# BIOCHEMICAL EFFECTS OF SEWAGE POLLUTION ON THE BENTHIC ORGANISM *Nerita polita*

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## DECLARATION

I, Joan Ndumi Munyasya, declare that this thesis is my original work and has not been presented for the award of a degree in any other university or for any other award

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## DEDICATION

To my family, John Muema, Elizabeth Kanini and James Munyasya. May the Almighty God bless you in ways you cannot envision.

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## ABBREVIATIONS AND ACRONYMS

| | |
|---|---|
| **ANOVA** | Analysis of Variance |
| **BOD** | Biological Oxygen Demand |
| **COD** | Chemical Oxygen Demand |
| **ICP-AES** | Inductively Coupled Plasma Atomic Emission Spectroscopy |
| **KMFRI** | Kenya Marine and Fisheries Research Institute |
| **NCAS** | National Council for Air and Stream Improvement |
| **NEPA** | National Environmental Protection Agency |
| **NRC** | National Research Council |
| **SLRI** | St. Lawrence River Institute |
| **TOC** | Total Organic Carbon |
| **TSS** | Total Suspended Solids |
| **UGCE** | University of Georgia Corporative Extension |
| **USEPA** | United States Environmental Protection Agency |

## ABSTRACT

Rapid urbanization has resulted into substantial increase in quantities of sewage, which is traditionally discharged into the ocean, causing a number of environmental problems including threats to oceanic lives as well as ground water contamination. So far, there is limited data in Kenya regarding the toxic effects of sewage on benthic organisms. The aim of this study was to investigate the biogeochemical effects of sewage pollution on the mollusc *Nerita polita* by determining its impacts on the condition factor and energy reserves. A total of 135 molluscs were exposed to varying sewage concentrations of between 5% - 50% over a three week period. The physicochemical parameters of raw sewage were simultaneously determined with those of the nine treatment media and they included pH, temperature, biological oxygen demand, chemical oxygen demand, total suspended solids, total organic carbon, dissolved inorganic nutrients and heavy metals. Condition factor of *Nerita polita* was determined weekly. After an exposure period of three weeks the molluscs were sacrificed and energy reserves determined. Raw sewage had phosphates and total organic carbon levels above permissible limits while dissolved oxygen was below limits. Iron, zinc and copper were within permissible limits while cadmium, lead and mercury were absent. Increase in sewage pollution resulted in elevation of inorganic nutrient contents (0.009-0.077 mg/L; 0.045-155.92 mg/L; and 0.017-1.99 mg/L) for ammonia, phosphates and nitrates respectively while at the same time lowering pH from 7.75 to 7.29 and dissolved oxygen from 5.62-2.38 mg/L. Tukey's post hoc analysis of pH at different sewerage concentrations indicated a statistically significant difference at 40% (7.44± 0.17; $p < 0.05$) and 50% (7.29 ± 0.10; $p < 0.001$) when compared to the control 0% (7.75 ± 0.03). pH levels for all the other treatments were insignificant ($p > 0.05$). Dissolved oxygen levels were statistically significant at $p < 0.05$ for concentrations of 15% (4.50±0.27 mg/L) and 20% (4.38±0.28 mg/L) while concentrations of 30% (4.10±0.39 mg/L), 40% (3.48±0.42 mg/L) and 50% (2.38±0.26 mg/L) were statistically significant at $p < 0.001$. Phosphates were significantly ($p < 0.001$) higher at concentrations of 15% (77.82±3.66 mg/L), 20% (88.69±2.67 mg/L), 30% (109.43±2.91 mg/L), 40% (144.43±2.94 mg/L) and 50% (155.92±2.74 mg/L) when compared to the control (0%; 0.05 ± 0.01mg/L). There was no statistical significant ($p > 0.05$) difference in temperature, ammonia, nitrates for all the nine treatment tanks. The relationship between the condition factor of *Nerita polita* and increasing sewage pollution was inconsistent and statistically insignificant ($r = 0.234$, $p > 0.05$, $r = 0.011$, $p > 0.05$ and $r = -0.453$, $p > 0.05$) for week one, two and three, respectively. Analysis of whole body tissues of *Nerita polita* indicated that lipid reserves (26.8 KJ) were highest in the mollusc tissues, followed by proteins (10.7 KJ) and glucose (1.1KJ) was least at the end of the experiment. Levels of the three biomolecules decreased with the increase in the pollution gradient. Glucose, lipid and protein concentrations in the mollusc tissues ranged from 29.6 -71.3 mg/L, 171-677mg/L and 338-445mg/L, respectively, along the increasing sewage gradient. Findings of this work suggest that energy reserves are sensitive bio indicators but that conditional factor is an unreliable bio indicator to assess acute sewage toxicity. In addition, increase in sewage pollution also leads to a decrease in the water quality and that sewage concentrations above 30% can have profound effects on *Nerita polita.*

# CHAPTER ONE

# INTRODUCTION

## 1.1 Background

Coastal areas are a home to more than half of the world's population and their major economic output is related to activities such as shipping, oil and gas development, coastal tourism and fisheries (Cicin-Sain and Belfiore, 2005). Pollution of the coastal ecosystems is a serious and growing problem. Indeed, different types of contamination including industrial, agricultural, domestic and shipping activities, are discharged into marine ecosystems, and may have a harmful impact on aquatic organisms (Freitas et al., 2012; Carreira et al., 2013). Kenya's coastal and marine ecosystems constitute a rich and diverse national asset that supports the livelihoods of coastal communities and contributes to the national economic development. Not only are marine environments a source of joy, they are also the habitat of rich ecosystems such as, mangrove swamps, coral reefs, sea grass meadows, rocky shores, estuaries, beaches, mudflats, and sand dunes. These habitats provide valuable socio-economic and ecological services, including protection from storm surges and habitats for critical biodiversity, sources of wood products, food and livelihood for local communities. They also provide numerous other irreplaceable ecosystem services (Zakai and Chadwick-Furman, 2002).

Urbanization and industrialization have persistently contaminated most of the natural environment, and consequently major cities around the globe are under a budding threat of pollution. Most of human activities produce large amounts of contaminants that pollute both marine and terrestrial environments.

In addition to exerting negative effects on the health of organisms living in these environments, they alsocause damage to the ecosystem and endangering species. Pollution from anthropogenic sources is certainly not a recent phenomenon and although it is in the general interest to minimize the effects of pollution on biota, the progress associated with extensive industrialization and use of sophisticated chemical processes unfortunately often collides with the goal of reducing pollution.Waste water and solid waste management in Kenya is vested on County governments. However, these authorities including Mombasa County government are facing a number of challenges such as lack of technical, human and financial capacities to effectively manage wastes. This is further aggravated by the fact that there have been no measures put in place to manage non-point sources of pollution, thus exposing the marine realm and the organisms that depend on it to a high risk of sewage pollution (Okuku et al., 2011).

Sewage can be defined as a cocktail of waste from food preparation, dish washing, garbage-grinding, toilets, baths, washing clothes, cleaning houses, showers and sinks (Okuku et al., 2011). Fresh domestic sewage has a slightly soapy and cloudy appearance depending on its concentration. Other sewage contents which are inorganic are chlorides, nitrogen and phosphorus as well as trace metals. Municipal sewage, which contains both domestic and industrial wastewater, may differ from place to place depending upon the type of industries and industrial establishments.

## 1.2 Problem statement

Marine pollution is an area of global concern owing to its adverse impacts on ecosystems and human health. The impacts of pollution are expected to rapidly expand to remote pristine coastal habitats including some highly sensitive ecosystems such as mangrove forests and coral reefs. One of the most serious and increasingly common sources of disturbance in coastal waters is the discharge and disposal of sewage effluents (Gray, 1982). Disposal of sewage is an environmentally harmful practice in Kenya. Sewage pollution is often manifested by elevated concentrations of inorganic nutrients (Fabricius, 2005). Responses of ecosystems to nutrient stress span a wide ambit of biological effects, including local declines in biodiversity, high rates of mortality, increase in benthic macro algae, cyanobacteria and algal blooms (Szmant, 2002; Fabricius and McCorry, 2006). Sewage also has adverse effects on human health. Risk of gastro-intestinal illnesses such as hepatitis and gastro-enteritis as well as development of facial swellings and rashes is likely to occur. This is especially for people that are involved in recreational activities in areas where sewage is discharged.

## 1.3 Justification

Despite the general understanding on effects of sewage, actual data on environmental impacts of sewage are lacking (Okuku et al., 2011). Society and managers require tools based on sound scientific knowledge to properly monitor, manage and protect such sensitive marine areas. Study of impacts at biochemical level is aimed at early detection of sewage effects before gross damage to the ecosystem. Effects of pollution at a biochemical level are more rapid than effects which are manifested at population levels.

This observation provides a rationale for the use of energy reserves at a biochemical level of organization, which functions as a rapid and sensitive measurement for slower, but ecologically more relevant responses to stressful environments. This study was carried out to investigate the biochemical effects of sewage pollution on the benthic mollusc *Nerita polita* by determining its impacts on the condition factor and energy reserves.

## 1.4 Research questions

i. Does sewage pollution alter the physicochemical properties of marine water?
ii. Does sewage pollution affect condition factor of *Nerita polita*?
iii. Does sewage pollution affect energy reserves of *Nerita polita*?

## 1.5 Null hypotheses

i. Sewage pollution does not alter the physicochemical properties of marine water.
ii. Sewage pollution does not affect condition factor and energy reserves of *Nerita polita*.

## 1.6 Objectives

### 1.6.1 General objective

i. To determine the effects of sewage on the physicochemical properties of marine water, condition factor and energy reserves of *Nerita polita*.

### 1.6.2 Specific objectives

i. To determine the effects of sewage on the physicochemical properties of marine water.

ii. To determine the effect of sewage on the condition factor of *Nerita polita*.

iii. To determine the effects of sewage pollution on energy reserves of *Nerita polita*.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 *Nerita polita* biology

*Nerita polita* is a marine gastropod mollusc in the family Neritidae. *Nerita polita* has a smooth and polished shell with variable color (marked with grey, or cream) and sometimes with axial growth rings. These rings are the principal source of information on the age and growth of molluscs. A growth annulus is assumed to be deposited each year (Lutz and Rhoads, 1980). The mollusc has a large muscular foot that is used for locomotion up and down the rocky cliffs as well as providing it with firm anchorage on the rocks during high tides.

**Figure 2.1: Photograph of *Nerita polita* anchored on a rock during low tide**

Distribution of *Nerita polita* is categorized as Indo-Pacific and it is found in Kenya, Madagascar, Mozambique and Tanzania (Richmond, 1997). *Nerita polita* habitat includes wet fine sandy shore and littoral fringe of rocky shores.

Due to their sedentary habitat, molluscs are exposed to a variety of environmental stressors, such as raw sewage and heavy metals especially in urban areas where human population and industrialization has been on the rise (Boening,1999). *Nerita polita* was the test organism of choice because they are highly abundant in tropical marine ecosystems and are filter feeders hence their tissues represent their environment well. In addition, molluscs including *Nerita polita*, are non-controversial as organisms for ecotoxicological research, especially as test animals and for environmental monitoring (Oehlmann and Oehlmann, 2003).


**Figure 2.2: Rocky shores where *Nerita polita* are found**

## 2.2 Effect of sewage pollution on aquatic life

One of the most serious sources of marine pollution in coastal waters is the discharge of sewage effluents (Schlacher et al., 2005). Sewage pollution causes eutrophication, which increases primary productivity and algal blooms, oxygen depletion, and reductions in quality of water, fish, coral, and other marine populations.

These changes in turn alter food web dynamics and the entire ecosystem. Sewage pollution has multiple detrimental effects on exposed organisms (Schlacher et al., 2007), and can alter key structural and functional attributes of ecosystems that are affected by wastewater loads (Pastorok and Bilyard, 1985). In comparison to other marine environments, access to intertidal ecosystems is usually easier, and these ecosystems are more amenable to management than sub-littoral benthic habitats (Espinosa et al., 2007). The composition of sessile communities such as molluscs is useful as baseline for ecological monitoring because such organisms are unable to avoid disturbances in the marine environment and thus, the composition of the community reflects their common history (Fa et al., 2002).

### 2.3 Impact of sewage disposal to marine ecosystems

Sewage is usually discharged into shallow coastal waters (Young-Jin and Rousseaux, 2001; McIntyre et al., 1995; Koop and Hutchings, 1996) and is one of the major stresses impacting coastal ecosystems. The potential deleterious effects of pollutants from sewage on the coastal environment are diverse and depend on volume of the discharge, the chemical composition and concentrations in the effluent (Owili, 2003). It also depends on the type of discharge for example whether it is amount of suspended solids or organic matter or hazardous pollutants like heavy metals and organo-chlorines, and the characteristics of the receiving waters (Canter, 1996; Nemerow and Dasgupta, 1991). High levels of soluble organics may cause oxygen depletion (Peter and Robin, 2002) with a negative effect on aquatic biota.

Contamination of the coastal water may result in changes in nutrient levels, abundance, biomass and diversity of organisms, bioaccumulation of organic and inorganic compounds and alteration of trophic interaction among species (Owili, 2003). Sewage effluent is harmful to sub-tidal organisms and the degree to which these communities are affected is determined primarily by the quantity and quality of effluent and the hydrography of the receiving waters. The effects of sewage effluent are most pronounced in the vicinity of the outlets and decrease progressively with increasing distance from the discharge points. Most of the small volumes of effluent discharge have ecological effects in the first 10 meters away from the outfall (Raffaelli and Hawkins, 1996).

### 2.3.1 Characteristics of raw sewage

Characterization of wastes water is essential for an effective waste management. It helps in the choice of treatment methods, deciding the extent of treatment, assessing the beneficial uses of wastes and utilizing the waste purification capacity of natural bodies of water in a planned and controlled manner (Raja et al., 2014). The most informative characteristics of sewage include pH, temperature, dissolved inorganic nutrients, dissolved oxygen, biochemical oxygen demand, chemical oxygen demand, total suspended solids, total organic carbon, and heavy metals (Raja et al., 2014). Biological oxygen demand (BOD) is a measure of the oxygen used by microorganisms to decompose waste (UGCE, 2010). If there is a large quantity of organic waste in the water supply, there will also be a lot of bacteria present. Subsequently, the demand for oxygen will be high so the BOD level will be high.

As the waste is dispersed through the aquatic environment, BOD levels will begin to decline (UGCE, 2010). Nitrates and phosphates in a body of water can contribute to high BOD levels as they can cause plant life and algae to grow quickly. This contributes to the organic waste in the water, which is then decomposed by bacteria. This results to high BOD levels. When BOD levels are high, dissolved oxygen (DO) levels decrease because the oxygen that is available in the water is being consumed by the bacteria. Since less dissolved oxygen is available in the water, fish and other aquatic organisms may not survive (UGCE, 2010). Decrease in dissolved oxygen subsequently raises pH and increases temperature. Chemical oxygen demand (COD) is the most popular alternative test to BOD for establishing the concentration of organic matter in wastewater samples. The COD test only takes a few hours to complete, giving it a major advantage over the 5-day BOD test.

The COD test results in a higher concentration than BOD concentration for the same wastewater sample since only organic compounds are consumed during BOD testing but in COD both organic and inorganic (UGCE, 2010). Total organic carbon (TOC) test is gaining popularity because it only takes 5 – 10 minutes to complete hence it is faster than COD. Suspended matter discharges may also be implicated in the depletion of DO, as well as other adverse aquatic impacts. Suspended matter, if it settles, can blanket the stream bed and block gravel spawning beds and, if organic, remove dissolved oxygen from the overlying water column. Suspended matter that does not settle may obstruct transmission of light into the water column, impairing aesthetics, as well as diminishing photosynthetic activity and the abundance of food to aquatic life (NCAS, 2013).

The most commonly encountered toxic heavy metals in waste water are arsenic, lead, iron, mercury and cadmium. Zinc, nickel, chromium and copper are less common (Varpula, 2013). Table 2.1 is a summary of some of the characteristics of raw sewage and their permissible limits.

**Table 2.1 : Parameters of untreated sewage and their permissible limits**

| Parameter | Limit |
|---|---|
| pH | 6.5-10 |
| Temperature | 30-50$^0$C |
| Dissolved oxygen | 4 mg/L |
| Biological Oxygen Demand | <20 mg/L |
| Chemical Oxygen Demand | <100 mg/L |
| Total Suspended Solids | <30 mg/L |
| Total Organic Carbon | <80 mg/L |
| Ammonia/Ammonium | <30 mg/L |
| Nitrates | <30 mg/L |
| Phosphates | <10 mg/L |
| Iron | <3.5 mg/L |
| Zinc | <2 mg/L |
| Copper | <0.5 mg/L |
| Lead, Cadmium and Mercury | <0.1 mg/L |

Extracted from the Jamaican National Sewage Effluent Standards, 1996

## 2.4 Nutrient enrichment of marine ecosystems

Organic nutrients entering the marine environment in sewage are usually utilized by aerobic bacteria. This process uses up oxygen causing anoxic conditions. Increased nutrient levels especially nitrogen and phosphorus containing materials lead to algal blooms (Pastorok and Bilyard, 1985). The resultant algal growth affects the photic zone depth, causing dissolved oxygen depletion (Danulat et al., 2002; Russo, 2002). Algal blooms, some of which may result in production of algal toxins, can be concentrated by filter feeders such as mussels and molluscs and then consumed by man and other animals thus contributing to risks for water and seafood quality and safety.

The toxins can cause illness ranging from a minor stomach upset to paralysis or heart attack. Increased nitrogen or phosphorus inputs from agricultural fertilizers or wastes and domestic sewage can dramatically change the aquatic community (SLRI, 2009). This situation has an effect of decreasing the amount of dissolved oxygen within the aquatic system. Plants found underneath the surface cannot add oxygen to the water since they become light starved and die. In addition, algae from the surface eventually die and sediment to the bottom where they decompose. Bacterial growth and decomposition on the bottom occurs at a high rate due to the abundance of organic matter from dead plant tissues and sewage (SLRI, 2009). Bacterial decomposition uses up dissolved oxygen which has an effect of creating anoxic conditions within the aquatic habitat.

Phosphates and nitrates occur in small amounts in all aquatic environments and are required to maintain the growth and metabolism of plants and animals. However, in excess amounts, these minerals can prove to be quite harmful (Gilman and Olvany, 2009). Sources of nitrates and phosphates include waste water treatment plants, untreated sewage discharged directly to the ocean, runoff from fertilized lawns and cropland, failing on-site septic systems, runoff from animal manure storage areas, and industrial discharges that contain corrosion inhibitors (USEPA, 2012). Nitrates are a form of nitrogen, which is found in several different forms in terrestrial and aquatic ecosystems. These forms of nitrogen include ammonia ($NH_3$), nitrites ($NO_2$) and nitrates ($NO_3$). Nitrates are essential nutrients, but in excess amounts they can cause significant water quality problems.

Together with phosphorus, nitrates in excess amounts can accelerate eutrophication, causing dramatic increases in aquatic plant growth and changes in biodiversity (USEPA, 2012). Excess nitrates can cause hypoxia and can become toxic to warm-blooded animals at higher concentrations (10 mg/L or higher) under certain conditions. The natural level of ammonia or nitrate in surface water is typically low (less than 1 mg/L). In the effluent of waste water treatment plants, it can range from 10-30 mg/L (USEPA, 2012).

Several studies on mineral levels in different bodies of water indicate that the levels of phosphates and nitrates heavily impact the overall health of the water and its inhabitants (Yanamadala, 2005). Ammonia is present in all natural waters, even if only at very low concentrations. It is derived either from the breakdown of organic nitrogen (mineralization) or by the reduction of nitrate. Nitrate and ammonia are the most common forms of nitrogen in aquatic systems. Ammonia is excreted by animals and produced during decomposition of plants and animals, thus returning nitrogen to the aquatic system. Ammonia is also one of the most important pollutants because it is relatively common but can be toxic, causing lower reproduction and growth, or death of fish and other aquatic life (Environment Canada, 1997).

### 2.5 Dissolved oxygen in aquatic ecosystems

Dissolved oxygen (DO) is the amount of oxygen measured in milligrams dissolved in one litre of water (Jack et al., 2009). Fish, macro-invertebrates, and other aquatic animals depend upon the oxygen dissolved in water.

Without this oxygen, they would suffocate. The ecological quality of the water depends largely upon the amount of oxygen the water can hold. Sources of dissolved oxygen include diffusion of oxygen from atmosphere, aeration of water as it flows over rocks and uneven surfaces, churning action of wind and waves of water and photosynthesis from aquatic plants. Dissolved oxygen depletion has shown lethal effects on physiological and behavioural changes in variety of organisms (Hughes and Ballintijin, 1968). If prolonged changes occur in dissolved oxygen levels in coastal waters, modification can also be expected in the local biotic community structure. Species intolerant of depressed oxygen will either die or try to avoid the environment, while tolerant species will survive in low dissolved oxygen levels.

Wave dominated coastal system is more susceptible to stratification and associated low dissolved oxygen because of low tidal mix. Life in the intertidal zone is particularly challenging since, in addition to the cyclic availability of oxygenated water (each tide cycle lasts 12 hours), organisms can also be challenged with multiple other stresses including desiccation, changes in salinity, and changes in temperature, sometimes including freezing; all of which can potentially change rapidly over the course of a single tidal cycle of immersion and emmersion (Bridges, 1994; Loomis, 1995). Anoxia tolerant molluscs show a two-phase response to declining oxygen tension. As tissue oxygen is depleted (such as during shell valve closure or during emmersion at low tide), organisms first enter a period of hypoxia. During this period, a graded increase in carbohydrate catabolism can occur that allows a compensatory increase in fermentative ATP output in order to maintain normal rates of ATP turnover.

However, as hypoxia deepens, a critical low oxygen tension is exceeded and further attempts at compensation are abandoned in favour of the initiation of conservation strategies. In this phase of severe hypoxia or anoxia, the rates of ATP production and ATP utilization are strongly suppressed and net metabolic rate drops to below 10% of the corresponding aerobic metabolic rate at the same temperature (Storey and Storey,1990). The critical $pO_2$ values that stimulate these transitions differ between species and contribute to the differential success of various species in hypoxic or polluted environments (deZwaan et al., 1992). Metabolic rate depression greatly extends the time that a fixed reserve of internal fuels can support survival and many marine molluscs can survive days or weeks of anoxia exposure.

## 2.6 pH in aquatic ecosystems

Most organisms have adapted to life in water of a specific pH and may die if it changes even slightly. The average pH of sea water is 8.2 (Chen and Durbin, 1994). The pH level can be affected by industrial waste, agricultural runoff or drainage from unmanaged mining operations.

### 2.6.1 Effects of pH on cellular metabolism of molluscs

The precise relationship between pH and metabolic rate depression in molluscs is yet to be established and has been reviewed by a number of researchers (Storey, 1993; Guppy et al.,1994; Hand and Hardewig, 1996).

Both intracellular and extracellular pH decrease during anaerobiosis in marine molluscs (Ellington, 1983; Walsh et al., 1984), but it is yet to be determined whether changes in pH play a role in initiating metabolic suppression. The change in pH during anoxia is typically a steady decline over a long time, sometimes extending to days,whereas the transition into the hypometabolic state during anaerobiosis occurs shortly after anoxia begins (Storey and Storey, 1990). Since pH change takes place gradually, it is unlikely to play a role in signaling metabolic suppression during anaerobiosis. Furthermore, anoxia-tolerant molluscs use strategies to minimize the acid load during anaerobiosis by accumulating neutral or volatile products, using shell bicarbonate for buffering (deZwaan, 1977; Storey and Storey, 1990), which argues against a signaling role for low pH.

However, a moderate decrease in pH during anoxia probably helps to create a metabolic context that favors metabolic depression (Busa and Nuccitelli, 1984). A lower pH environment may facilitate other actions such as enzyme binding with subcellular structural elements, enzyme reaction rates, the relative activities of protein kinases versus protein phosphatases, and changing protein stability (Storey, 1988; Hand and Hardewig, 1996; Schmidt and Kamp, 1996; Sokolova et al., 2000).

### 2.7 Temperature in aquatic ecosystems

Temperature affects many physical, biological and chemical processes, e.g. the amount of oxygen that can be dissolved in water and the sensitivity of organisms to toxic wastes, parasites and diseases.

Some factors that affect water temperature include changes in air temperature and currents. Wastes discharged into water can also affect temperature if the effluent processing or treatment temperature is substantially different to the background water temperature. For example, discharges of water used for cooling in industrial processes can be considerably warmer than the water into which they are discharged. Temperature and dissolved oxygen are inter-related. Temperature affects the amount of dissolved oxygen that water can hold. Solubility of gases such as oxygen, carbon dioxide, and nitrogen decreases as the temperature rises (Manjare et al., 2010).

## 2.8 Molluscs as bio indicators

Chemical analysis of the contaminants in marine water has limitations in that it does not provide reliable evidence of the integrated influence and possible toxicity of such pollutants to the organisms and ecosystem (Zhou et al., 2008; Adjei et al., 2010). Bio monitoring has therefore been applied in a number of places to determine the deleterious effects of sewage pollution. It involves the use of indicators, indicator species or indicator communities to detect changes in water and sediments quality. The presence or absence of a bio indicator is usually interpreted to reflect environmental conditions. The most commonly used bio indicators include benthic macro invertebrates such as molluscs, fish or algae (Oehlmann and Oehlmann, 2003). Molluscs are a group of invertebrates that includes squid, octopuses, cuttlefish, nudibranchs, snails, slugs, sea hares, mussels, clams, oysters, scallops, among others. Molluscs have assumed a major role in monitoring contaminants worldwide (Lauenstein, 1995; Baumard et al., 1998; Boening, 1999).

They have successfully been used to obtain information on the quality of terrestrial, marine and fresh water ecosystems and to quantify the exposure and effects of contaminants in the environment (Markert et al., 1999). Their use as bio monitors and bio indicators provides information on the quantities of pollutants sequestered in the organisms and corresponding effects induced. Additionally, molluscs are preferred as good bio indicators because:

i. They are abundant and widespread in all marine and fresh water ecosystems worldwide.

ii. Majority of molluscs' exhibit limited mobility or are completely sessile as adults therefore, they represent contamination of their habitat ideally.

iii. They are relatively large and therefore easy to handle. Consequently, they can be used both under laboratory and field conditions for active and passive bio monitoring.

iv. Molluscs lack an exoskeleton therefore, they are in direct contact with the ambient medium (water or soil) therefore chemicals can be taken up not only from their diet but additionally via their integument including respiratory organs in aquatic species. This results in greater accumulation potency for contaminants.

v. Molluscs exhibit a limited ability to excrete pollutants, metabolise organic chemicals or to physiologically inactivate toxic metals i.e. through formation of metallothionein (Lee, 1985; Berger et al., 1995). As a result, molluscs have high bioaccumulation capacity for pollutants and can exhibit negative impacts of pollutants at lower environmental concentrations than other invertebrates.

## 2.9 Impact of sewage pollution on condition factor of molluscs

Condition factor is an indicator of health of aquatic organisms (Froese, 2006). It reflects the feeding habits as well as the energy consumption of the mollusc and therefore stress in *Nerita polita* can be estimated by determination of the body condition factor. Stress responses are indicative of multiple stressors on the organisms as toxicants deplete energy reserves through detoxification processes, thus reducing the amounts meant for growth and reproduction. This is a situation that reduces the overall fitness of the organism (Lucas and Berninger, 1985), leading to retarded growth and poor tissue condition. Condition factor is sensitive to pollution and may provide stress levels resulting from exposures. However, it is affected by a number of additional environmental stressors next to pollutants such as salinity, temperature, infestation with parasites and food under field conditions where such parameters are not easily controlled (Oehlmann and Oehlmann, 2003). Under controlled laboratory conditions, such indices have provided useful information on particular stressors.

Use of condition factor, as an index, provides a way of determining the effect of pollutants or serve as a measure of aquatic pollution by sewage though it is less sensitive than biochemical parameters. Indices are designed to communicate information about the current status, and when recorded over time, can yield valuable information about changes or trends (NRC, 2000). Even though a single index may provide a good overview of the status of benthic environments, a universal index that works in all systems is unfeasible, since benthic communities are complex and geographically diverse (Engle and Summers, 1999; Dauvin et al., 2006).

The use of several indices is therefore recommended for providing evaluation of the benthic community health and preferentially in association with other parameters (Salas et al., 2006; Carvalho et al., 2006).

## 2.10 Impact of sewage pollution on energy reserves of molluscs

Use of energy reserves as a biochemical parameter has received much attention as it is more comprehensive and conclusive. This is the most sensitive stress biomarker in an organism (Widdows et al., 1995). It is an integrative measure of energy status of an organism at a particular time. It also responds to chemical stressors in the environment. Energy reserves in an organism are known to provide a sensitive measure of stress in organism rather than the direct measurement of growth (Smolders et al., 2003). Stress due to exposure of pollutants will directly or indirectly influence the energy reserves of an organism (Voets et al., 2006). Molluscs require energy to surpass any physiological stress resulting from sewage toxicity. Energy reserves in *Nerita polita* are quantified as carbohydrates, proteins, and lipids content. *Nerita polita* use carbohydrates as the main source of energy for their metabolic processes.

### 2.10.1 Carbohydrates reserves of molluscs

Carbohydrates are an important source of energy. Polysaccharides function to store the energy and form the structural components. Glycogen as the primary energy reserve in molluscs drives many physiological processes and can be utilized at anoxia and scarcity of food (Bayne et al., 1976; Gabbot, 1983; Bayne et al.,1985; Hummel et al., 1988).

Glycogen is utilized mainly as the source of energy in different invertebrates (deZwaan and Zandee, 1972; Hummel et al.,1989). Glycogen has been used as an indicator of physiological conditions in mussels after the exposure to the different pollutants (Hamelraad and Holwerda, 1990). During periods of stress, depletion of glucose occurs first followed by lipids and lastly proteins. Mandal and Ghose (1970) observed a reduced level of glycogen in the digestive gland of the snail, *Achatina fulica*, due to pollutant stress caused by calcium arsenate. Thurnberg and Manchester (1972) demarcated glycogen as an immediate source of energy when required. The biomolecules act as sensitive indicators of stress (Peter, 1973).

#### 2.10.1.1 Regulation of carbohydrate metabolism during anoxia

Under aerobic conditions, organisms can make use of lipid, carbohydrate or amino acids as fuels for respiration with considerable variation between species and organs in the relative importance of different fuel types. Under anoxic conditions, however, carbohydrates become the primary substrate because the oxidation of hexose phosphates to triose phosphates produces ATP in substrate-level phosphorylation events. Although the yield of ATP is low compared with that available from the complete oxidation to $CO_2$ and $H_2O$ by the tricarboxylic acid cycle, anoxia tolerant species have capitalized on this pathway with adaptations that maximize the length of time that fermentative metabolism can sustain survival (Larade and Storey, 2002).

Among anoxia-tolerant molluscs, adaptive strategies include large tissue stores of fermentable fuels, coupling of glycolysis to additional substrate-level phosphorylation reactions to increase the ATP output per hexose phosphate, production of alternative end products to lactic acid that are either volatile or less acidic so that cellular homeostasis is least perturbed by acid build-up during long term anoxia, and strong metabolic rate depression that greatly lowers the rate of ATP utilization by tissues to a rate that can be sustained over the long periods of anoxia (Larade and Storey, 2002).

### 2.10.2 Lipids reserves of molluscs

Lipid content is an essential organic constituent of the tissues of all animals, and plays a key role in energy metabolism. Lipids are major sources of metabolic energy and essential compounds for formation of cell and tissue membranes (Sargent, 1995).

Molluscs generally contain large amounts of lipids in comparison with proteins and carbohydrates and have high energy contents and thus more costly in energetic terms (Salman and Nasar, 2013). Lipid content of stressed organisms declines due to the energy requirement to eliminate toxins.

### 2.10.3 Proteins reserves of molluscs

Proteins are the most abundant organic molecules of any living system and form the basis of structure and function of life. Proteins are associated with enzyme synthesis, transport, and regulation of metabolism, structural elements and storage. They hence represent an important biochemical constituent in molluscs. Both increases and decreases in protein levels have been observed in organisms exposed to extreme stress.

De Coen and Janssen (2003) and El-Sheekh et al. (2000) reported increased protein levels at low pollutant concentrations, but decreased protein levels at high concentrations respectively. Under anoxic conditions, the mRNA substrate available for translation may be reduced. Gene expression and protein synthesis are costly processes that require many resources, not only large amounts of ATP for energy but supplies of nucleotide and amino acids substrates and sustained amounts of the transcriptional and translational machinery (enzymes, ribosomes, tRNAs).

ATP limitation during anoxia is probably the first and strongest reason for the suppression of nuclear transcription rates (Larade and Storey, 2002). It is expected that organisms would conserve their resources during anaerobiosis by strongly suppressing the rates of transcription and translation of most genes (Larade and Storey, 2002). Against a background of generally reduced gene transcription, those genes whose transcription is specifically up-regulated during anoxia stand out as genes whose protein products are likely to play very important roles in anoxia (Larade and Storey, 2002). Smolders et al. (2003) indicated that low to moderate levels of pollution trigger increased protein synthesis (for instance for detoxification) while glycogen and lipids are still sufficiently available as energy reserves.

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 Study approach

A laboratory bioassay that involved exposure of *Nerita polita* to serially diluted sewage was conducted at KMFRI laboratory to determine impacts of sewage on aquatic ecosystem. A laboratory setting of nine serial dilutions of sewage was preferred because large-scale effects on ecosystem results in a challenging research environment as most of the influencing parameters rather than pollution can be controlled or accounted for in the control tank. In order to achieve measurable impacts, the exposure period was adjusted in the trial phase with an attempt of achieving the most appropriate exposure period. The dilution levels were set to be as environmentally relevant as possible. Higher exposure levels above the environmental concentration in Mombasa were included to mimic expected future increase of sewage inputs into the coastal areas.

## 3.2 Molluscs sampling and collection

In order to control genetic variation and prior contamination history, all molluscs were collected randomly from Mama Ngina Drive (4°4'36"S 39°40'17"E), a suburb in Mombasa County (Figure 3.1). This site was identified during pre-sampling periods and preferred due to the abundance of *Nerita polita* in this site as well as the high flushing rate of the coastal waters. Collection of the molluscs *Nerita polita* was done by hand (Figure 3.2) during low tide and the samples were immediately transported to the laboratory in aerated bags.


**Figure 3.1: Map of sampling location, Mama Ngina Drive (KMFRI Data Resource Center)**

**Figure 3.2: Mollusc collection by hand during low tide**


**Figure 3.3: Mollusc *Nerita polita* in their natural habitat**

Sample collection by hand was preferred because the molluscs are usually firmly anchored to the rocks and well camouflaged with their habitat. A sharp-pointed tool is necessary to assist in their removal too. The species were identified *'in situ'* (Figure 3.3) with the help of the mollusc album of the seashores of Eastern Africa. The molluscs that could not be properly identified due to epiphytic alga or fauna were rejected. Immediately after identification and collection, molluscs were transferred inside fresh plastic containers and then transported to the laboratory. In the laboratory, all samples were washed with running distilled water and were then sorted. The purpose of sorting them was to ensure that molluscs of approximately the same size (2-3 centimetres) were the only ones that were used in the sewage exposure experiment. Each mollusc's size was estimated by measuring the shell length (Guerra-Garcı´a et al., 2004) using vernier callipers (Figure 3.4) and the total weight was determined by a calibrated weighing scale (Figure 3.5).

**Figure 3.4: Determination of *Nerita polita* length using Vernier callipers**


**Figure 3.5: Determination of mollusc *Nerita polita* total weight using a calibrated scale.**

### 3.3 Sewage exposure experiment

*Nerita polita* were first acclimated to laboratory conditions (Figure 3.6). Since the concentration of sewage differs with the tide cycles with the highest impact occurring during low tides, serial dilutions were made to cover environmentally relevant concentrations. The molluscs bioassay consisted of nine different concentrations in 19 Litre glass aquariums as follows: Control (Tank 1) had 0% sewage whereas Tank 2, Tank 3, Tank 4, Tank 5, Tank 6, Tank 7, Tank 8 and Tank 9 had 5%, 7.5%, 10%, 15%, 20%, 30%, 40% and 50% of sewage in a total volume of 15 litres respectively, as shown in figure 3.7. Tanks 1-4 represented the possible sewage concentrations during high and low tides, whereas tanks 5-9 were included to represent any possible future increase in discharge of sewage. Sewage was obtained from KMFRI sewage ejector pipe draining in to the Indian Ocean. Fifteen molluscs of length 2-3 centimetres were used in each test aquarium and the experiment ran for three weeks.

**Figure 3.6: *Nerita polita* in a treatment tank**

**Figure 3.7: Sewage exposure experiment in the Wet Lab**

### 3.4 Physico-chemical analysis

In addition to the mollusc study, several physico-chemical parameters were measured in the treatment media of each treatment tank. The treatment media of each aquarium was changed daily. Water samples were taken in all the aquariums. Water chemistry measurements for ammonia, nitrates and phosphates were determined on alternate days after changing of treatment media. Temperature, pH, and dissolved oxygen were determined daily before the treatment media from each aquarium after the treatment media from the aquarium was changed. Water temperature, dissolved oxygen as well as pH were measured in each aquarium using a digital YSI probe (Professional plus®) (Figure 3.9). The probe was inserted in each treatment tank containing the molluscs, sewage and marine water. The probe was allowed two minutes to read and display the readings.


**Figure 3.8: Digital YSI probe.**

The YSI probe gave readings for pH, dissolved oxygen and temperature simultaneously. After readings are obtained from one treatment tank, the probe was rinsed using distilled water and the process repeated for all the subsequent tanks. The physicochemical analysis of the raw sewage were also determined along with the treatment media.

## 3.5 Dissolved inorganic nutrients

### 3.5.1 Ammonia

Ammonium was determined by following the modified procedure of Parsons et al. (1984). This is an indophenol method, which relies on the measurement of an indophenol colouration formed by ammonium in the presence of sodium nitroprusside after oxidation with hypochlorite and phenol in an alkaline citrate solution.

A 50 ml of sample was dispensed into an Erlenmeyer flask and 2 ml of the phenol solution was added. This was followed by immediate addition of 2 ml of nitroprusside solution and then 5 ml of oxidising solution (100 ml of alkaline solution and 25 ml hypochlorite solution). Absorbance was read in a 1 cm cuvette at 630 nm after at six hours. Standard curve was produced using ammonium sulphate.

### 3.5.2 Nitrates

Dissolved nitrates were determined following the reduction of nitrates to nitrites by the use of a reduction column containing copper coated cadmium fillings. The nitrite ion reacted with sulphanilamide under acidic conditions to form a diazo-compound that when coupled with N (1-naphthyl)-ethylenediamine will form a red-purple dye. This coloration was measured spectrophotometrically at a wave-length of 543 nm. Analytical grade potassium nitrate was used for the preparation of working standard solution. To the 100 ml sample, 2.0 ml of the stock ammonium chloride solution was added. The solutions were mixed by gently swirling and then 5 ml of the solution dispensed at the top of the column. The sample was allowed to pass through but with care not to allow the level to drain to the surface of the cadmium. The remainder of the sample was added to the column. The first 40 ml passing through the column was collected and discarded. The next 50 ml was collected and set aside for further analysis. To each of the reduced 50 ml samples, 1ml of sulfanilamide reagent was added and mixed by swirling. It was allowed to stand for 2 to 8 min. and then 1 ml of the naphthyl-ethylenediamine reagent added. The sample was allowed to stand between10 minutes and 2 hours, and then its absorbance read at 543 nm.

### 3.5.3 Phosphates

The method employed was adapted from Strickland and Parsons (1968). It involved the formation of a complex between soluble reactive phosphate, molybdic acid, ascorbic acid and trivalent antimony. The absorbance of the resulting blue coloured complex was measured spectrophotometrically at a wavelength of 885 nm. Analytical grade potassium dihydrogen phosphate was used for the preparation of calibration standards. To a 100 ml sample, 10 ml of mixed reagent (ammonium molybdate, sulphuric acid, ascorbic acid and potassium antimonyl-tartrate solutions) was added and mixed at once. After 5 minutes, the absorbance at 885nm of the sample was measured.

## 3.6 Biological oxygen demand

The sample was filled in an airtight bottle and incubated at 20°C for 5 days. The dissolved oxygen (DO) content of the sample is determined before and after five days of incubation. The dissolved oxygen of triplicate samples of raw sewage was determined using a digital YSI probe and then placed in air tight bottles and incubated in the dark for 5 days at $20^0$C. The residual dissolved oxygen was determined after incubation period of 5 days using the digital probe again. The $BOD_5$ was calculated from the difference between dissolved oxygen at day 5 and day 1.

## 3.7 Chemical oxygen demand

This test measures the oxygen required to oxidize organic matter in water and waste water samples by the action of strong oxidizing agents under acid conditions. A 0.5 ml sample of raw sewage was taken in triplicates.

It was placed in three 15 ml COD digestion tubes (pre-washed with dilute $H_2SO_4$) and this was followed by addition of 2.5 ml standard potassium dichromate digestion reagent slowly and they were allowed to mix. That was followed by addition of 3.5 ml sulfuric acid reagent through sides of the tubes and let to go to the bottom. The tubes were capped and the contents mixed and cooled. The tubes were then transferred to the pre-heated COD digester at 150 $^0$C and digested for 2 hours. Three blanks were run by substituting sample (raw sewage) with distilled water and sample protocol as in sample analysis followed. The contents of the COD digestion tube were transferred into a 100 ml beaker. Distilled water was added to make the volume to 50 ml followed by the addition of 2 drops of ferroin indicator and finally titration against 0.05 M ferrous ammonium sulfate solution**.**

### 3.8 Total suspended solids

Water samples are filtered through pre-weighed filters and the residue collected on the filter is dried to constant weight. The measured weight change is the total suspended solids in the volume of sample. A dried and pre-weighed 0.45µm filter paper was used to filter the sample and the filtrate was collected in a conical flask. The filter paper was oven dried at105 $^0$C for one hour and was put in incubator for 24 hours. The change in the weight was the weight of the total suspended solids.

### 3.9 Total organic carbon

In the total organic carbon test, organic carbon is converted to carbon dioxide ($CO_2$) and measured with a carbon analyzer. Total organic carbon was analysed by the USEPA method 415.3 (Potter and Wimsatt, 2003). A 0.350 g of homogenized sample was analyzed by placing it on a clean, carbon-free combustion boat. Each sample boat was treated with phosphoric acid drop by drop until the sample stopped "bubbling" and was completely moist with acid. The sample was placed in an oven set at 40°C for 24 hours and then transferred to an oven set at 105°C. Once the sample was dry, the boat was placed on the rack and loaded onto the carbon analyzer.

### 3.10 Heavy metal analysis

This was done using the inductively coupled plasma atomic emission spectroscopy (ICP-AES). It is an emission spectrophotometric technique, exploiting the fact that excited electrons emit energy at a given wavelength as they return to ground state. A 10 ml waste water sample was pumped into the nebulizer via the peristaltic pump. The nebulizer generated an aerosol mist and injected humidified argon gas into the chamber along with the sample. This mist accumulated in the spray chamber, where the largest mist particles settled out as waste and the finest particles were subsequently swept into the torch assembly. Approximately 1% of the total solution eventually entered the torch as a mist, whereas the remainder was pumped away as waste. The fine aerosol mist containing argon gas and sample was injected vertically up the length of the torch assembly into the plasma. The plasma was viewed horizontally by an optical channel.

Light emitted from the plasma was focused through a lens and passed through an entrance slit into the spectrometer and synchronized with the wavelength being diffracted by the grating. The wavelengths that wish to be observed were entered into the computer as the grating sequentially moves to the specified wavelengths. The energy intensity at each wavelength was measured to provide a quantitative result.

### 3.11 Condition factor

This index has traditionally been used by shell fisheries biologists to quantify mollusc's physiological condition. The condition factor (K) was calculated as follows using Fulton's mathematical formula (Ricker, 1971).

$$K = 100 \frac{W}{L^3}$$

Where: K = Condition factor

L = Length of mollusc in centimeters

W =Weight of mollusc in grams

100 = Factor to bring K close to unity

### 3.12 Mollusc homogenisation

At the end of the experiment, fifteen *Nerita polita* from the same exposure tanks were pooled together and homogenised using a home blender. The homogenate was further homogenized using a homogenizer (Homgen Plus®).

The homogenate was kept frozen at -60°C for further analysis of energy reserves concentrations (glucose, lipids and proteins).

### 3.13 Glucose analysis

Body glucose content was determined using the Anthrone method ( Hedge and Hofreiter, 1962). Carbohydrates are dehydrated using concentrated sulfuric acid to form a furfural. Furfural condenses with anthrone to form a blue-green complex that can be measured spectrophotometrically at a wavelength of 630 nm. A 100 mg of the sample was weighed into a boiling tube and hydrolyzed by keeping it in a boiling water bath for three hours with 5 ml of 2.5N HCL. The contents were cooled to room temperature after which neutralization with solid sodium carbonate took place until the effervescence ceased. The next step was to make up the volume to 100 ml and centrifuge at 4472 g-value. The supernatant was collected and 0.5 and 1 ml aliquots for analysis were taken in duplicates. Standards were prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard. The volume of the standards and samples was made up to 1 ml in all the tubes by adding distilled water then 4 ml of anthrone reagent subsequently. The tubes were heated for eight minutes in a boiling water bath and cooled rapidly and the absorbance read at 630 nm. A standard calibration graph was prepared by plotting concentration of the standard versus absorbance. From the graph the amount of glucose present in the sample tube was calculated.

### 3.14 Lipid analysis

Lipid content was determined by dissolving homogenized tissue in chloroform (Bligh and Dyer, 1959). This is a simple standard method that uses chloroform/methanol-based protocols that include phase partitioning into the organic layer. These protocols work relatively well for a wide variety of physiologically relevant lipids. The main advantage of this procedure is that it determines total lipids in a sample and samples can be analysed directly with no pre-drying required. A 500 µl volume of chloroform was added to the 200 µl of homogenate. Samples were mixed and 500 µl methanol and 200 µl deionized water were added and shaken. The mixture was centrifuged for 5 minutes at 4472 g-value. The top phase of methanol and water and the thin layer between the two phases were removed. All lipids dissolved in the remaining bottom phase. A 100 µl volume of the bottom part was transferred to clean glass tubes. Standard curve concentration was made from tri-palmitine 5 mg/ml stock solution. Concentration of 0, 1250, 2500, 3750, 5000 µg/ml tri-palmitine was made for the standard curve. A 500 µl aliquot of concentrated sulphuric acid was added to all test tubes (the samples and the standard curve) and incubated in a hot oven for 15 minutes at 200°C. The glass tubes were removed from the oven and left to cool on ice. 1 ml of distilled water was added to all glass tubes and mixed gently. The mixture was transferred in duplicates and read in the spectrophotometer at 400nm wavelength. Based on the absorbance result and concentration of the standard solutions, the lipid concentration was calculated using the regression equation of the corresponding standard curve.

### 3.15 Protein analysis

For protein determination, sample homogenates were analyzed using the Kjeldahl method (Figure 3.8) as recently described by Shaw (2006). Kjeldahl method is accomplished in three steps: digestion (conversion of nitrogen in the sample to ammonia), distillation (separation of the ammonia from the digestate and collection for analysis), and titration (quantification of the ammonia and calculation of the initial protein concentration). A sample of 5 grams was homogenized, weighed and transferred into digestion tubes. The tubes were placed on the digestor at $450^0$C for digestion. This was followed by careful addition of 15ml of concentrated sulfuric acid and 1 kjeldahl tab to each of the digestion tubes. Digestion was done at $450^0$C for 45 minutes. After digestion, the tubes were left to cool. The tube contents were then removed and put in the steam distillation unit. Distillion was done for 4 minutes. The distillation converted $NH_4^+$ to $NH_3$ using an alkali (NaOH). The ammonia released was thereafter steam distilled into a receiver solution containing boric acid and indicator solution of 0.1g methyl red and 0.1g Bromocresol green in 100 ml ethanol. Titration of the the distillate with 0.02 M hydrochloric acid until a blue end point is achieved followed. The volume of acid consumed in titration was noted and used for protein calculations.

**Figure 3.9: Kjeldahl apparatus comprising distillation and titration units, digester and scrubber**

### 3.16 Total energy available

Energy available was calculated by summing the energy values for the different reserves. The enthalpy of combustion for each energy reserve is 24000 J per one gram of protein, 16000 J per one gram of glucose and 39500 J per one gram of lipid (Chebbaki et al., 2010).

### 3.17 Data management and statistical analysis

The mean lengths, weight and condition factor of molluscs as well as the physicochemical properties of sewage and marine water were expressed as mean±standard deviation. Pearson product moment correlation was obtained using Minitab 16. This gave *r* and *p* values that were used to interpret the relationships between energy reserves, condition factor, physicochemical parameters and dissolved inorganic nutrients and the increasing sewage pollution.

In addition, multiple comparisons between the different treatment tanks were determined using ANOVA followed by Tukey's test with bonferroni adjustment for the physicochemical parameters, lengths and weights. The statistical significance was considered at $p < 0.05$.

# CHAPTER FOUR

# RESULTS

## 4.1 Mean values of various parameters of raw sewage

Table 4.1 shows the parameters of the raw sewage. The values of the various parameters are averages of each parameter over the study period.

**Table 4.1: Means of various parameters in raw sewage used for sewage exposure experiment**

| Parameter | Mean values |
|---|---|
| pH | 7.21±0.17 |
| Temperature | 27.37±0.68$^0$C |
| Dissolved oxygen | 1.57±0.04 mg/L |
| Biological oxygen demand | 1.38+0.07 mg/L |
| Chemical oxygen demand | 2.99±0.05 mg/L |
| Total suspended solids | 7.00±3.00 mg/L |
| Total organic carbon | 84.00±3.00 mg/L |
| Ammonia | 2.29±0.02 mg/L |
| Nitrates | 8.36±0.01 mg/L |
| Phosphates | 280.49±3.15 mg/L |
| Iron | 0.44±0.01 mg/L |
| Zinc | 0.02±0.01mg/L |
| Copper | 0.10±0.01 mg/L |
| Lead, Mercury and Cadmium | Not detected |

Results are expressed as mean±standard deviation of triplicates.

The pH and temperature were within their permissible limits in sewage effluents given in table 2.1. The dissolved oxygen in the raw sewage was below the expected minimum value of 4 mg/L and this posed a direct danger to aquatic organisms in the treatment tanks. The biological oxygen demand was 1.38±0.07 mg/L and this was within the permissible value of 20 mg/L.

The chemical oxygen demand was seen to be almost twice the amount of biological oxygen demand at 2.99±0.05 mg/L but was also within the permissible limits in sewage effluents. Total suspended solids results ranged below the permissible limit of 30 mg/L as they were measured at 7±3.00 mg/L. The total organic carbon was seen to be above the permissible limit of 80 mg/L to a measurable quantity of 84 mg/L. Such results superseding the acceptable limits were also observed with the phosphates. The measured value of phosphates in the raw sewage was 280.49±3.15 mg/L which is 28 times higher than the allowable limit of 10 mg/L. Ammonia and nitrates levels were 2.29±0.02 mg/L and 8.36±0.01 mg/L, respectively, both of which were within permissible limits. The heavy metals detected in the raw sewage were zinc, iron and copper. They were all within the permissible limits of 2.0, 3.5 and 0.5 mg/L, respectively.

## 4.2 Effects of sewage pollution on the physicochemical properties of marine water

Table 4.2 summarises water quality (pH, dissolved oxygen, temperature, ammonia, nitrates and phosphates) in the nine treatment tanks containing varying levels of sewage pollution. The values in table are the averages of each parameter in each treatment tank over the study period. Ammonia concentration in the control tank was low (0.01±0.01 mg/L) and as the sewage concentration increased, there was a gradual increase in the ammonia levels. The highest levels of ammonia were recorded in tank 9 (0.08±0.02 mg/L) which is about 8 times higher than the control. The correlation between ammonia and sewage levels was significantly positive ($r = 0.994$; $p < 0.05$).

Initially the nitrate levels followed the same pattern as ammonia with the control tank containing a low concentration of 0.02±0.01 mg/L and the concentration increasing with the increase in the pollution gradient. However, there was a sharp spike in tank 5 and this increase continued with the highest levels being recorded in tank 9 (1.99±1.45 mg/L) which had nitrate levels that were over 100 times above those of the control tank. Pearson coefficient indicates a significant positive correlation between nitrates and pollution gradient ($r$ = 0.903; $p$ < 0.05). The phosphates followed a similar pattern to that of nitrates in that their levels increased with increase in pollution gradient and that there was a sharp spike in tank 5 (77.82±3.66 mg/L). The highest levels of phosphates were recorded in tank 9 (155.92±2.74 mg/L) which was over 3,000 times higher than that of the control. Pearson correlation indicated a significant positive relationship between the phosphates and the pollution gradient (r = 0.950; p < 0.05).

As in table 4.2, the control tank had the highest pH at 7.75±0.03 while tank 9 had the lowest pH of 7.29±0.10 during the experiment. The trend of the water pH was opposite that of the temperature in that there was a gradual drop in pH with increase in sewage concentration, Pearson correlation indicated that there was significant negative correlation between pH and sewage concentration ($r$ = -0.965; $p$ < 0.05). The water temperature for the nine treatment tanks during the experiment was averaged and presented in table 4.2. Tank 1 recorded the least temperature of 24.30±0.46$^0$C while the tank with the highest pollution (tank 9) recorded the highest temperature of 25.10±0.46$^0$C.

This showed that water temperature of the treatment media increased along the pollution gradient. There was a significant positive correlation between temperature and sewage concentration ($r = 0.978$; $p < 0.05$). Dissolved oxygen followed a similar pattern as that observed with pH in that it gradually decreased with the increase in the sewage concentration.

The highest levels of dissolved oxygen were observed in tank 1 with 5.62±0.27 mg/L and the lowest levels were in tank 9 with 2.38±0.26 mg/L. The amount of oxygen in tank 9 was less than half of that measured in the control tank. Pearson correlation also showed a significant negative correlation between dissolved oxygen and the sewage concentration ($r = -0.981$; $p < 0.05$).

Tukey's post hoc analysis of pH at different sewerage concentrations indicated a statistically significant difference at 40% (7.44± 0.17; $p < 0.05$) and 50% (7.29 ± 0.10; $p < 0.001$) when compared to the control 0% (7.75 ± 0.03). pH levels for all the other treatments were insignificant ($p > 0.05$) (Table 4.2). Dissolved oxygen levels were statistically significant at $p < 0.05$ for concentrations of 15% (4.50±0.27 mg/L) and 20% (4.38±0.28 mg/L) while concentrations of 30% (4.10±0.39 mg/L), 40% (3.48±0.42 mg/L) and 50% (2.38±0.26 mg/L) were statistically significant at $p < 0.001$. Phosphates were significantly ($p < 0.001$) higher at concentrations of 15% (77.82±3.66 mg/L), 20 % (88.69±2.67 mg/L), 30% (109.43±2.91 mg/L), 40% (144.43±2.94 mg/L) and 50% (155.92±2.74 mg/L) when compared to the control (0%; 0.05 ± 0.01mg/L).

There was no statistical significant ($p > 0.05$) difference in temperature, ammonia, nitrates for all the nine treatment tanks.

**Table 4.2: Effects of sewage pollution on the physicochemical properties of marine water**

| Treatment tank | Sewage concentration | pH | Temp ($^{o}$ C) | Dissolved oxygen (mg/L) | Ammonia (mg/L) | Nitrates (mg/L) | Phosphates (mg/L) |
|---|---|---|---|---|---|---|---|
| 1 | 0% | 7.75±0.03 | 24.34±0.46 | 5.62±0.27 | 0.01±0.01 | 0.02±0.01 | 0.05±0.01 |
| 2 | 5% | 7.70±0.03 | 24.40±0.49 | 5.26±0.26 | 0.01±0.05 | 0.02±0.01 | 0.26±0.17 |
| 3 | 7.5% | 7.70±0.01 | 24.42±0.48 | 4.97±0.24 | 0.02±0.04 | 0.02±0.01 | 0.36±0.26 |
| 4 | 10% | 7.70±0.01 | 24.50±0.44 | 4.81±0.19 | 0.02±0.05 | 0.03±0.01 | 0.47±0.30 |
| 5 | 15% | 7.66±0.06 | 24.56±0.42 | 4.50±0.27* | 0.03±0.07 | 1.25±0.91 | 77.82±3.66** |
| 6 | 20% | 7.60±0.08 | 24.60±0.39 | 4.38±0.28* | 0.03±0.05 | 1.47±0.20 | 88.69±2.67** |
| 7 | 30% | 7.57±0.04 | 24.70±0.34 | 4.10±0.39** | 0.04±0.05 | 1.72±1.26 | 109.43±2.91** |
| 8 | 40% | 7.44±0.17* | 24.80±0.37 | 3.48±0.42** | 0.06±0.02 | 1.85±1.38 | 144.43±2.94** |
| 9 | 50% | 7.29±0.10** | 25.12±0.46 | 2.38±0.26** | 0.08±0.02 | 1.99±1.45 | 155.92±2.74** |

Results are expressed as mean± standard deviation of triplicates. The *p* values are for multiple comparisons between the control and other treatments: * $p < 0.05$, ** $p < 0.001$.

### 4.3 Effect of sewage pollution on the growth of *Nerita polita*

Figure 4.1 is a photograph of two *Nerita polita* molluscs collected from Mama Ngina Drive in Mombasa County. The lengths and weights of all 135 molluscs used in this study were determined on a weekly basis. The individual values of length and weight are presented in appendix 1 and 2 respectively while the means are shown in tables 4.3 and 4.4. For the control tank, the mean length of the molluscs was fairly constant across the experimental period (29.60±2.37 mm, 30.06±2.94 mm, and 30.02±2.99 mm for week one, two and three respectively). The same pattern was also observed across the other pollution gradients for example in tank 9, it remained approximately 29 millimetres over the three weeks period. Tukey's analysis indicated that there was no statistical ($p > 0.05$) difference in length of the molluscs in the various sewer concentrations for both week one and two. However, week three indicated a significant ($p < 0.05$) difference in the length of the molluscs at 15% and 50%. Weekly comparisons of the lengths did not show any statistical difference ($p > 0.05$).

The mean weight of the molluscs over the three week period per treatment tank was also fairly constant. In the control tank, the average weight was seen to increase from8.857±2.24 grams in week 1, 9.07±2.66 grams in week 2 and 9.472±2.89 grams in week 3. In tank 9, the mean weight of the molluscs was 7.445±1.40 grams in week 1 but there was a slight decrease in weight in week 2 and 3 at 7.337±1.76 grams and 6.736±1.44 grams respectively. Multiple comparison of the mean weight of molluscs showed no statistical ($p > 0.05$) difference in the different sewer concentrations in the tanks. A similar trend was observed in the comparison over the three weeks period.

**Table 4.3: Mean length in millimetres of *Nerita polita* molluscs exposed to concentrations of sewage over a three week period**

| Treatment tank | Sewage concentration | Week 1 | Week 2 | Week 3 |
|---|---|---|---|---|
| 1 | 0% | 8.857±2.24 | 9.07±2.66 | 9.472±2.89 |
| 2 | 5% | 8.866±2.46 | 8.507±2.30 | 8.245±2.32 |
| 3 | 7.5% | 7.891±1.96 | 7.608±1.71 | 7.557±1.62 |
| 4 | 10% | 8.203±2.52 | 8.146±2.45 | 8.089±2.26 |
| 5 | 15% | 6.836±1.47 | 6.736±1.59 | 6.611±1.57* |
| 6 | 20% | 7.934±1.81 | 7.892±1.89 | 7.643±1.86 |
| 7 | 30% | 8.296±1.85 | 7.754±1.61 | 7.237±1.87 |
| 8 | 40% | 8.212±2.38 | 7.978±2.25 | 7.832±2.43 |
| 9 | 50% | 7.445±1.40 | 7.337±1.76 | 6.736±1.44* |

Results are expressed as mean±standard deviation for N=15. The *p* values are for multiple comparisons between the control and other treatments: * $p < 0.05$, ** $p < 0.001$.

**Table 4.4: Mean weight in grams of *Nerita polita* molluscs exposed to concentrations of sewage over a three week period**

| Treatment tank | Sewage concentration | Week 1 | Week 2 | Week 3 |
|---|---|---|---|---|
| 1 | 0% | 29.603±2.37 | 30.057±2.94 | 30.017±2.99 |
| 2 | 5% | 29.500±2.21 | 29.473±2.73 | 29.444±2.65 |
| 3 | 7.5% | 28.649±2.40 | 28.293±2.57 | 28.26±2.33 |
| 4 | 10% | 28.850±3.21 | 28.857±3.01 | 28.273±3.63 |
| 5 | 15% | 27.420±2.68 | 27.410±2.21 | 27.39±2.06 |
| 6 | 20% | 29.097±2.66 | 28.900±2.39 | 28.21±3.14 |
| 7 | 30% | 28.423±2.02 | 28.413±1.86 | 28.38±2.08 |
| 8 | 40% | 28.970±3.25 | 28.603±3.47 | 28.403±3.55 |
| 9 | 50% | 28.277±1.89 | 28.085±2.15 | 28.446±2.10 |

Results are expressed as mean±standard deviation for N=15. Multiple comparisons between the control and other treatments showed non-significant difference.

**Figure 4.1: *Nerita polita* molluscs photographed in the laboratory**

### 4.4 Effects of sewage pollution on the condition factor (K) of *Nerita polita*

In order to obtain the condition factor values, the individual condition factor of each mollusc in the treatment tank was determined and averaged. Table 4.5 summarises the effect of sewage pollution on condition factor of *Nerita polita* in the nine treatment tanks. In the control tank, the condition factor was 0.34±0.37 in week one, 0.34±0.08 in week two and 0.35±0.09 in week three. In the tank containing 50% sewage, the condition factor was 0.33±0.03 in week one, 0.38±0.23 in week two and 0.292±0.05 in week three. Pearson correlation analysis between the K and increasing sewage pollution showed no distinct trend between the increasing sewage pollution and the condition factor over the study period was not statistically significant. In week 1, the statistics were ($r$ = 0.234, $p$ > 0.05) for week 1, ($r$ = 0.011, $p$ > 0.05) in week 2 and week 3 also showed a similar trend ($r$ = -0.453, $p$ > 0.05).

**Table 4.5: Effects of sewage pollution on the condition factor (K) of *Nerita polita* over a three week period**

| Treatment tank | Sewage concentration | Week 1 | Week 2 | Week 3 |
|---|---|---|---|---|
| 1 | 0% | 0.34 ±0.37 | 0.34± 0.08 | 0.35±0.09 |
| 2 | 5% | 0.34 ±0.05 | 0.33± 0.03 | 0.32±0.06 |
| 3 | 7.5% | 0.35 ±0.13 | 0.35±0.11 | 0.33 ±0.02 |
| 4 | 10% | 0.33±0.21 | 0.38± 0.23 | 0.37±0.14 |
| 5 | 15% | 0.35±0.13 | 0.34 ±0.11 | 0.33 ±0.09 |
| 6 | 20% | 0.34±0.12 | 0.34±0.12 | 0.36±0.13 |
| 7 | 30% | 0.36±0.05 | 0.34±0.09 | 0.33±0.11 |
| 8 | 40% | 0.34±0.09 | 0.34±0.12 | 0.38±0.21 |
| 9 | 50% | 0.33±0.03 | 0.38±0.23 | 0.29±0.05 |

Results are expressed as mean±standard deviation for N=15.

### 4.5 Effect of sewage pollution on glucose levels in the tissues of *Nerita polita*

Figure 4.2 shows the effects of sewage pollution on glucose levels in the tissues of the mollusc *Nerita polita* following three weeks of exposure. The general trend was that there was a decrease in the glucose levels in the tissues of molluscs with increase in the pollution gradient. The highest concentration of glucose was recorded in control tank at 71.3 mg/L while the lowest concentration was found in the molluscs in tank 9 (29.6 mg/L), which represents a 140% decreases across the pollution gradient. Pearson coefficient analysis between the glucose levels and increasing sewage pollution indicated a highly significant negative correlation ($r$ = -0.999: $p < 0.05$).

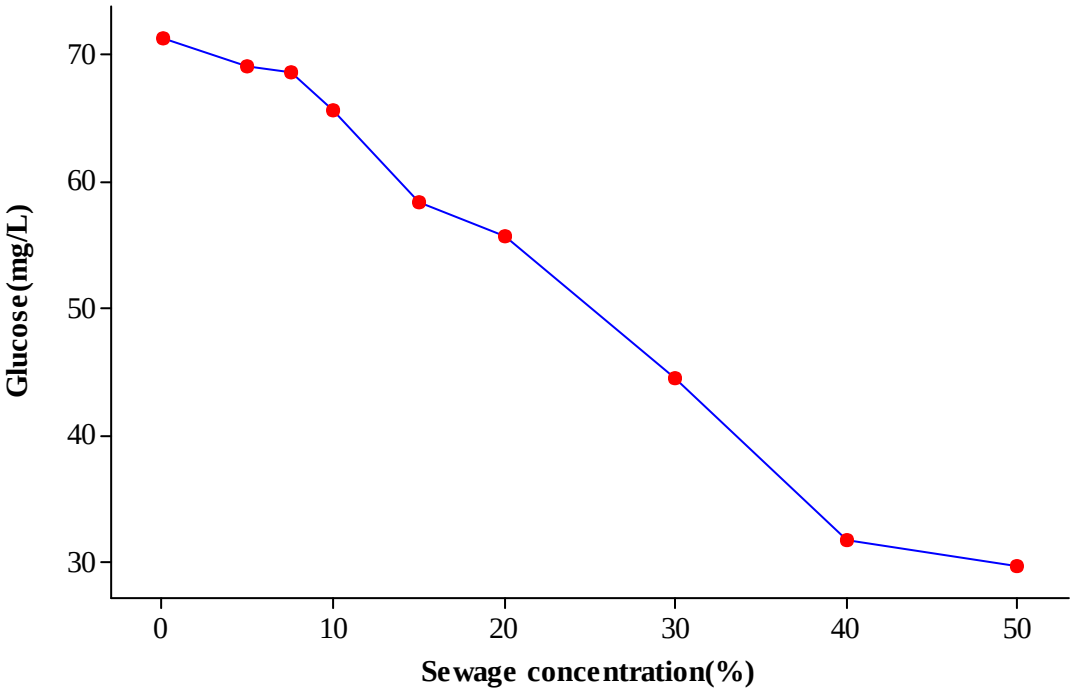


**Figure 4.2: Relationship between glucose levels in the tissues of *Nerita polita* and the pollution gradient**

### 4.6 Effect of sewage pollution on lipid levels in the tissues of *Nerita polita*

The trend of the relationship between lipid levels in tissues of *Nerita polita* and increasing sewage pollution was similar to that observed with glucose in that increase in sewage pollution was accompanied by decreasing levels of lipids in the tissues of the molluscs (Figure 4.3). The highest levels in lipids were recorded in the molluscs harvested in the control tank (677 mg/L) and the least levels were those in the in the tank containing 50% sewage (171 mg/L) which translates to a four-fold difference. Pearson coefficient correlation analysis confirms the existence of a significant inverse relationship between the two variables ($r$ = -0.914: $p < 0.05$).

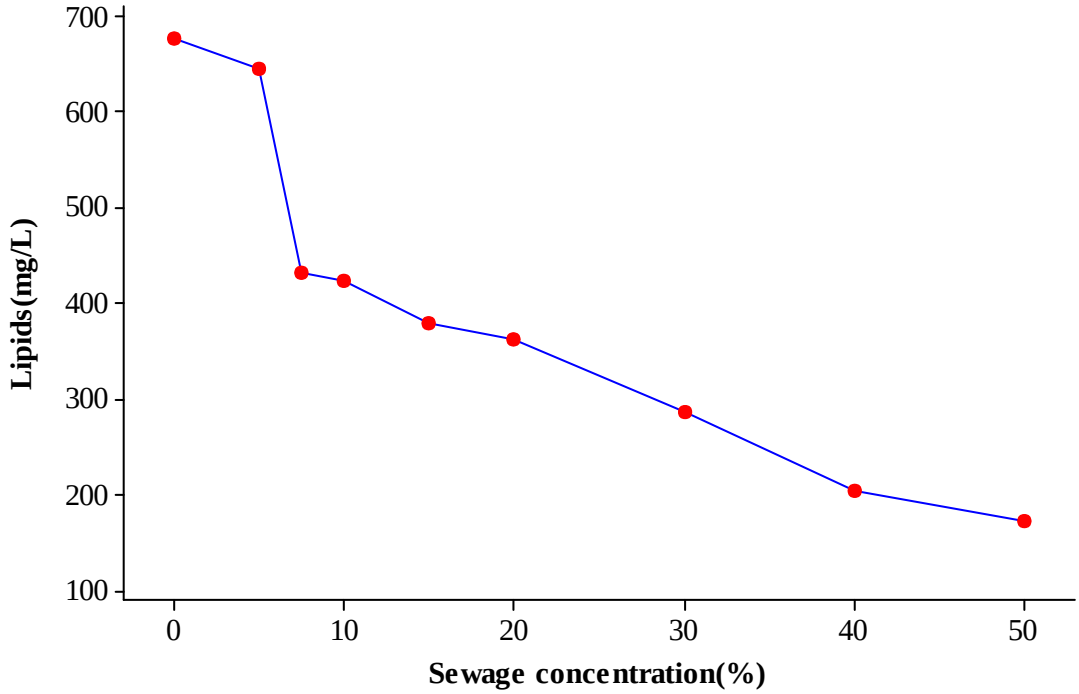


**Figure 4.3: Relationship between lipid levels in the tissues of *Nerita polita* and the pollution gradient**

### 4.7 Effect of sewage on the protein concentration in the tissues of *Nerita polita*

Just like with glucose and lipids, increase in sewage pollution was also accompanied by lowering in levels of protein in the tissues of *Nerita polita*. Figure 4.4 shows that in the control tank (0%), the protein levels were 445 mg/L and in the treatment tank with the highest sewage pollution the levels were 338 mg/L, which is a 1.3 fold difference. Pearson correlation indicated a statistically significant relationship between the two variables ($r$ = -0.97, $p < 0.05$).

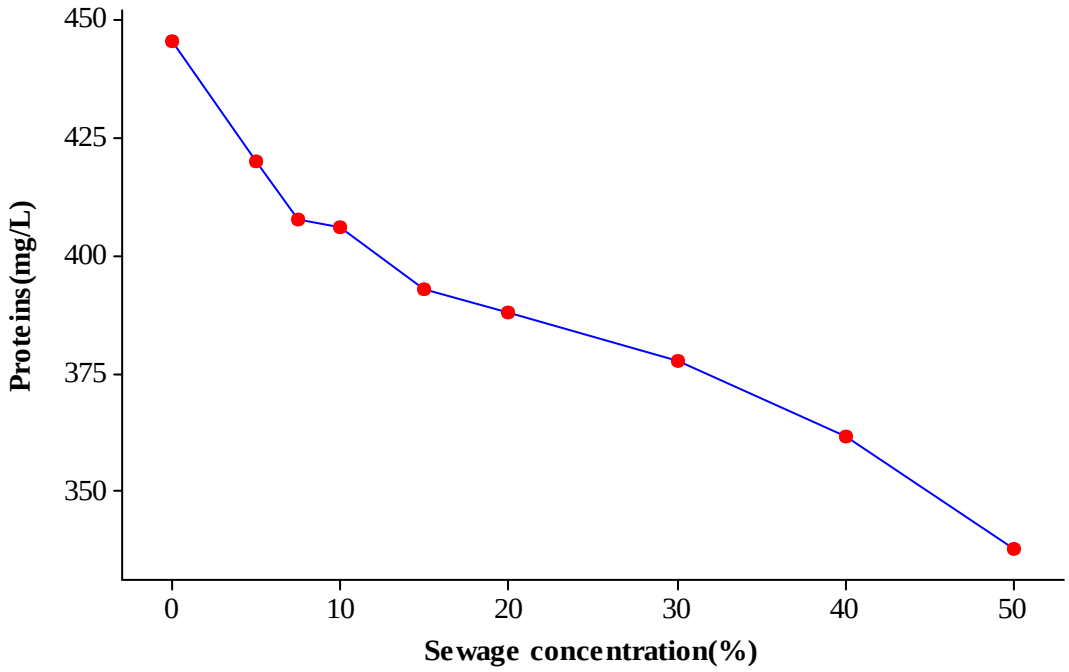


**Figure 4.4: Relationship between protein levels in the tissues of *Nerita polita* and the pollution gradient**

### 4.8 Effects of sewage on the total energy available in the tissues of *Nerita polita*

Table 4.6 summarizes the total energy available in joules in the tissues of *Nerita polita* subjected to a sewage pollution gradient. These values were obtained by summing the individual energy values of glucose, lipids, and proteins of all the molluscs in each treatment tank. The major source of energy for *Nerita polita* was the lipids, followed by proteins while glucose provided the least. For glucose, the amount of energy available to *Nerita polita* in control tank was 1.1 KJ but only 0.5 KJ was available in the tank with the highest pollution; for proteins, it was 11 KJ in the control tank versus 8 KJ in the tank with the highest pollution, while for lipids it was 27 KJ in the control tank versus 7 KJ available in the tank with the highest pollution.

Likewise, the total energy available in the molluscs decreased with the increase in sewage pollution. The control tank recorded the highest levels of total energy of 39 KJ while tank 9 with the highest level of pollution had the least amount of energy (15 KJ). The difference in energy levels in the two tanks is by a factor of 2.6. Pearson correlation indicates a statistically significant negative correlation between energy levels and increasing pollution ($r$ = -0.93: $p < 0.05$).

**Table 4.6: Changes in the energy levels in the whole body tissues of *Nerita polita* exposed to various sewage concentrations after the three week experimental period**
Results are expressed as a summation for N=15

| Treatment tank | Sewage concentration | Glucose (Joules) | Lipid (Joules) | Protein (Joules) | Total energy (Joules) |
|---|---|---|---|---|---|
| 1 | 0% | 1141 | 26753 | 10690 | 38585 |
| 2 | 5% | 1105 | 25474 | 10085 | 36664 |
| 3 | 7.5% | 1097 | 17069 | 9782 | 27949 |
| 4 | 10% | 1049 | 16751 | 9748 | 27550 |
| 5 | 15% | 933 | 14989 | 9429 | 25352 |
| 6 | 20% | 890 | 14296 | 9311 | 24498 |
| 7 | 30% | 711 | 11321 | 9059 | 21093 |
| 8 | 40% | 507 | 8029 | 8673 | 17209 |
| 9 | 50% | 474 | 6780 | 8101 | 15356 |

# CHAPTER FIVE

# DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

## 5.1 Discussion

Wastes from sewage treatment plants, paper manufacturing, food processing and other industries can substantially affect the levels of dissolved oxygen, pH, temperature and nutrients of the natural environment of aquatic organisms  The extent of pollution can be determined through chemical analysis of water, use of condition factor and bio monitoring (Shanmugam et al., 2007; Udo, 2013; Zhou et al., 2008). Chemical analysis is the most direct approach and it involves determination of the physicochemical properties of the exposure media. The most informative physicochemical parameters of water are temperature, pH, oxygen and dissolved inorganic nutrients (Espinosa et al., 2007; Jack et al., 2009; Patil et al., 2012), and they formed the basis of this study.

Phosphates and total organic carbon were above the permissible limits in the raw sewage. High levels of phosphates have been known to cause algae bloom thus enhancing eutrophication. Total organic carbon in sewage is easily oxidised to produce carbon dioxide gas that has an acidification role in the receiving waters. Therefore, since the TOC was above the permissible limit, it could have contributed to decline of pH along the pollution gradient. The levels of COD were higher than the BOD. This was in agreement with studies carried out by UGCE (2010) that suggested that the potassium dichromate used in the COD test oxidises both organic and inorganic substances in raw sewage which results in a higher COD than BOD in the same sample.

Since both the BOD and COD were below the standard limits, then the microbial activity in the sewage exposure tanks was minimal. The temperature of water is important because most of the physical, chemical and biological characteristics of the aquatic life are directly affected by it. Temperature has been shown to affect the solubility of dissolved gases, rate of growth of aquatic plants, algae, and other marine organisms and metabolic rate of organisms. Many aquatic organisms require a specific temperature range, so changing that range makes them stressed hence lowering their resistance to pollutants, diseases and parasites. In this study, water temperature was found to increase with the increase in sewage concentration. Subsequently, the increase in raw sewage concentration was accompanied by an increase in levels of the organic matter. Decomposition of the organic matter results in generation of heat that warmed water to higher temperatures. Additionally, there is release of carbonic acid that was responsible for lowering the pH from 7.74 in unpolluted water to 7.28 in the marine water containing 50% sewage.

Most organisms have adapted to life in water of a specific pH and so small changes in pH may endanger them as they may not survive or reproduce outside that range. According to Johnson et al. (1999), pH of 6.5–8.2 is optimal for most aquatic organisms. Thus, although there was a marginal drop in the pH of the treatment media across the pollution gradient the values were still within the optimal range. Oxygen is essential for respiration and in marine organisms it is available in the dissolved form.. The depletion of dissolved oxygen in marine ecosystem can eventually lead to hypoxia which has profound effects on marine organisms.

The internationally acceptable minimal permissible levels of dissolved oxygen for aquatic life are 4 mg/L (Shanmugam et al., 2007). Levels below 4 mg/L put stress on aquatic life and levels of less than 2 mg/L may result in death of molluscs (Moore, 1991). In this study, there was a significant decrease in dissolved oxygen with the increase in sewage pollution, in the tanks containing 40% and 50% sewage, the oxygen levels were reduced to 3.5 mg/L and 2.4 mg/L, respectively. So sewage concentrations of 40% and above are likely to cause stress to the mollusc *Nerita polita* and this can have profound effect on the development and survival of the species.

Temperature of water has long been known to decrease the ability of water to dissolve oxygen. Studies by USEPA (2006) showed that sea water can hold a dissolved oxygen concentration of 9.0 mg/L at 10°C, but the concentration drops to 7.3 mg/L when the temperature increases to 20°C. In addition, when sewage decomposes the increased microbial activity uses up oxygen from the surrounding water and this leads to the reduction in the amount of dissolved oxygen (Patil et al., 2012). Therefore, it can be concluded that the decrease in levels of the dissolved oxygen in the sewage treated marine water was attributed to the decomposition of the organic material present in the treatment media.

Due to lack of toxicity data with marine organisms the minimum permissible limit for ammonia in salty water is still controversial. Nevertheless, based on acute and chronic toxicity data the tentative limits for ammonia have been set at 0.035 mg/L (USEPA, 1989).

Boardman et al. (2004) suggested that these levels should be significantly increased from 0.035 mg/L to 0.081 mg/L. In the present study, the ammonia levels ranged from 0.01 mg/L in the control tank to 0.08 mg/L in the tank with the highest pollution. Therefore, based on the USEPA criterion it is concluded that *Nerita polita* is safe up to a sewage concentration of 20%, where ammonia levels were 0.03 mg/L, but at sewage concentrations of 30% and above the ammonia levels in the treatment tanks were above the minimum recommended limits thus making the mollusc vulnerable. Likewise, on the basis of the Boardman criterion the ammonia concentrations in all treatment media were within the allowable limits including one in which the marine water was substituted with half sewage. These results indirectly support the adjusted high limit values of ammonia as there was no recorded impairment on the growth of *Nerita polita* even in the treatment tank with an ammonia concentration of 0.08 mg/L.

Nitrogen is an essential nutrient required by all living organisms for building protein. In aquatic ecosystems, the usable form of nitrogen is ammonia and nitrate. As aquatic plants and animals die, ammonia is oxidized to nitrates. Consequently, the concentration of nitrates in fresh water and marine systems is higher than that of ammonia (Rabalais et al., 2002), an observation that is consistent with the findings of this study, where the levels of nitrates ranged from 0.02 mg/L to 1.99 mg/L while those of ammonia were lower and ranged from 0.01 mg/L to 0.08 mg/L. The internationally accepted maximum high limit for nitrates in portable water is 10 mg/L (Shanmugam et al., 2007).

The Canadian Council of Ministers of the Environment (2003) recommended water quality criteria ranging of 2.9–3.6 mg/L to protect fresh water and marine life, while Camargo et al. (2005) proposed a maximum level of 2 mg/L for the protection of sensitive aquatic animals. In this study the highest levels of nitrate was 1.99 mg/L and thus within permissible limits.

Due to its biological role, phosphorous is an essential macro-mineral for terrestrial plants and for marine phytoplankton, algae and sea-grasses (Paytan and Mclaughlin, 2011). However, excess of phosphates mainly contributed by sewage pollution and agricultural runoff, can be problematic causing eutrophication (Sharp, 1991), which in turn poses problems of hypoxia and anoxia to marine life and thus affecting aquatic resources. A review of the literature indicates that there is insufficient information available on acute aquatic toxicity assessment of phosphates. Based on a recent study by Kim et al. (2013), phosphates with a nominal concentration above 100 mg/L possessed no direct toxicity to aquatic organisms. So it requires very high levels of phosphorous to express toxicity in marine organisms. In this study, the concentration of phosphate increased with the increase in sewage pollution. Sewage concentration of 15% and above recorded very high levels of phosphate above 77 mg/L, and these are likely to cause algae bloom leading to eutrophication and depletion of dissolved oxygen in water. Several studies have reported mass mortalities of marine organisms arising from hypoxia (Diaz and Roserberg 1995; Jack et al., 2009). Out of a total of 135 molluscs used there were only three mortalities reported that occurred in the treatment media with the highest pollution.

The level of dissolved oxygen in this tank was 2.38 mg/L which is below the recommended levels of 4 mg/L. Therefore these deaths were most likely caused by hypoxia due to the reduction of dissolved oxygen to levels that were of a risk to marine life. By virtue as choice organisms, *Nerita polita* are suitable organisms for bio monitoring due to their ability to accumulate toxins without death for long periods. Therefore a single mortality is significant. In terms of acute toxicity, this study suggests that *Nerita polita* is at risk at a sewage concentration above 30% and that the greatest risk is contributed by reduced oxygen levels.

Condition factor (K) is a somatic biomarker that indicates the health and well being of an individual. Studies done by King (2007) proposed that the greater the weight of limpet, the greater its condition. These variations in the condition of limpets may reflect variations in food abundance and reproductive stage. Condition factor is determined using the weight and length of the mollusc. Out of the two parameters, weight is expected to vary more than the length because weight of the *Nerita polita* can be directly interfered by other factors such as reduction of food readily available for the mollusc in its environment among others. Toxins in sewage affect the growth of *Nerita polita* by changing their metabolism and thus requiring the mollusc to increase energy for maintaining homeostasis rather than growth and reproduction. Condition factor of *Nerita polita* was determined using the fulton's condition index. This study revealed that fulton's index was inconsistent despite a consistent increasing pollution gradient. This implies that condition factor is not an informative tool for studying acute toxicity in *Nerita polita.*

Bio monitoring is a technique used to assess the environment based on analysis of an individual organism's contents. The markers used are principally toxins and biological changes. The latter is in form of energy reserves as reported by Nahrgang et al. (2013). Indicator species used in bio monitoring are mainly the benthic macro invertebrates such as the mollusc *Nerita polita*. The major energy reserves for the molluscs are carbohydrates, lipids and proteins. Demands for the energy reserve in marine organisms are not constant, and they are affected by exogenous factors such as food availability, temperature, pH, dissolved oxygen and sewage pollution. Here the analysis of whole body tissues of *Nerita polita* showed that lipid concentration were the highest, followed by protein and carbohydrates was least. These findings are consistent with observations of other molluscs collected in Euphrates River (Salman and Nasar, 2013). When investigations were made for the effects of sewage pollution on the biochemical composition of the tissues, it was noticed that the carbohydrate, lipid and protein contents of the whole body tissues showed a decrease with the increase in the sewage concentration.

Glucose is the immediate source of energy for *Nerita polita.* When the molluscs are subjected to sewage toxicity, glucose is the first energy reserve to be used to provide energy for detoxification processes. Decrease in glucose reserves is probably due to glycogenolysis and utilization of glucose to meet increased metabolic cost. In response to carbohydrate depletion, molluscs use other substrates to obtain the energy needed for its maintenance through gluconeogenesis such as lipids.

Lipids are the second best energy producers next to carbohydrates and they are mainly used in stress conditions. The lipid reserves were also seen to decrease as the sewage concentration in the treatment media increased. This observation is in agreement with a previous study by Capuzzo et al. (1984) which observed reduction in triacylglycerol synthesis and decreased mobilization of essential fatty acids to phospholipid pools at the biochemical level due to contaminants. The regulation of global protein synthesis represents an important adaptive strategy for a response to cellular stress (Ivanina, 2009).

Proteins are the last source of energy for a mollusc in the absence of glucose and lipids. Just like the previous two biomolecules, there was evidence of decline of protein concentration in the tissues as the sewage concentration increased. Reduction of protein contents of *Nerita polita* under sewage toxicity stress may be attributed to use of various aminoacids in various catabolic reactions as well as muscle wasting indicating a starvation response. It is also possible that the decrease in both lipids and proteins can be attributed partly due to their utilization in cell repair due to corrosion of plasma membranes by some toxicants in sewage such as heavy metals (Harper, 1983) that were detected in the raw sewage.

Changes in physicochemical parameters have also been seen to have an influence on the energy reserves of *Nerita polita*. Elevated temperatures as reported by Bayne et al. *(*1973) reduce the growth of soft tissues. Consequently, molluscs grown at high temperatures are expected to be of a lower physical condition.

In severe hypoxia, the rates of ATP production and ATP utilization are strongly suppressed and net metabolic rate drops to below 10 % of the corresponding aerobic metabolic rate at the same temperature (Storey and Storey, 1990). Decrease in pH affects the energy metabolism in mollusc mitochondria. This decrease produces an increase of $H^+$ concentration in mitochondrial matrix thus increasing ATP production. Throughout the study, pH and temperature more or less remained within the optimal range when compared to dissolved oxygen levels hence one can quickly assume that the effect of decrease in total energy is mainly due to decrease in dissolved oxygen.

## 5.2 Conclusions

- Increase in sewage pollution resulted in increase in water temperature and elevated nutrient contents; while at the same time causing a reduction in pH and dissolved oxygen. It is clear from the study that the effects of elevated nutrients levels, declining pH and dissolved oxygen levels and increasing temperatures distorted the normal environment of *Nerita polita* and this finally led to enhanced metabolic expenditure in response to increased sewage concentrations.
- This study has evidenced that sewage is as threat to *Nerita polita*. Sewage levels above 15% have potential of causing chronic toxicity while those above 30% are likely to cause severe toxicity.
- The relationship between the condition factor of *Nerita polita* and increasing sewage pollution was inconsistent. This exposed a gap in the role of condition factor in acute toxicity thus making condition factor an unreliable biomarker of sewage pollution in short term studies.

- Analysis of whole body tissues of *Nerita polita* indicated that lipid reserves were the highest, followed by proteins and glucose was least. The level of these biomolecules in whole tissues was found to decrease with the increase in the pollution gradient due to the additional energy costs of maintaining homeostasis which led to trade-offs in terms of availability of energy for growth. Thus energy reserves of *Nerita polita* are good bio-indicators that can inform the state of the marine environment even in short term studies.

## 5.3 Recommendations

- Raw sewage that is discharged into shallow marine realm, especially at the vicinity of outlets, should not be allowed to rise above 20% as it is likely to affect the marine environment which in turn endangers benthic organisms including *Nerita polita*.
- Regular monitoring of the marine water realm to warn of any unwanted trends possibly by combining bio monitoring and physiochemical analysis.
- Industries should dilute sewage and /or treat it before it is released into the marine realm.

## 5.4 Suggestions for further studies

- Setting up a similar study but extending the duration of the experiment to beyond three weeks so as to capture the effect of sewage pollution in long term studies and the condition factor of *Nerita polita*.

- Setting of experiments in the field because in the laboratory, most conditions are controlled hence the results are not always representative of the reality in the field.

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# APPENDICES

## Appendix 1: Lengths (mm) of all molluscs used in sewage exposure experiment

| **WEEK 1** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Tank 1** | **Tank 2** | **Tank 3** | **Tank 4** | **Tank 5** | **Tank6** | **Tank 7** | **Tank 8** | **Tank 9** |
| 1 | 31 | 30 | 30.9 | 29.1 | 28 | 30.45 | 28 | 26.55 | 30.25 |
| 2 | 31.75 | 27 | 28.65 | 26.55 | 33 | 31 | 30 | 29.65 | 33.65 |
| 3 | 27 | 30.65 | 30.45 | 30.8 | 27 | 27.35 | 27.4 | 26.9 | 29.5 |
| 4 | 26.75 | 30.6 | 31.65 | 35.75 | 26 | 24.35 | 32.4 | 31.3 | 26.55 |
| 5 | 30.35 | 28 | 27.55 | 32.2 | 28 | 29.15 | 27.45 | 29.75 | 26.6 |
| 6 | 29.45 | 31.75 | 31.9 | 24.9 | 31.2 | 28.65 | 27.25 | 28.85 | 27.5 |
| 7 | 32.65 | 30.9 | 32 | 27.45 | 26.15 | 34.65 | 30.75 | 27.6 | 34.4 |
| 8 | 26.2 | 31.4 | 28.45 | 28.65 | 24.9 | 30.85 | 30.65 | 31 | 33 |
| 9 | 35 | 29.2 | 30.1 | 27 | 31.25 | 27.75 | 28 | 30.95 | 29.35 |
| 10 | 29.1 | 28.8 | 25.15 | 29.55 | 26.75 | 31.15 | 26.85 | 29 | 29.25 |
| 11 | 29.45 | 34.4 | 24.55 | 32.5 | 26.15 | 29.35 | 29.15 | 25.25 | 22.7 |
| 12 | 26.55 | 30 | 27 | 24.5 | 21.6 | 26 | 30.85 | 25.75 | 31.25 |
| 13 | 30.85 | 25.85 | 26.23 | 29.4 | 26.35 | 29.65 | 25.45 | 27.25 | 28.2 |
| 14 | 28.85 | 27.5 | 26.15 | 30.85 | 27.35 | 31.45 | 26.15 | 26.75 | 28.85 |
| 15 | 29.1 | 26.45 | 29 | 23.55 | 27.6 | 24.65 | 26 | 27.6 | 23.5 |
| | | | | | | | | | |
| **WEEK 2** | | | | | | | | | |
| | **Tank 1** | **Tank 2** | **Tank 3** | **Tank 4** | **Tank 5** | **Tank6** | **Tank 7** | **Tank 8** | **Tank 9** |
| 1 | 36.1 | 29 | 25.1 | 30.85 | 32.5 | 27.2 | 27.2 | 30 | 29.55 |
| 2 | 31.1 | 27.45 | 30.25 | 24.65 | 24.5 | 31.45 | 27.65 | 31.1 | 27.85 |
| 3 | 36 | 34.65 | 25.9 | 25 | 26 | 30 | 30.6 | 26.65 | 34.15 |
| 4 | 29.5 | 31.15 | 31.9 | 29 | 30.1 | 31 | 25.85 | 30.1 | 26 |
| 5 | 28.6 | 27.25 | 28.65 | 29.1 | 28.65 | 27.15 | 30 | 24.35 | 30 |
| 6 | 30 | 25.8 | 26.35 | 35.1 | 27.65 | 30.75 | 28.8 | 25.35 | 34.2 |
| 7 | 26.35 | 30.15 | 32 | 27 | 27.5 | 33.65 | 30 | 29.4 | 27.35 |
| 8 | 27.45 | 31.25 | 27.65 | 24 | 25.65 | 24.45 | 28.15 | 30.75 | 29 |
| 9 | 28.4 | 28.8 | 27 | 26.65 | 26 | 29 | 32.55 | 26 | 31.55 |
| 10 | 31.5 | 25.55 | 32.45 | 30 | 28.55 | 28.8 | 28.35 | 28 | 22.25 |
| 11 | 27.45 | 27.65 | 24.4 | 29.65 | 25.65 | 29.75 | 28.15 | 27.23 | 26.85 |
| 12 | 28.3 | 34.2 | 31.35 | 31.55 | 27.1 | 28.6 | 26.6 | 29.6 | 23 |
| 13 | 33.1 | 26.65 | 27.7 | 27.75 | 26.2 | 30 | 26.15 | 29 | 32.25 |
| 14 | 30 | 31.1 | 26.6 | 33.1 | 30.45 | 26.7 | 30 | 29 | 29.4 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 15 | 27 | 31.45 | 27.1 | 29.45 | 24.65 | 25 | 26.15 | 24.75 | 25.65 |
| | | | | | | | | | |
| **WEEK 3** | | | | | | | | | |
| | **Tank 1** | **Tank 2** | **Tank 3** | **Tank 4** | **Tank 5** | **Tank6** | **Tank 7** | **Tank 8** | **Tank 9** |
| 1 | 35.35 | 27.3 | 28.85 | 29.6 | 25.95 | 30.75 | 26.35 | 28.3 | 26.85 |
| 2 | 28.35 | 27.55 | 28.65 | 27.2 | 28.45 | 34.35 | 29 | 26.1 | 31.45 |
| 3 | 31.85 | 31.6 | 32.15 | 30.55 | 29.8 | 21.15 | 25.6 | 32.65 | 29.35 |
| 4 | 27 | 32.2 | 31.55 | 21.2 | 24 | 28.55 | 26.6 | 34 | 28.8 |
| 5 | 28.85 | 26.65 | 30 | 31.2 | 27.35 | 29.4 | 28 | 26 | 31.8 |
| 6 | 33.55 | 30.45 | 27.85 | 26.4 | 29.8 | 24.75 | 25.45 | 27.35 | 27 |
| 7 | 33.3 | 25.2 | 31.15 | 33 | 32.65 | 31 | 30.5 | 25.1 | 25.75 |
| 8 | 26.65 | 28 | 25 | 29.3 | 26.1 | 31 | 31 | 28.45 | 27.65 |
| 9 | 29.15 | 28.45 | 26.6 | 35.5 | 26.85 | 24.55 | 32.55 | 29.65 | 30.45 |
| 10 | 27 | 25.65 | 30.45 | 24.35 | 28.1 | 29.55 | 29.35 | 34.4 | 24.95 |
| 11 | 28.65 | 30.45 | 24.2 | 29.65 | 26 | 27.45 | 28 | 30 | 29.85 |
| 12 | 29.7 | 30.85 | 27.2 | 23.65 | 27.6 | 28.45 | 30.75 | 29.35 | 27.45 |
| 13 | 35.55 | 30.56 | 26.55 | 29.55 | 25.95 | 26.65 | 28.45 | 22 | |
| 14 | 28.3 | 31.75 | 26.15 | 24.95 | 26.25 | 29.55 | 28 | 30 | |
| 15 | 27 | 35 | 27.55 | 28 | 26 | 26 | 26.1 | 22.7 | |

**Appendix 2: Weights (grams) of all molluscs used in sewage exposure experiment**

**WEEK 1**

| | Tank 1 | Tank 2 | Tank 3 | Tank 4 | Tank 5 | Tank6 | Tank 7 | Tank 8 | Tank 9 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 9.1301 | 13.3938 | 5.5064 | 7.8706 | 10.754 | 6.5671 | 10.8047 | 8.973 | 9.3829 |
| 2 | 12.7791 | 6.2218 | 8.778 | 6.7043 | 5.424 | 8.5039 | 10.7043 | 11.6071 | 9.5167 |
| 3 | 5.716 | 10.3641 | 6.4165 | 9.6989 | 6.1823 | 9.0951 | 8.0566 | 8.0962 | 7.1027 |
| 4 | 6.8156 | 10.9532 | 11.6787 | 14.2396 | 8.1625 | 8.5944 | 11.0653 | 6.076 | 9.0913 |
| 5 | 9.6971 | 6.7121 | 7.2162 | 10.4588 | 7.2637 | 6.38213 | 7.6732 | 6.5733 | 6.754 |
| 6 | 8.6206 | 9.1181 | 6.4814 | 4.7635 | 7.014 | 10.271 | 6.493 | 7.2213 | 5.466 |
| 7 | 10.7934 | 10.0061 | 10.2566 | 7.2028 | 7.832 | 11.6265 | 10.7491 | 12.8478 | 7.9911 |
| 8 | 6.3354 | 10.4454 | 7.6468 | 7.7882 | 5.6061 | 4.7794 | 9.6856 | 10.2049 | 8.9432 |
| 9 | 14.7001 | 8.6826 | 7.345 | 5.9243 | 5.6527 | 7.9784 | 7.7507 | 7.7894 | 5.624 |
| 10 | 7.1924 | 7.1748 | 10.7323 | 8.4704 | 7.6697 | 7.422 | 6.2571 | 8.992 | 6.0169 |
| 11 | 7.4102 | 12.2302 | 4.8572 | 11.1465 | 5.0167 | 9.4062 | 8.5207 | 4.2464 | 7.0551 |
| 12 | 6.645 | 10.6233 | 10.2063 | 5.3558 | 6.3634 | 7.9434 | 8.4948 | 10.3419 | 8.2023 |
| 13 | 8.5809 | 5.4151 | 8.0565 | 8.7093 | 5.7798 | 9.03 | 6.2807 | 8.6848 | 7.9711 |
| 14 | 8.5835 | 6.2649 | 6.7007 | 9.848 | 8.2191 | 5.955 | 5.5169 | 7.4645 | 7.4275 |
| 15 | 9.8628 | 5.3911 | 6.47 | 4.863 | 5.6006 | 5.4507 | 6.3893 | 4.0542 | 5.1251 |

**WEEK 2**

| | Tank 1 | Tank 2 | Tank 3 | Tank 4 | Tank 5 | Tank6 | Tank 7 | Tank 8 | Tank 9 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 13.8152 | 7.8769 | 8.7029 | 10.225 | 5.0355 | 8.1745 | 6.3454 | 8.2988 | 6.7746 |
| 2 | 8.621 | 6.4963 | 8.5973 | 5.1368 | 7.5005 | 12.4752 | 7.479 | 5.8734 | 8.2674 |
| 3 | 9.32 | 13.0201 | 6.5046 | 5.3505 | 7.7721 | 8.5619 | 5.9134 | 10.2593 | 6.505 |
| 4 | 6.8229 | 10.1742 | 9.79 | 8.047 | 5.1702 | 7.7861 | 6.0661 | 11.9622 | 10.1873 |
| 5 | 8.5557 | 7.0633 | 6.8127 | 7.5974 | 7.4055 | 8.4106 | 6.9549 | 7.8061 | 8.3971 |
| 6 | 12.2968 | 5.2284 | 10.3932 | 13.3392 | 8.5629 | 5.3419 | 5.818 | 7.1361 | 6.5849 |
| 7 | 8.4124 | 9.3488 | 10.4377 | 6.0942 | 11.1544 | 9.8026 | 9.8052 | 6.6749 | 6.9358 |
| 8 | 6.4636 | 9.5319 | 7.5733 | 4.7191 | 5.4338 | 9.2516 | 8.3497 | 7.2819 | 10.0042 |
| 9 | 9.3263 | 9.9232 | 8.8468 | 5.9914 | 5.5689 | 4.7185 | 10.7032 | 8.3923 | 10.0237 |
| 10 | 6.6089 | 5.334 | 5.2069 | 8.5297 | 6.7492 | 9.1743 | 8.5811 | 11.338 | 7.5542 |
| 11 | 7.4243 | 6.8804 | 4.7706 | 7.6537 | 6.254 | 7.1089 | 7.2979 | 8.8162 | 3.654 |
| 12 | 9.536 | 12.3296 | 6.7569 | 11.4889 | 7.3744 | 7.2016 | 10.8571 | 7.5905 | 5.7239 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 13 | 15.3949 | 5.9436 | 6.4419 | 8.0579 | 6.3594 | 6.2755 | 7.9487 | 4.1194 | 5.7345 |
| 14 | 7.2093 | 8.8299 | 6.4345 | 11.3737 | 5.4024 | 8.2234 | 7.9502 | 10.1099 | 6.1369 |
| 15 | 6.2438 | 9.6271 | 6.8449 | 8.5824 | 5.2974 | 5.8773 | 6.2426 | 4.0078 | 7.574 |
| | | | | | | | | | |
| **WEEK 3** | | | | | | | | | |
| | **Tank 1** | **Tank 2** | **Tank 3** | **Tank 4** | **Tank 5** | **Tank6** | **Tank 7** | **Tank 8** | **Tank 9** |
| 1 | 16.3469 | 6.6855 | 6.9882 | 8.6237 | 7.3427 | 8.4429 | 6.4271 | 8.0153 | 5.9995 |
| 2 | 8.8021 | 8.7535 | 7.3084 | 5.757 | 10.9004 | 8.3588 | 4.6888 | 7.2507 | 9.2507 |
| 3 | 14.8241 | 9.0844 | 10.3004 | 10.5795 | 6.6646 | 6.2893 | 10.4275 | 11.2283 | 7.7379 |
| 4 | 9.2428 | 11.8459 | 9.9042 | 8.1191 | 5.987 | 4.3197 | 4.2022 | 8.4913 | 6.0009 |
| 5 | 7.6211 | 6.6325 | 7.8235 | 9.4485 | 7.4133 | 7.0424 | 8.9721 | 8.7325 | 9.3887 |
| 6 | 9.4288 | 9.4127 | 7.1727 | 6.2938 | 8.7959 | 7.608 | 7.8127 | 12.7595 | 6.5528 |
| 7 | 6.7446 | 4.9989 | 10.4991 | 10.1922 | 5.3763 | 11.8852 | 9.194 | 6.6412 | 6.6294 |
| 8 | 7.4566 | 9.8029 | 5.3128 | 8.0702 | 5.188 | 9.6787 | 7.5651 | 7.776 | 5.3183 |
| 9 | 9.1194 | 7.2448 | 6.3631 | 13.1687 | 5.3153 | 6.4359 | 10.3647 | 10.0122 | 7.1315 |
| 10 | 10.3673 | 5.1754 | 8.8819 | 4.9336 | 5.4824 | 7.8161 | 7.0928 | 3.7237 | 5.2803 |
| 11 | 6.0245 | 5.8325 | 4.9595 | 9.7748 | 5.196 | 7.3918 | 7.0157 | 6.0101 | 7.0624 |
| 12 | 7.3766 | 9.7154 | 7.5944 | 5.0958 | 5.5159 | 5.7166 | 5.3395 | 4.0793 | 4.4847 |
| 13 | 12.4576 | 5.9919 | 6.7838 | 8.3558 | 5.3718 | 9.6414 | 5.6668 | 10.0938 | |
| 14 | 9.5068 | 9.5116 | 6.4242 | 5.3422 | 7.0044 | 8.6537 | 7.9644 | 7.0849 | |
| 15 | 6.758 | 12.9843 | 7.0401 | 7.573 | 7.6172 | 5.3637 | 5.8286 | 5.583 | |