878	0.961	0.935	0.977	1.015	1.000
	0.1	0.064	0.065	0.065	0.842	0.738	0.708	1.000	0.885	0.854	1.006	0.929	0.925	1.068	0.995	0.955
	1	0.064	0.064	0.063	0.770	0.758	0.823	0.946	0.916	0.968	0.985	0.958	0.974	1.073	0.988	1.031
	Control	0.072	0.071	0.070	0.853	0.85	0.82	1.091	1.089	1.114	1.083	1.082	1.086	1.211	1.166	1.177
	Solvent Control	0.065	0.064	0.069	0.787	0.776	0.746	0.896	0.883	0.893	0.948	0.942	0.96	0.978	0.994	0.992

Table 1.5: Average absorbance (600nm) of Isolate 1 in the different Naphthalene concentrations at the different times

Isolate 1 NAPHTHALENE AVERAGE						
Toxicant Concentration (µg/L)	Time	t0	t24	t48	t72	t144
	0.025	0.064	0.762	0.960	0.966	1.015
	0.05	0.063	0.748	0.908	0.925	0.997
	0.1	0.065	0.763	0.913	0.953	1.006
	1	0.064	0.784	0.943	0.972	1.031
	Control	0.071	0.841	1.098	1.084	1.185
	Solvent Control	0.066	0.770	0.891	0.950	0.988

Table 1.6: Total absorbance (600nm) of Isolate 1 in the different Naphthalene concentrations at the different times after the effect of the Solvent control has been subtracted

Isolate 1 NAPHTALENE AVERAGE						
Toxicant Concentration (µg/L)	Time	t0	t24	t48	t72	t144
	0.025	0.059	0.690	0.752	0.832	0.819
	0.05	0.058	0.677	0.701	0.791	0.801
	0.1	0.060	0.691	0.706	0.820	0.809
	1	0.059	0.712	0.736	0.839	0.834
	Control	0.071	0.841	1.098	1.084	1.185

Table 1.7: The Standard Deviation of the absorbance values of Isolate 1 in the different Naphthalene concentrations at the different times

Isolate 1 Naphthalene Standard Deviation						
Toxicant Concentration (µg/L)	Time	t0	t24	t48	t72	t144
	0.025	0.002	0.025	0.021	0.014	0.029
	0.05	0.001	0.008	0.035	0.042	0.019
	0.1	0.001	0.070	0.077	0.046	0.057
	1	0.001	0.035	0.026	0.014	0.043
	Control	0.001	0.018	0.014	0.002	0.023
	Solvent control	0.003	0.021	0.007	0.009	0.009

2. Isolate 2

Table 2.1: Absorbance (600nm) of Isolate 2 in the different Atrazine concentrations at the different times in triplicate

Isolate 2 ATRAZINE (TRIPLICATE)																
Toxicant Concentration (µg/L)	Time	t0			t24			t48			t72			t144		
	0.1	0.063	0.063	0.064	1.292	1.434	1.468	1.366	1.507	1.439	1.248	1.308	1.354	0.792	0.79	0.758
	0.2	0.064	0.065	0.065	1.274	1.496	1.388	1.605	1.534	1.373	1.293	1.269	1.402	1.029	0.863	1.349
	0.4	0.064	0.063	0.063	1.35	1.052	1.281	1.431	1.298	1.507	1.221	1.166	1.267	0.872	0.72	0.871
	1	0.066	0.064	0.064	1.591	1.409	1.369	1.404	1.421	1.372	1.27	1.179	1.208	0.815	0.796	0.859
	Control	0.076	0.072	0.071	1.511	1.524	1.554	1.681	1.73	1.725	1.599	1.633	1.661	0.911	0.866	1.037

Table 2.2: Average absorbance (600nm) of Isolate 2 in the different Atrazine concentrations at the different times

Isolate 2 ATRAZINE AVERAGE						
Toxicant Concentration (µg/L)	Time	t0	t24	t48	t72	t144
	0.1	0.063	1.398	1.437	1.303	0.780
	0.2	0.065	1.386	1.504	1.321	1.080
	0.4	0.063	1.228	1.412	1.218	0.821
	1	0.065	1.456	1.399	1.219	0.823
	Control	0.073	1.530	1.712	1.631	0.938

Table 2.3: The Standard Deviation of the absorbance values of Isolate 2 in the different Atrazine concentrations at the different times

	Isolate 2 Atrazine Standard deviation					
Toxicant Concentration (µg/L)	**Time**	**t0**	**t24**	**t48**	**t72**	**t144**
	0.1	0.001	0.093	0.071	0.053	0.019
	0.2	0.001	0.111	0.119	0.071	0.247
	0.4	0.001	0.156	0.106	0.051	0.087
	1	0.001	0.118	0.025	0.046	0.032
	Control	0.003	0.022	0.027	0.031	0.089

Table 2.4: Absorbance (600nm) of Isolate 2 in the different Naphthalene concentrations at the different times in triplicate

Isolate 2 NAPHTHALENE (TRIPLICATE)																
Toxicant Concentration (µg/L)	Time	t0			t24			t48			t72			t144		
	0.025	0.063	0.063	0.065	1.431	1.588	1.561	1.262	1.222	1.388	1.197	1.174	1.178	0.788	0.772	0.835
	0.05	0.066	0.063	0.063	1.43	1.345	1.528	1.384	1.497	1.228	1.16	1.168	1.221	0.786	0.801	0.839
	0.1	0.064	0.065	0.064	1.386	1.383	1.583	1.337	1.412	1.139	1.237	1.082	1.187	0.858	0.736	0.775
	1	0.064	0.064	0.064	1.655	1.301	1.46	1.293	1.399	1.255	1.186	1.169	1.192	0.81	0.778	0.865
	Control	0.072	0.073	0.072	1.649	1.683	1.703	1.63	1.713	1.735	1.521	1.576	1.577	0.873	0.903	0.853
	Solvent Control	0.064	0.063	0.065	1.385	1.355	1.448	1.303	1.399	1.318	1.162	1.151	1.135	0.733	0.728	0.719

Table 2.5: Average absorbance (600nm) of Isolate 2 in the different Naphthalene concentrations at the different times

Isolate 2 NAPHTHALENE AVERAGE						
Toxicant Concentration (µg/L)	**Time**	**t0**	**t24**	**t48**	**t72**	**t144**
	0.025	0.064	1.527	1.291	1.183	0.798
	0.05	0.064	1.434	1.370	1.183	0.809
	0.1	0.064	1.451	1.296	1.169	0.790
	1	0.064	1.472	1.316	1.182	0.818
	Control	0.072	1.678	1.693	1.558	0.876
	Solvent Control	0.064	1.396	1.340	1.149	0.727

Table 2.6: Total absorbance (600nm) of Isolate 2 in the different Naphthalene concentrations at the different times after the effect of the Solvent control has been subtracted

Isolate 2 NAPHTHALENE AVERAGE						
Toxicant Concentration (µg/L)	Time	t0	t24	t48	t72	t144
	0.025	0.055	1.244	0.938	0.774	0.649
	0.05	0.056	1.152	1.017	0.774	0.659
	0.1	0.056	1.168	0.943	0.760	0.640
	1	0.056	1.190	0.963	0.774	0.668
	Control	0.072	1.678	1.693	1.558	0.876

Table 2.7: The Standard Deviation of the absorbance values of Isolate 2 in the different Naphthalene concentrations at the different times

Isolate 2 Naphthalene Standard Deviation						
Toxicant Concentration (µg/L)	Time	t0	t24	t48	t72	t144
	0.025	0.001	0.084	-0.087	0.012	0.033
	0.05	0.002	0.092	-0.135	0.033	0.027
	0.1	0.001	0.115	-0.141	0.079	0.062
	1	0.000	0.177	-0.075	0.012	0.044
	Control	0.001	0.027	-0.055	0.032	0.025
	Solvent control	0.001	0.047	-0.052	0.014	0.007

3. Isolate 3

Table 3.1: Absorbance (600nm) of Isolate 3 in the different Atrazine concentrations at the different times in triplicate

Isolate 3 ATRAZINE (TRIPLICATE)																
Toxicant Concentration (µg/L)	Time	t0			t24			t48			t72			t144		
	0.1	0.059	0.064	0.065	1.023	1.049	1.04	1.182	1.196	1.218	1.116	1.113	1.121	0.718	0.713	0.719
	0.2	0.064	0.065	0.066	0.98	1.009	1.01	1.136	1.154	1.171	1.21	1.158	1.19	1.071	1.034	1.061
	0.4	0.063	0.064	0.068	1.018	0.985	1.016	1.153	1.141	1.154	1.065	1.051	1.06	0.724	0.717	0.726
	1	0.068	0.066	0.066	1.053	1.011	1.026	1.221	1.156	1.158	1.154	1.068	1.066	0.774	0.724	0.726
	Control	0.082	0.072	0.075	1.192	1.177	1.178	1.404	1.376	1.434	1.393	1.351	1.401	0.959	0.942	0.933

Table 3.2: Average absorbance (600nm) of Isolate 3 in the different Atrazine concentrations at the different times

Isolate 3 ATRAZINE AVERAGE						
Toxicant Concentration (µg/L)	Time	t0	t24	t48	t72	t144
	0.1	0.063	1.037	1.199	1.117	0.717
	0.2	0.065	1.000	1.154	1.186	1.055
	0.4	0.065	1.006	1.149	1.059	0.722
	1	0.067	1.030	1.178	1.096	0.741
	Control (no atrazine)	0.076	1.182	1.405	1.382	0.945

Table 3.3: The Standard Deviation of the absorbance values of Isolate 3 in the different Atrazine concentrations at the different times

Isolate 3 Atrazine Standard deviation						
Toxicant Concentration (µg/L)	Time	t0	t24	t48	t72	t144
	0.1	0.003	0.013	0.018	0.004	0.003
	0.2	0.001	0.017	0.018	0.026	0.019
	0.4	0.003	0.019	0.007	0.007	0.005
	1	0.001	0.021	0.037	0.050	0.028
	Control	0.005	0.008	0.029	0.027	0.013

Table 3.4: Absorbance (600nm) of Isolate 3 in the different Naphthalene concentrations at the different times in triplicate

Isolate 3 NAPHTHALENE (TRIPLICATE)																
Toxicant Concentration (µg/L)	Time	t0			t24			t48			t72			t144		
	0.025	0.07	0.07	0.07	1.35	1.403	1.647	1.207	1.212	1.333	1.112	1.089	1.217	0.881	0.904	0.992
	0.05	0.071	0.071	0.071	1.28	1.514	1.517	1.279	1.285	1.33	1.128	1.162	1.175	0.818	0.839	0.904
	0.1	0.07	0.07	0.07	1.459	1.499	1.51	1.316	1.284	1.284	1.192	1.151	1.15	0.961	0.824	0.839
	1	0.07	0.075	0.07	1.425	1.567	1.357	1.273	1.301	1.261	1.217	1.184	1.153	1.021	0.945	0.922
	Control	0.077	0.076	0.077	1.855	1.91	1.837	1.529	1.578	1.608	1.451	1.474	1.536	1.227	1.244	1.311
	Solvent Control	0.066	0.067	0.062	1.109	1.114	1.117	1.265	1.26	1.251	1.136	1.133	1.148	0.708	0.705	0.716

Table 3.5: Average absorbance (600nm) of Isolate 3 in the different Naphthalene concentrations at the different times

Isolate 3 NAPHTHALENE AVERAGE						
Toxicant Concentration (µg/L)	Time	t0	t24	t48	t72	t144
	0.025	0.070	1.467	1.251	1.139	0.926
	0.05	0.071	1.437	1.298	1.155	0.854
	0.1	0.070	1.489	1.295	1.164	0.875
	1	0.072	1.450	1.278	1.185	0.963
	Control	0.077	1.867	1.572	1.487	1.261
	Solvent Control	0.065	1.113	1.259	1.139	0.710

Table 3.6: Total absorbance (600nm) of Isolate 3 in the different Naphthalene concentrations at the different times after the effect of the Solvent control has been subtracted

Isolate 3 NAPHTHALENE AVERAGE						
Toxicant Concentration (µg/L)	Time	t0	t24	t48	t72	t144
	0.025	0.058	0.713	0.938	0.791	0.375
	0.05	0.059	0.683	0.985	0.807	0.303
	0.1	0.058	0.735	0.982	0.816	0.324
	1	0.060	0.696	0.965	0.837	0.412
	Control	0.077	1.867	1.572	1.487	1.261

Table 3.7: The Standard Deviation of the absorbance values of Isolate 3 in the different Naphthalene concentrations at the different times

Isolate 3 Naphthalene Standard Deviation						
Toxicant Concentration (µg/L)	Time	t0	t24	t48	t72	t144
	0.025	0.000	0.158	-0.071	0.068	0.059
	0.05	0.000	0.136	-0.028	0.024	0.045
	0.1	0.000	0.027	-0.018	0.024	0.075
	1	0.003	0.107	-0.021	0.032	0.052
	Control	0.001	0.038	-0.040	0.044	0.044
	Solvent control	0.003	0.004	-0.007	0.008	0.006

4. Isolate 4

Table 4.1: Absorbance (600nm) of Isolate 4 in the different Atrazine concentrations at the different times in triplicate

Isolate 4 ATRAZINE (TRIPLICATE)																	
Toxicant Concentration (µg/L)	Time	t0			t24			t48			t72			t144			
	0.1	0.06	0.061	0.062	1.316	1.479	1.434	1.615	1.445	1.412	1.486	1.286	1.237	1.185	1.011	1.039	
	0.2	0.06	0.062	0.06	1.399	1.476	1.521	1.119	1.323	1.115	1.074	1.17	1.061	0.858	0.933	0.852	
	0.4	0.063	0.064	0.061	1.116	1.267	1.211	1.226	1.23	1.221	1.12	1.257	1.164	0.874	0.943	0.894	
	1	0.06	0.062	0.061	1.088	1.118	1.338	1.344	1.214	1.232	1.197	1.213	1.163	1.045	0.909	0.927	
	Control	0.07	0.07	0.074	1.671	1.809	1.648	1.778	1.676	1.634	1.639	1.509	1.551	1.402	1.303	1.326	

Table 4.2: Average absorbance (600nm) of Isolate 4 in the different Atrazine concentrations at the different times

Isolate 4 ATRAZINE AVERAGE						
Toxicant Concentration (µg/L)	Time	t0	t24	t48	t72	t144
	0.1	0.061	1.410	1.491	1.336	1.078
	0.2	0.061	1.465	1.186	1.102	0.881
	0.4	0.063	1.198	1.226	1.180	0.904
	1	0.061	1.181	1.263	1.191	0.960
	Control	0.071	1.709	1.696	1.566	1.344

Table 4.3: The Standard Deviation of the absorbance values of Isolate 4 in the different Atrazine concentrations at the different times

Isolate 4 Atrazine Standard deviation					
Toxicant Concentration (µg/L)	Time	t0	t24	t48	t72	t144
	0.1	0.001	0.084	0.109	0.132	0.093
	0.2	0.001	0.062	0.119	0.060	0.045
	0.4	0.002	0.076	0.005	0.070	0.036
	1	0.001	0.137	0.070	0.026	0.074
	Control	0.002	0.087	0.074	0.066	0.052

Table 4.4: Absorbance (600nm) of Isolate 4 in the different Naphthalene concentrations at the different times in triplicate

Isolate 4 NAPHTHALENE (TRIPLICATE)																
Toxicant Concentration (µg/L)	Time	t0			t24			t48			t72			t144		
	0.025	0.065	0.065	0.064	1.441	1.444	1.268	1.315	1.254	1.209	1.201	1.18	1.126	1.025	1.011	0.97
	0.05	0.065	0.065	0.063	1.411	1.398	1.366	1.219	1.376	1.245	1.149	1.24	1.161	0.963	1.026	0.996
	0.1	0.064	0.061	0.063	1.522	0.446	1.526	1.283	0.561	1.384	1.15	0.525	1.244	1.002	0.529	1.031
	1	0.064	0.065	0.069	1.554	1.41	1.44	1.318	1.225	1.198	1.239	1.143	1.116	1.024	0.992	0.964
	Control	0.073	0.078	0.074	1.918	1.853	1.804	1.804	1.568	1.595	1.616	1.436	1.434	1.454	1.267	1.296
	Solvent Control	0.063	0.062	0.059	1.454	1.415	1.447	1.253	1.166	1.309	1.192	1.113	1.207	0.986	1.028	0.999

Table 4.5: Average absorbance (600nm) of Isolate 4 in the different Naphthalene concentrations at the different times

Isolate 4 NAPHTHALENE AVERAGE					
Toxicant Concentration (µg/L)	Time	t0	t24	t48	t72	t144
	0.025	0.065	1.384	1.259	1.169	1.002
	0.05	0.064	1.392	1.280	1.183	0.995
	0.1	0.063	1.165	1.076	0.973	0.854
	1	0.066	1.468	1.247	1.166	0.993
	Control	0.075	1.858	1.656	1.495	1.339
	Solvent Control	0.061	1.439	1.243	1.171	1.004

Table 4.6: Total absorbance (600nm) of Isolate 4 in the different Naphthalene concentrations at the different times after the effect of the Solvent control has been subtracted

Isolate 4 NAPHTHALENE AVERAGE						
	Time	**t0**	**t24**	**t48**	**t72**	**t144**
Toxicant Concentration (µg/L)	**0.025**	0.051	0.965	0.846	0.844	0.667
	0.05	0.051	0.972	0.867	0.859	0.660
	0.1	0.049	0.745	0.663	0.648	0.519
	1	0.052	1.048	0.834	0.841	0.659
	Control	0.075	1.858	1.656	1.495	1.339

Table 4.7: The Standard Deviation of the absorbance values of Isolate 4 in the different Naphthalene concentrations at the different times

Isolate 4 Naphthalene Standard Deviation						
Toxicant Concentration (µg/L)	**Time**	**t0**	**t24**	**t48**	**t72**	**t144**
	0.025	0.001	0.101	-0.053	0.039	0.029
	0.05	0.001	0.023	-0.084	0.049	0.032
	0.1	0.002	0.622	-0.449	0.391	0.282
	1	0.003	0.076	-0.063	0.065	0.030
	Control	0.003	0.057	-0.129	0.105	0.101
	Solvent control	0.002	0.021	-0.072	0.051	0.022

5. Isolate 5

Table 5.1: Absorbance (600nm) of Isolate 5 in the different Atrazine concentrations at the different times in triplicate

Isolate 5 ATRAZINE (TRIPLICATE)																
Toxicant Concentration (µg/L)	Time	t0			t24			t48			t72			t144		
	0.1	0.064	0.062	0.064	0.984	0.962	0.99	1.164	1.145	1.207	1.058	1.056	1.099	0.664	0.655	0.671
	0.2	0.063	0.063	0.064	0.948	0.946	0.945	1.196	1.18	1.128	1.263	1.254	1.033	1.11	1.084	0.642
	0.4	0.064	0.064	0.064	0.961	0.971	0.935	1.129	1.151	1.119	1.026	1.046	1.016	0.654	0.669	0.657
	1	0.063	0.063	0.062	1.009	0.97	0.954	1.212	1.15	1.14	1.11	1.056	1.039	0.708	0.672	0.659
	Control	0.071	0.071	0.071	1.093	1.096	1.09	1.334	1.303	1.335	1.291	1.249	1.323	0.841	0.834	0.836

Table 5.2: Average absorbance (600nm) of Isolate 5 in the different Atrazine concentrations at the different times

Isolate 5 ATRAZINE AVERAGE						
Toxicant Concentration (µg/L)	Time	t0	t24	t48	t72	t144
	0.1	0.063	0.979	1.172	1.071	0.663
	0.2	0.063	0.946	1.168	1.183	0.945
	0.4	0.064	0.956	1.133	1.029	0.660
	1	0.063	0.978	1.167	1.068	0.680
	Control	0.071	1.093	1.324	1.288	0.837

Table 5.3: The Standard Deviation of the absorbance values of Isolate 5 in the different Atrazine concentrations at the different times

Isolate 5 Atrazine Standard deviation						
Toxicant Concentration (µg/L)	Time	t0	t24	t48	t72	t144
	0.1	0.001	0.015	0.032	0.024	0.008
	0.2	0.001	0.002	0.036	0.130	0.263
	0.4	0.000	0.019	0.016	0.015	0.008
	1	0.001	0.028	0.039	0.037	0.025
	Control	0.000	0.003	0.018	0.037	0.004

Table 5.4: Absorbance (600nm) of Isolate 5 in the different Naphthalene concentrations at the different times in triplicate

Isolate 5 NAPHTHALENE (TRIPLICATE)																
Toxicant Concentration (µg/L)	Time	t0			t24			t48			t72			t144		
	0.025	0.065	0.065	0.064	1.038	1.008	1.017	1.11	1.072	1.114	0.982	0.95	0.987	0.645	0.623	0.642
	0.05	0.064	0.068	0.064	1.017	1.008	1.01	1.095	1.081	1.079	0.963	0.941	0.939	0.62	0.604	0.604
	0.1	0.066	0.067	0.065	1	0.978	0.988	1.085	1.079	1.077	0.957	0.945	0.938	0.611	0.603	0.598
	1	0.071	0.066	0.072	1.016	1.021	1.028	1.172	1.094	1.108	1.004	0.973	0.982	0.645	0.623	0.642
	Control	0.079	0.075	0.079	1.123	1.102	1.12	1.291	1.214	1.224	1.177	1.133	1.137	0.799	0.78	0.804
	Solvent Control	0.064	0.065	0.063	1.07	1.078	1.077	1.184	1.15	1.161	1.065	1.036	1.047	0.657	0.648	0.648

Table 5.5: Average absorbance (600nm) of Isolate 5 in the different Naphthalene concentrations at the different times

Isolate 5 NAPHTHALENE AVERAGE						
Toxicant Concentration (µg/L)	Time	t0	t24	t48	t72	t144
	0.025	0.065	1.021	1.099	0.973	0.637
	0.05	0.065	1.012	1.085	0.948	0.609
	0.1	0.066	0.989	1.080	0.947	0.604
	1	0.070	1.022	1.125	0.986	0.637
	Control	0.078	1.115	1.243	1.149	0.794
	Solvent Control	0.064	1.075	1.165	1.049	0.651

Table 5.6: Total absorbance (600nm) of Isolate 5 in the different Naphthalene concentrations at the different times after the effect of the Solvent control has been subtracted

Isolate 5 NAPHTHALENE AVERAGE						
Toxicant Concentration (µg/L)	Time	t0	t24	t48	t72	t144
	0.025	0.051	0.981	1.021	0.873	0.493
	0.05	0.052	0.972	1.007	0.848	0.466
	0.1	0.052	0.949	1.002	0.847	0.461
	1	0.056	0.982	1.047	0.887	0.493
	Control	0.078	1.115	1.243	1.149	0.794

Table 5.7: The Standard Deviation of the absorbance values of Isolate 5 in the different Naphthalene concentrations at the different times

Isolate 5 Naphthalene Standard Deviation						
Toxicant Concentration (µg/L)	Time	t0	t24	t48	t72	t144
	0.025	0.001	0.015	-0.023	0.020	0.012
	0.05	0.002	0.005	-0.009	0.013	0.009
	0.1	0.001	0.011	-0.004	0.010	0.007
	1	0.003	0.006	-0.042	0.016	0.012
	Control	0.002	0.011	-0.042	0.024	0.013
	Solvent control	0.001	0.004	-0.017	0.015	0.005

6. Isolate 6

Table 6.1: Absorbance (600nm) of Isolate 6 in the different Atrazine concentrations at the different times in triplicate

Isolate 6 ATRAZINE (TRIPLICATE)																
Toxicant Concentration (µg/L)	Time	t0			t24			t48			t72			t144		
	0.1	0.064	0.062	0.064	1.049	1.013	1.035	1.226	1.209	1.249	1.077	1.11	1.144	0.706	0.714	0.741
	0.2	0.063	0.063	0.064	1.01	1.027	1.026	1.198	1.202	1.223	1.201	1.045	1.246	1.081	0.683	1.063
	0.4	0.064	0.064	0.064	1.039	1.022	1.051	1.202	1.199	1.213	1.018	1.032	1.055	0.683	0.682	0.686
	1	0.063	0.063	0.062	1.02	1.03	1.01	1.273	1.209	1.184	1.134	1.038	1.034	0.71	0.709	0.741
	Control	0.07	0.073	0.07	1.12	1.155	1.153	1.361	1.305	1.314	1.247	1.167	1.227	0.761	0.761	0.792

Table 6.2: Average absorbance (600nm) of Isolate 6 in the different Atrazine concentrations at the different times

Isolate 6 ATRAZINE AVERAGE						
Toxicant Concentration (µg/L)	Time	t0	t24	t48	t72	t144
	0.1	0.063	1.032	1.228	1.110	0.720
	0.2	0.063	1.021	1.208	1.164	0.942
	0.4	0.064	1.037	1.205	1.035	0.684
	1	0.063	1.020	1.222	1.069	0.720
	Control (no atrazine)	0.071	1.143	1.327	1.214	0.771

Table 6.3: The Standard Deviation of the absorbance values of Isolate 6 in the different Atrazine concentrations at the different times

Isolate 6 Atrazine Standard deviation						
Toxicant Concentration (µg/L)	Time	t0	t24	t48	t72	t144
	0.1	0.001	0.018	0.020	0.034	0.018
	0.2	0.001	0.010	0.013	0.105	0.225
	0.4	0.000	0.015	0.007	0.019	0.002
	1	0.001	0.010	0.046	0.057	0.018
	Control	0.002	0.020	0.030	0.042	0.018

Table 6.4: Absorbance (600nm) of Isolate 6 in the different Naphthalene concentrations at the different times in triplicate

Isolate 6 NAPHTHALENE (TRIPLICATE)																
Toxicant Concentration (µg/L)	Time	t0			t24			t48			t72			t144		
	0.025	0.063	0.064	0.065	1.064	1.047	1.122	1.167	1.134	1.196	1.007	0.991	1.06	0.668	0.668	0.713
	0.05	0.07	0.065	0.066	1.055	1.067	1.054	1.186	1.177	1.172	1.012	1.008	1.003	0.651	0.649	0.656
	0.1	0.066	0.064	0.064	1.041	1.081	1.014	1.164	1.208	1.176	0.991	1.042	1	0.664	0.687	0.652
	1	0.066	0.063	0.065	1.063	1.033	1.006	1.227	1.173	1.147	1.056	0.987	0.964	0.674	0.656	0.645
	Control	0.074	0.072	0.071	1.177	1.182	1.178	1.295	1.288	1.327	1.149	1.113	1.187	0.747	0.735	0.814
	Solvent Control	0.065	0.064	0.063	1.107	1.083	1.109	1.194	1.195	1.18	1.042	1.056	1.042	0.637	0.642	0.637

Table 6.5: Average absorbance (600nm) of Isolate 6 in the different Naphthalene concentrations at the different times

Isolate 6 NAPHTHALENE AVERAGE						
Toxicant Concentration (µg/L)	Time	t0	t24	t48	t72	t144
	0.025	0.064	1.078	1.166	1.019	0.683
	0.05	0.067	1.059	1.178	1.008	0.652
	0.1	0.065	1.045	1.183	1.011	0.668
	1	0.065	1.034	1.182	1.002	0.658
	Control	0.072	1.179	1.303	1.150	0.765
	Solvent Control	0.064	1.100	1.190	1.047	0.639

Table 6.6: Total absorbance (600nm) of Isolate 6 in the different Naphthalene concentrations at the different times after the effect of the Solvent control has been subtracted

Isolate 6 NAPHTHALENE AVERAGE						
Toxicant Concentration (µg/L)	Time	t0	t24	t48	t72	t144
	0.025	0.056	0.998	1.052	0.916	0.556
	0.05	0.059	0.979	1.065	0.905	0.525
	0.1	0.056	0.966	1.069	0.908	0.541
	1	0.056	0.955	1.069	0.899	0.532
	Control	0.072	1.179	1.303	1.150	0.765

Table 6.7: The Standard Deviation of the absorbance values of Isolate 6 in the different Naphthalene concentrations at the different times

Isolate 6 Naphthalene Standard Deviation						
Toxicant Concentration (µg/L)	Time	t0	t24	t48	t72	t144
	0.025	0.001	0.039	-0.031	0.036	0.026
	0.05	0.003	0.007	-0.007	0.005	0.004
	0.1	0.001	0.034	-0.023	0.027	0.018
	1	0.002	0.029	-0.041	0.048	0.015
	Control	0.002	0.003	-0.021	0.037	0.043
	Solvent control	0.001	0.014	-0.008	0.008	0.003

7. *E.coli*

Table 7.1: Absorbance (600nm) of *E.coli* in the different Atrazine concentrations at the different times in triplicate

E.coli ATRAZINE (TRIPLICATE)																
Toxicant Concentration (µg/L)	Time	t0			t24			t48			t72			t144		
	0.1	0.063	0.064	0.064	1.324	1.468	1.421	1.435	1.543	1.449	1.242	1.34	1.244	1.19	1.138	1.282
	0.2	0.06	0.061	0.061	1.46	1.517	1.491	1.581	1.455	1.621	1.481	1.528	1.537	1.383	1.305	1.464
	0.4	0.061	0.063	0.062	1.33	1.48	1.359	1.457	1.356	1.47	1.304	1.19	1.298	1.161	1.074	1.271
	1	0.062	0.061	0.06	1.43	1.344	1.326	1.437	1.495	1.159	1.276	1.301	1.055	1.026	1.082	1.024
	Control	0.071	0.07	0.072	1.72	1.726	1.74	1.66	1.683	1.653	1.557	1.523	1.544	1.476	1.52	1.568

Table 7.2: Average absorbance (600nm) of *E.coli* in the different Atrazine concentrations at the different times

E.coli ATRAZINE AVERAGE						
Toxicant Concentration (µg/L)	Time	t0	t24	t48	t72	t144
	0.1	0.064	1.404	1.476	1.275	1.203
	0.2	0.061	1.489	1.552	1.515	1.384
	0.4	0.062	1.390	1.428	1.264	1.169
	1	0.061	1.367	1.364	1.211	1.044
	Control	0.071	1.729	1.665	1.541	1.521

Table 7.3: The Standard Deviation of the absorbance values of *E.coli* in the different Atrazine concentrations at the different times

***E.coli* Atrazine Standard deviation**						
Toxicant Concentration (µg/L)	**Time**	**t0**	**t24**	**t48**	**t72**	**t144**
	0.1	0.001	0.073	0.059	0.056	0.073
	0.2	0.001	0.029	0.087	0.030	0.080
	0.4	0.001	0.080	0.062	0.064	0.099
	1	0.001	0.056	0.180	0.135	0.033
	Control	0.001	0.010	0.016	0.017	0.046

Table 7.4: Absorbance (600nm) of *E.coli* in the different Naphthalene concentrations at the different times in triplicate

***E.coli* NAPHTALENE (TRIPLICATE)**															
Toxicant Concentration (µg/L)	Time	t0			t24			t48			t72			t144		
	0.025	0.063	0.063	0.064	1.402	1.454	1.503	1.385	1.409	1.406	1.188	1.26	1.196	1.134	1.209	1.088
	0.05	0.063	0.064	0.064	1.513	1.53	1.383	1.435	1.464	1.394	1.241	1.296	1.222	1.168	1.203	1.166
	0.1	0.064	0.063	0.064	1.47	1.331	1.434	1.382	1.348	1.433	1.176	1.202	1.255	1.114	1.14	1.203
	1	0.064	0.068	0.062	1.339	1.35	1.392	1.331	1.345	1.398	1.21	1.147	1.131	0.993	1.024	0.991
	Control	0.072	0.073	0.072	1.731	1.73	1.748	1.687	1.674	1.67	1.519	1.521	1.524	1.481	1.497	1.479
	Solvent Control	0.063	0.064	0.062	1.423	1.42	1.431	1.437	1.432	1.454	1.142	1.098	1.216	0.864	0.96	0.998

Table 7.5: Average absorbance (600nm) of *E.coli* in the different Naphthalene concentrations at the different times

***E.coli* NAPHTHALENE AVERAGE**						
Toxicant Concentration (µg/L)	**Time**	**t0**	**t24**	**t48**	**t72**	**t144**
	0.025	0.063	1.453	1.400	1.215	1.144
	0.05	0.064	1.475	1.431	1.253	1.179
	0.1	0.064	1.412	1.388	1.211	1.152
	1	0.065	1.360	1.358	1.163	1.003
	Control	0.072	1.736	1.677	1.521	1.486
	Solvent Control	0.063	1.425	1.441	1.152	0.941

Table 7.6: Total absorbance (600nm) of *E.coli* in the different Naphthalene concentrations at the different times after the effect of the Solvent control has been subtracted

E.coli NAPHTHALENE AVERAGE						
	Time	**t0**	**t24**	**t48**	**t72**	**t144**
Toxicant Concentration (µg/L)	**0.025**	0.054	1.141	1.164	0.845	0.599
	0.05	0.054	1.164	1.195	0.884	0.634
	0.1	0.054	1.100	1.152	0.842	0.607
	1	0.055	1.049	1.122	0.793	0.458
	Control	0.072	1.736	1.677	1.521	1.486

Table 7.7: The Standard Deviation of the absorbance values of *E.coli* in the different Naphthalene concentrations at the different times

E.coli Naphthalene Standard Deviation						
Toxicant Concentration (µg/L)	**Time**	**t0**	**t24**	**t48**	**t72**	**t144**
	0.025	0.001	0.051	-0.013	0.039	0.061
	0.05	0.001	0.080	-0.035	0.038	0.021
	0.1	0.001	0.072	-0.043	0.040	0.046
	1	0.003	0.028	-0.035	0.042	0.019
	Control	0.001	0.010	-0.009	0.003	0.010
	Solvent control	0.001	0.006	-0.012	0.060	0.069