INFLUENCE OF POLLUTION ON DIVERSITY AND NUTRITIONAL COMPOSITION OF AQUATIC EDIBLE INSECTS FROM LAKE VICTORIA, KENYA

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DECLARATION AND APPROVAL

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The thesis is my original work and has not been presented for an award of a diploma or conferment of a degree in any other University or institution.

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APPROVAL

This thesis has been submitted with our approval as the University supervisors.

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DEDICATION

This thesis is dedicated to my parents, The Late Alex Naftali Okoti Obulemire Okusimba and Mrs. Phanice Khanali Okoti; to my husband and partner in life ways Mr. Paul Boiyo Chemabus and to my gifted Son Rhoyce Kiptarus Boiyo and only gifted daughter Lauren Cherop Boiyo.

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ABSTRACT

Freshwater pollution has become a significant global environmental issue, impacting biodiversity and ecosystem services. Winam Gulf of Lake Victoria is greatly affected by pollution from human activities and natural forces. However, little is known about the impacts of pollution on the diversity and nutritional value of edible insects in Winam gulf. This study was therefore conducted with the main objective of determining the effect of pollution on the diversity and nutritional status of the aquatic insects, particularly Chironomus species inhabiting Winam Gulf. The targets of the study included understanding the quality of water, how it impacts the distribution and genetic variation of insects, and the impact of pollution on the nutritional value of aquatic insects. 6 sampling stations were established within Winam Gulf: Kisumu Bay, Kendu Bay, Homa Bay, Maboko and Ndere Islands, and several fish landing beaches. Pollutants like heavy metals (arsenic, mercury, lead, and cadmium), nutrients (NH_4-N, NO_3-N, NO_2-N, TN, TP, PO_4-P), physic-chemical parameters (temperature, pH, conductivity, dissolved oxygen, and turbidity) were analyzed from water and sediment samples. Morphological and molecular approaches used, and the indices of Simpson, Shannon-Weaver, and Pielou's calculated in studying the diversification of aquatic insects. Their genetic variation was measured using the molecular marker from the CO1 gene, and nutritional content, including amino acids, vitamins, fatty acids, micronutrients and macronutrients determined using spectrophotometric analysis. Data analysed, expressed Mean ±SE. Analysis of Variance (ANOVA), Tukey's post hoc test employed. Associations between variables analyzed by Pearson's Correlation Coefficient, Canonical Correspondence Analysis (CCA), and Principal Component Analysis (PCA). The results revealed significant differences in water quality parameters at the six stations. Specifically, Kisumu Bay and Homa Bay recorded a relatively high level of pollutants, NO_2, NO_3, PO_4-P, and heavy metals, compared to other areas like, Maboko and Ndere Islands, more isolated from human activities, exhibited lower pollution levels. 383 aquatic insects were captured, comprising 6 orders, 16 families, 19 genera and 19 species. The highest diversity was observed in Kisumu Bay, despite high pollution levels, suggesting the presence of pollution-tolerant species. Chironomus spp. dominated the insect community in more polluted sites, indicating their resilience to environmental stress. Molecular analysis identified three *Chironomus* species: *Chironomus transvaalensis* (Kisumu Bay), *Chironomus pseudothummi* (Kendu Bay and Homa Bay). In contrast, another unidentified species was found in Ndere Island. This means these species were diverse genetically, showing their adaptability to pollution. Amino acid concentrations varied significantly across sites, with arginine, valine, methionine, and isoleucine showing higher levels in less polluted areas -Ndere Island. Pollution resulted in changes in the fatty acid profile, with palmitic, stearic, and oleic acids, abundant, with significant differences in concentration between polluted and less polluted sites. Omega-3 and omega-6 fatty acids were detected though in smaller quantities, in more polluted zones, indicating the effect of pollution on nutritional quality of Chironomus spp. Finally, the study ascertained that pollution significantly impacts both the diversity and nutritional status of Chironomus spp.in Lake Victoria. Despite this, Chironomids, as bio indicators, showed resilience to pollution, but their nutritional quality, particularly fatty acids and amino acids, was compromised in highly polluted sites which had implications on safety and nutritional value as a food source. Hence, the urgent need for pollution management and recommended continuous monitoring of water quality, adoption of sustainable practices to reduce pollution and suggested the potential for cultivating Chironomids in controlled environments to ensure their safety as an alternative food source, contributing to food security in the region.

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ABBREVIATIONS/ ACRONYMS

AOAC	Official Methods of Analysis of Association of Official Analytical Chemists
APHA	American Public Health Association
ANOVA	Analysis of Variance
BOD	Biochemical Oxygen Demand
BLAST	Basic Local Alignment Search Tool
CH	Chironomus
COD	Chemical Oxygen Demand
CTD	Conductively-Temperature-Depth Profiling Systems
CV-AFS	Cold Vapor Absorption Technique
DNA	Deoxyribonucleic Acid
DO	Dissolved oxygen
EPA	Environmental Protection Agency
FAO	Food and Agriculture Organization
GC/MS	Gas Chromatography-mass spectrometry
GF-AAS	Graphite furnace Atomic Absorption spectrophotometer
GIS	Geographical Information System
H_2O_2	Hydrogen Peroxide
HNO_3	Nitric acid
HP-LC	High- Performance Liquid Chromatography
IARC	International Agency for Research on Cancer
ICP-MS	Inductively Coupled Plasma- Mass Spectrometry
ICP-AES	Inductively Coupled Plasma Emission Spectrometry
KPHC	Kenya Population and Housing Census
KWS	Kenya Wildlife Service
LVBC	Lake Victoria Basin Commission

LVEMP	Lake Victoria Environmental Management Plan
MEGA	Molecular Evolutionary Genetics Analysis
MUFA	Monounsaturated Fatty Acids
mt-DNA	Mitochondrial DNA
NADH	Nicotinamide Adenine Dinucleotide
NCBI	National Center for Biotechnology Information
NO_2-N	Nitrite
NO_3-N	Nitrate
OUT	Operational taxonomic units
PCR	Polymerase Chain reaction
PO_4-P	Soluble phosphorous
PUFA	Polyunsaturated Fatty Acids
PVC	Polyvinyl chloride
UNEDESA	United Nations Department of Economic and Social Affairs
USA	United States
USF	Unsaturated Fatty acids
UV	Ultraviolet
r-DNA	Recombinant DNA molecules
r-RNA	Predominant RNA
RNA	Ribonucleic acid
SFA	Saturated fatty acids
TN	Total Nitrogen
TP	Total phosphorous
WHO	World Health Organization

CHAPTER ONE
INTRODUCTION

1.1 Background Information

Globally, water pollution has been identified as an invisible crisis threatening societies and economies (CDP et al., 2019). In the year 2000, 4 million human deaths were associated with the utilization of polluted water (WHO & UNICEF, 2000). Some species of mammals and fish were threatened to near extinction in inland waters, and this was associated with water pollution (Bashir et al., 2020; Mace et al., 2010; UN WWAP 2003; Vie et al., 2009). Despite this, human livelihood and economies still depend on water for sustainable development (WWAP, 2015). Safe water is vital for socioeconomic development, healthy ecosystems, and human survival. Indeed, pollution of freshwater bodies is a growing concern worldwide (Bashir et al., 2020; Bogardi et al., 2020; Gleik et al., 2001).

Lake Victoria, the world's second-largest freshwater lake is in grave danger of pollution (Nyamweya et al., 2023; Awange, 2020). The Lake is surrounded by some of the major East African cities and faces the pressure of a progressively increasing population of about 53 million in 2020 (Gebreegziabher et al., 2024). According to the International Union for Conservation of Nature (IUCN) report in 2018, out of 234 native freshwater fish species in the Lake Victoria, 86 were classified as threatened, and pollution was highlighted as a danger to 90.2% of the Lake's native fish species, (IUCN, 2018). The Lake has undergone an ecological crisis engineered by human-induced activities and climate change. Currently, the Lake is faced with environmental perturbations, over-exploitation of resources, and habitat disruption of the landscape through projects such as dams, and climate change (Bănăduc et al., 2022; Lukman,2024). The threats synchronously affect biodiversity by altering and modifying the flow and creating environmental gradients, affecting the water quality. Consequently, the population changes in taxa richness, abundance, diversity, physiology, functionality, and biochemistry, which also affects the nutritional components of edible biota.

The Lake Victoria ecosystem is affected by natural bio-geophysical processes, which include increased eutrophication, acidification, and input of toxic pollutants (Gikuma-Njuru et al., 2005). The Lake currently suffers from effects of catchment land use and riparian vegetation, coupled with downstream sedimentation, nutrient loading, and siltation of organic and inorganic

materials, which negatively lead to variations in dynamics of biodiversity. Other anthropogenic activities which pollute the Lake and affect water quality include industrialization, sewage discharge, and other domestic activities. The cumulative effect of the activities influences ecosystem productivity, population dynamics, species composition, and the genetic diversity of the aquatic flora and fauna (Li et al., 2020; Sjöqvist & Kremp, 2016; Correia & Lopes, 2023). In addition, anthropogenic activities have led to massive biodiversity dysfunction and alteration of community structure and functions (McFadden et al., 2023; Gao et al., 2023).

Similarly, the pollution-linked decline in population dynamics of both vertebrates and invertebrates utilized as food and feeds by riparian communities has been reported (Rather et al., 2016). The dynamics also negatively affect the ecological integrity of the entire Lake Victoria (Rather et al., 2016). This calls for a nature-based solution to biodiversity conservation (World Water Development Report, 2021). Most previous research studies have concentrated on spatial assemblages of macroinvertebrates, with little attention to aquatic insects. This implies that little work has been done to survey aquatic insects in Lake Victoria. Therefore, data on the spatial and temporal analysis of pollution indicator species such as insects remain obscured. Analysis of submerged larval stages of insects, population, genetic diversity and nutritional composition may offer information on pollution status for sustainable management of such water bodies (Holt & Miller, 2010; Horiike et al., 2016). Indeed, previous studies on insect larval stages of growth have indicated that the larval stages are diverse, ultrasensitive, and rich, which are perfect biomonitoring tools (Padmanabha et al., 2020).

This study evaluated the impact of pollution on diversity and nutritional composition of aquatic insects of Lake Victoria. In order to achieve this goal, the study investigated spatial variation in water quality to ascertain the pollution status of Winam Gulf of Lake Victoria. Secondly, the influence of pollution on the distribution and genetic diversity of aquatic larval insect assemblages in Winam Gulf, was assessed. A morphological approach was employed to understand insect populations, and a molecular approach was used to understand the genetic diversity. Nutritional profiles of chironomids, a pollution-tolerant species was also determined. Identification of insects using morphological features has previously used, but was found to be inconsistent in resolving taxonomic incongruence (Tihelka et al., 2021; Simon et al., 2012; Li et al., 2019; Wipfler et al., 2019). A molecular approach was then employed to confirm and delineate the cryptic features. DNA barcoding using molecular markers such as rDNA Internal

Transcribed Spacers regions (ITS1 and ITS2), cytochrome oxidase subunit 1 (Cox-1), NADH dehydrogenase subunit 1 (nadh1), and Cytochrome b oxidase (cytb) have been recommended in in studies of phylogenetic relationships between insect species (Zembrzuski & Anderson, 2018; Gokhman, 2018; Fu et al., 2014). However, analyses and phylogeny of the mitochondrial Cytochrome c oxidase 1 (Cox-1) gene were found to be more efficient as a diagnostic tool due to the presence of universal primer conserved regions, ease of amplification, and sufficient variability between closely related species (Mathieu et al., 2020). Further, mitochondrial markers detailed phylogeny among closely related groups since mt-DNA is inherited maternally evolves rapidly, and nucleotide substitutions occur at neutral sites (Zhang et al., 2021; Dowling &Wolff, 2023). Therefore, partial sequences from the cytochrome oxidase subunit I (Cox-1) gene sequences can confer advantages to infer phylogenetic relationships in a spatial-temporal study of edible insect species within an aquatic ecosystem (Usman et al., 2020; Dähn et al., 2024; Charul et al., 2023).

Chironomid insects are a delicacy to people living along the shores of Lake Victoria. In different formulations, the insects are administered to malnourished children, added as flour for baking cakes, used as fish food and baits, used for medicinal value, and associated with cultural beliefs (Otieno et al., 2023; Kambani et al., 2022; Ayieko & Oriaro, 2008). Despite their significance, whether the pollution of Lake Victoria affects the people consuming the chironomids remains a puzzle. The nutritional profiles of chironomids were qualitatively and quantitatively evaluated to provide information on the worthiness of chironomids as food and animal feed as an alternative nutritious food source. This was done to designate chironomids, among other aquatic insects, with superior nutrients to food (FAO, 2010). The study sought to understand if pollution affected the nutritional profiles and define the implications for the human population through the fish diet or in direct eating, a fact that is not fully elucidated. The systems theory, ecological systems theory, environmental theory (NRCC, 1989), and evolutionary theory were used to explain the impact of pollution on the diversity and nutritional composition of aquatic edible insects. A Driver- Pressure-State Impact Response (DPSIR) framework was adopted to describe the interactions in the Lake Victoria ecosystem. This was meant to explain the effect of pollution as a pressure and stressor (Independent variable) affecting the water quality parameters (physical-chemical parameters, nutrients, and heavy metals-dependent variables) and how, in turn, the parameters influence the aquatic insect diversity and the nutritional profiles (dependent

variable). The effect of heavy metal toxicity was also explained. Analytical techniques were employed in analyses.

This dissertation explained the extent to which pollution affects the aquatic insect species populations and the complete functioning of the Lake ecosystem, which directly or indirectly affects the livelihood of the riparian communities. Further, the study explains how the seafood-edible aquatic insects are a conduit through which humans get intoxicated and suffer from related ailments. Hence, the need for early diagnosis of an ailing lake system due to pollution prompts the legislative decision on environmental protection to ensure food security and healthy aquatic systems. The study, therefore, recommends biomonitoring of the marine systems.

1.2 Statement of the Problem

Water pollution is a menace to Lake Victoria's biodiversity and ecosystem, causing substantial risks to human utilization. The pollutants originate from both point and nonpoint sources, which include industrial wastes, domestic wastes, solid wastes, agricultural wastes (fertilizer and pesticides) and emerging pollutants like antibiotics. Pollutants change water quality, affecting biodiversity, particularly the benthic macroinvertebrates like insects. Consequently, impact of pollutants is realized in population and trophic dynamics, alteration in physiology, biochemistry, and the nutritional status of aquatic benthic macroinvertebrates such as chironomids. Pollutants cause an ecological imbalance in biodiversity. Some find their way up the trophic levels and eventually affect people. Chemical hazards, even at deficient levels, can persist, accumulate, and magnify at higher levels of the food chains. The flow of the toxic pollutants in biodiversity renders their use unsafe, particularly as food and feed. This results in environmental gradients in parameters, which lead to diversification in the ecosystem as the fauna and flora develop characteristics that are adaptable to changes. Evolutionary divergence may occur due to genetic variation attributed to chemical gradients and can only be evaluated using molecular techniques.

Nutritional components of aquatic insects are commonly affected as pollutants are ingested and absorbed through the body, resulting in changes in physiology and biochemistry, although this has has attracted cursory attention. Early warning systems for detection of pollutants are necessary to minimize the effects on biodiversity to ensure ecosystem integrity. Pollution tolerant species such as chironomids are necessary for biomonitoring. The species are ubiquitous in polluted waters because of many remarkable adaptations that allow them to survive and

reproduce due to dynamic ecosystems attributed to climate change and human activities. Hence, there is a need to establish the status of pollution and its influence on aquatic insects' diversity and nutritional components. This will inform the policymakers to project on the restoration, management and conservation of the affluent economic resource of freshwater ecosystem-dependent community livelihoods. The study will recommend the utilization of cultivated chironomids for livelihood.

1.3 General Objective

To assess the influence of pollution on diversity and nutritional profiles of aquatic edible insects and the implications on food security in Winam Gulf, Lake Victoria

1.3.1 Specific Objectives

1. To determine the spatial variations in water quality parameters of selected inshore and offshore sites in Winam Gulf.
2. To assess the influence of pollution on the distribution and genetic diversity of aquatic edible insect species in Winam Gulf.
3. To evaluate the effects of pollution on the nutritional status of selected aquatic edible insects, such as non-biting midge(chironomids), as food and animal feeds.

1.4. Null Hypotheses

1. There are no significant associations in water quality parameters of selected inshore and offshore sites in the Winam Gulf
2. There are no significant associations between pollution of the Winam Gulf and the distribution and genetic diversity of aquatic edible insect species
3. There are no significant associations between the pollution of Winam Gulf and the nutritional status of the selected aquatic edible insects- non-biting midges

1.5. Justification

The rate of water pollution of Lake Victoria is alarming putting over 40 million people at risk. Pollution of the lake significantly impacts human health, social and economic systems, the environment and the Earth's systems' functioning. Pollution touches all parts of the planet. In 2019, approximately 9 million deaths were reported which were linked to pollution (Fuller et al., 2022). Pollutants such as pharmaceutical compounds, heavy metals, and industrial dye compounds in environmental samples, display hazardous effects on humans, animals and plants.

Anthropogenic activities such as eforestation, industrialization, agricultural activities (use of fertilizers and pesticides), waste disposal accelerate lake pollution. Human-induced activities include rubbish and fecal matter, maritime traffic, fuel spillages and land encroachment due to urbanization. Climate change factors include changing weather patterns where the frequency, intensity, and duration result in extreme storms and seasonal precipitation patterns, flooding leading to overflows of sewer systems, which can lead to disease outbreaks from water-borne pathogens like bacteria, acidification and global warming. These contaminants are sources that end up or are discharged into lakes through points or are diffused, causing water quality changes and creating environmental gradients. The pollutants cause nutrient loading and sedimentation, change the nature of sediment by toxication, reduce the wetlands through encroachment, and change the water quality parameters, causing ecosystem imbalance. The changes affect aquatic life's functioning, physiology, biochemistry, population dynamics, and trophic dynamics.

Changes in water quality affect aquatic insect species richness, abundance, distribution and diversity in insect populations. The insects adjust to the changes through behavioral, functional, physiological, and ecological means. Natural selection occurs, and in the worst scenario, the most sensitive species may become extinct while some may undergo diversification for survival. Only species that are tolerant to the harsh conditions may survive. The changes affect the aquatic life as a whole. Hence, insect diversities need to be understood, for they occupy a critical ecological niche. Pollutants like chemical hazards have unique characteristics, including toxicity, persistence, accumulation, and biomagnification up the food chains. Some insects also absorb pollutants through their body, while others ingest them through food. The effects are realized through the change in biochemistry and physiology, affecting the nutritional components, which renders human food and animal feed unsafe. Therefore, carefully monitoring these pollutants in environmental samples is critical. Researching water pollution helps to understand the sources, impacts, and mitigation strategies for different contaminants, ultimately leading to protecting and preserving freshwater resources. Knowledge about pollution will provide facts and information about the state and trends in ambient environmental quality, pollutant emissions, and exposure rates. This knowledge is then used to improve pollution-related policies to protect the public and the environment from high pollution levels.

In society, water pollution causes significant adverse health outcomes in humans, wild and domestic animals, and plants. For instance, non-infectious diseases such as cancer-related and

some respiratory illnesses are associated with chemical hazards, which include heavy metals (As, Hg, Cd, Pd) and Persistent organic pollutants (POPs). The vulnerable populations include people living in crowded, non-formal settlements, lacking safe drinking water, children, and pregnant mothers. This affects the achievement of sustainable goals.

Public awareness of the sources of pollutants that lead to the degradation of water and the environment will enable us to prompt government action for environmental protection. These will go a long way in saving money, reducing toxic materials, and promoting efficient utilization of raw materials, resulting in a healthy population. Indeed, a cleaner environment can reduce the adverse health impacts on local populations from illnesses, diseases, and cancer risks associated with pollution exposure. Pollution prevention is equivalent to disease prevention. In addition, this will ensure food security through using aquatic resources for food and animal feed and promote the aquaculture of aquatic insects, particularly Chironomus larvae, for livelihood. Pollution control offers an essential solution to maintain the environment, limit climate change, prevent impacts on human health, and eventually reduce financial burdens.

1.6. Significance of the study

Water pollution, an environmental problem, significantly impacts human health, water resources, and ecosystems and affects the beneficial uses of water bodies, such as drinking, habitat, irrigation, and recreation. Research on water pollution is essential for several reasons. Firstly, analysis of water quality determined by physicochemical parameters, nutrients, and heavy metal presence provides valuable information on the pollution status of Winam Gulf. This is because water quality issues are a significant challenge humanity faces in the twenty-first century, with chemical pollution being a critical concern. Secondly, the universe of chemicals described by the EPA that occur in the environment is vast, and the selective lists of regulated chemicals might need to capture the most significant risks to the environment or human health. Therefore, assessing heavy metals in water, sediment, and insect samples will aid in the determination of the flow of chemical hazards up the food chain and food web. Indeed, heavy metal pollution in lakes is associated with population dynamics and trophic dynamics and also with detrimental effects on human health, which include developmental retardation, kidney damage, and various cancers. In addition, water pollution due to chemical hazards may alter the biota's physiology, biochemistry, and functionality, resulting in evolutionary divergences and convergences in an attempt to adapt to environmental dynamics. Molecular studies in diversity assessment of

aquatic insect communities correlated to the effects of insect species due to environmental influence. The data obtained from these current studies will provide a forecast of the impact of pollution on the aquatic ecosystem, provide possible evidence of evolutionary divergence amongst the insects, and serve as a benchmark for early detection of chemical hazardous components in our ecosystem and form policies on mitigation measures. Researching water pollution helps to understand the sources, impacts, and mitigation strategies for different contaminants, ultimately leading to protecting and preserving freshwater resources. The data obtained will help make legislative decisions on monitoring pollution in aquatic ecosystems to ensure safe and healthy systems.

1.7 Scope of the study

The current research study looked at water pollution in Lake Victoria and its impact on aquatic insects, and it analyzed the effect on nutritional components in chironomids. The study was confined to Winam Gulf, also referred to as Nyanza Gulf, also called Kavirondo Gulf, the Kenyan side of Lake Victoria known for the many inflows of rivers, highly populated with approximately 35 million people depending on the Lake for livelihood, known for a hive of activities like industries, trade, transport and whose shorelines are known for the urban centers like Kisumu City, Homabay, Kendu Bay, Port Victoria etc. Sampling was done on September 8^{th} - 11^{th}, 2020. The main objective was to assess the influence of pollution on the diversity and nutritional composition of edible aquatic insects. In this attempt, the study evaluated the pollution status of the Winam Gulf by determining the physico-chemical parameters, nutrients, and heavy metals. The study then assessed the taxa richness, abundance, distribution, and diversity of all the aquatic insects in the gulf.

Further analyses were done on a selected edible aquatic insect-chironomids, also referred to as non-biting midges, available in almost all the study sites and also a pollution-tolerant species that coupled up as a bioindicator of pollution for phylogenetic analyses and analyses of nutritional components—quantitative and qualitative analyses of nutritional components *Chironomus* spp. Larvae were assessed for the gulf's amino acids, vitamins, fatty acids, and macro and micronutrients. The study only looked at spatial variations among the six sampling sites: Kisumu Bay, Kendu Bay, Homa Bay, Maboko Island, Ndere Island, and Fish Landing Beaches. The study applied four theories: systems theory, ecological systems theory, ecological theory, and evolutionary. The study identified pollution as an independent variable, and dependent variables

included taxa diversities and nutritional components. The water quality analyses were done using analytical techniques, and aquatic insect diversities were done following two approaches: morphological and molecular, and dietary components were analyzed using spectrophotometry techniques. Data analyses were done using the PAST statistical tool software version 4.03. The data was expressed as a mean±(S.D.), ANOVA was used to establish variations amongst the sampling stations, and the Turkey post hoc test was used to separate the means Associations determined by Spearman's rank and person correlation coefficient. Data is displayed in figures and tables. This study limited spatial differences within Winam Gulf of Lake Victoria, Kenya.

CHAPTER TWO
LITERATURE REVIEW

2.0 Introduction

The human population is increasing, projected to rise to approximately 10 billion by 2050 (U.N., 2019; Van Huis et al., 2013), with the highest increase envisioned in Sub-Saharan Africa (United Nations., 2019). Consequently, an increasing demand for food puts pressure on the available resources, particularly those of terrestrial origin. Additionally, the growing population poses a fundamental challenge to accessibility to food and overall human welfare in low and middle-income economies (Vilar-Compte et al., 2021; Clapp, 2020). Moreover, the demand for nutritious food to address malnutrition remains a significant challenge, particularly in the developing world. The sustainability of the available food sources remains a challenge, given climate changes and the increasing levels of environmental contamination. Therefore, there is a need for alternative, safe, and sustainable food sources to alleviate food insecurity and associated vulnerabilities to risks for future generations. This calls for historical aspects of eating insects as food entomophagy (Van Huis & Itterbreek, 2019; Svanberg & Berggren, 2021; Olivadese & Dindo, 2023). Consequently, there has been an increase in interest in using insects as food and feed, as a combative strategy for socioeconomic purposes, and to promote sustainable food security. Despite this, few studies have explored using aquatic insects as food and feed.

2.1. Food Security

Food security is defined as when all people, at all times, have physical and economic access to sufficient safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life (Food and Agriculture Organization of the United Nations, 1996). Food security has continuously experienced significant physical force exerted by climate change, increasing global population, unpredictable food prices, and environmental stressors. Strategies to alleviate food insecurity include and are not limited to providing alternative nutritious food sources that are affordable, accessible, and sustainable (Van Huis et al., 2013). The use of insects as food and feed has been a subject that has aroused much interest recently (Meyer-Rochow et al., 2021), particularly the use of aquatic insects (Van Huis et al., 2013).

The food security base of the lacustrine communities in Lake Victoria Gulf depends on alternative protein-rich food sources such as edible insects. However, the lake ecosystem is compromised by water pollutants from the riparian environment due to human-induced activities

*(*MARINE, 2010; L.V.B.C., 2011). The pollutants compromise the health of the aquatic communities and ecosystems (Awange & Awange, 2018; Cheruiyot *et al.*, 2011; Cheruiyot & Muhandiki, 2014) dependent upon millions of people for food, fisheries, water, aquaculture activities, and livelihood, among others. Aquatic insects form a base in the food chains and webs. However, they are susceptible and dynamic due to environmental variations, climate change, and anthropogenic pressure due to the growing population (Harvey et al., 2023). The insect's ecological role as food, feed, and sustainability remains unclear (Van Huis, 2020). This is attributed to the exposure of aquatic insects to chemical hazards, hefty metals, which are toxic and persistent, have the potential for bioaccumulation and biomagnification, and are challenging to track through feeding and the environment. This influences their morphological and molecular diversity. Heavy metal pollution impacts the nutritional status, causing adjustments in nutritive contents and compromising the insects' superiority as food and feed (Gall et al., 2015). In addition, the insects offer an avenue for the flow of heavy metals into the food chains and food web), which raises questions about future generations' health due to their accumulative and bio-magnification properties. Given these facts, this research explores the potential use of edible aquatic insects as alternative food and feed sources for people in riparian communities, in which aquaculture activities form essential components of livelihoods coupled with their use as bioindicators of heavy metal pollution. Hence, the current research sought to define the pollution status determined by the water quality and the effects on aquatic insects' diversity and nutritional composition as food and feed.

2.2. Water Quality

Water quality parameters are indicators that include physical parameters, chemical parameters, and nutrients. Physical water quality parameters include eight principle indicators: electrical conductivity, salinity, total dissolved solids, turbidity, temperature, color, and taste and odor (Hassan, 2020). Chemical water parameters include pH, acidity, alkalinity, hardness, chlorine, and dissolved oxygen. Ammonia (NH^{3+}) and Ammonium (NH^{4+}) are among the primary forms of nitrogen in natural waters. Nitrate (NO^{3-}) is another primary form of nitrogen in lakes and streams (Hassan, 2020). Phosphates (containing PO_4^{3-}) are the most common form of phosphorus in natural waters.

2.2.1. Physical and Chemical Parameters, Nutrients, and Biodiversity

Freshwater ecosystems are under threat (Olagunju *et al*., 2019; Grill *et al*., 2019; Awange, 2018) due to global population pressure (F.A.O, 2013; U.N.D.E.S.A., 2017*).* This is attributed to anthropogenic activities, including industrialization, urbanization, pollution, agricultural-related activities, and climate change. Lake Victoria, the second-largest freshwater lake, is no exception. The changes have negatively affected the lake ecosystem, compromising the water quality despite being a repository of the livelihood of over 35 million people. Water quality varies with environmental conditions and is exacerbated by climate change (Sitoki *et al.*, 2010; Ogutu *et al*., 2014) and anthropogenic activities.

Water quality parameters are abiotic components that influence population dynamics in aquatic biota populations (Khaliq *et al*., 2014). Besides, water quality components result in environmental gradients affecting morphological, physiological, and behavioral characteristics to develop adaptability to ecological variability. This directly or indirectly affects the food chains and food webs. Hence, it results in environmental imbalance, threatening the sustainability of the ecosystem.

Human-induced activities, which cause water pollution from point and non-point sources, considerably change the physical and chemical characteristics and the nutrient loads. The changes are associated with dynamics in taxa richness, species abundance, distribution, and diversity (Strayer & Dudgeon, 2010). For instance, studies on phytoplankton in Nyanza Gulf have shown that phytoplankton growth is influenced by temperature, light, and nutrient levels (Sitoki *et al*., 2010*).* Water chemistry has also been observed to correlate strongly with phytoplankton assemblages. These factors restructure the distribution and phytoplankton community, an essential food for aquatic insects and fish. In turn, the effects are higher in the trophic levels (Misiko *et al*., 2014*).* In other studies, temperature, electrical conductivity, pH, and current velocity (Fontaínhas-Fernandes et al., 2008; Mireji *et al*., 2008) have been observed to influence the flora and fauna—nonetheless, Chironomids, a pollution-tolerant spp. Respond to a wide range of environmental parameters, which include D.O., TA, pH, salinity, temperature, availability in food, depth, and even organic and inorganic pollution, including heavy metals.

2.2.2. Heavy Metals Concentration in Water, in Sediment and Edible Aquatic Insects

Among the metals, the toxic, systemic, and carcinogenic metals such as arsenic, cadmium, chromium, and mercury are some of the metals that are of most concern to the public (WHO, 1996; Watson *et al*., 1990). These metals are reported to cause multiple organ damage even if exposed to lower concentrations (Kumar *et al*., 2014). They argue that heavy metals are rising due to the discovery of their use in industrial, domestic, agricultural, and technological equipment in the 21st century by Hamdy *et al.*, 2012. These are the main human activities that contribute to generating maternal sources. These metals are essential in physiological and biochemical action, although high excess is harmful (Golovanova, 2008; Albergoni & Piccinni,1983). However, some persist covert in a water environment because of low detection, even though some may be vitiating even at low concentrations. Exposure to stressors may alter the insect physiology of the molecular and biochemical parts of the insect to the environment. The effects put into play are taxa traits, which are helpful instruments in identifying and observing environmental variability regarding climate change and other natural or even artificial activities. Taxa traits are more effective than traditional indexes (Holt *et al*., 2011) in detecting shifts, including pollution.

Heavy metal exposure is through oral, inhalation, dermal, and ingestion. S.D.W.A. (1996) has set the standard for drinking water at <10µg/l while the concentration in foods such as i.e. plants and animals is 20-140ng/kg. The most significant exposure occurs through diet at approximately 50 ug/day. Food chains and water are two essential ways of exposure (Tchounwou *et al*., 2012). The sources are agricultural wastes, industrial wastes, effects of urbanization, domestic wastes, and sewage wastes. Their availability depends on the physical factors, which include temperature, and the biological factors, which include providing information on species interaction, trophic interactions, and physiological factors (Jaishankar *et al.,* 2014). Their toxicity levels vary with their biological species, genetic characteristics, and nutritional status (Abernathy et al., 1997). Heavy metals can interact with cellular organelles, D.N.A., and rRNA nucleus, protein, and lipid membranes and induce gene mutations (Li *et al.*, 2009), Cellular signal transduction, transcription activation, oncogene amplification, and recombination (Leaner *et al*., 2007). These effects break up integrated structures and functions. These effects are destructive and permanent, accumulating and concentrating in the food chain. This is a concern in our potential alternative source of protein, the edible aquatic insects, to fight malnutrition and

the health of future generations. Due to the health and sustainable consumption of edible aquatic insects, there is a need to identify and alert pollutants early on. These edible insects are beneficial in indicating a change in the environment since they bring forth significant changes. Undefined Sustainability and health can be achieved through biomonitoring and biomarking aquatic systems' pollution status.

2.3. Edible Aquatic Insects

Aquatic insects are approximately 76,000 species (William & William,2017), and about 2000 species, approximately 15%, are edible (Zhao et al., 2021). They revealed that the larval stage was vital for consumption, especially in Mexico, China, Thailand, India, and Venezuela. In Africa, approximately 470 species of edible insects were out of which 256 were from Central Africa, 100 from East Africa, 8 from Northern Africa and 17 from Kenya (I.C.P.E., 2015). Further studies in the Kisumu area of Kenya report that termites, lake flies, and crickets are the most common edible insects (Kelemu et al., 2015). Insects are known for their high protein content, an average of 59.55%; high nutritive value, an average of 51.60% of essential amino acids, with Leucine being the most abundant and Methionine, Cytosine, and Tryptophan the least (Zhao et al., 2021). Aquatic insects have rich fatty acids - omega three and omega 6. Few studies have focused on fatty acid content's qualitative and quantitative aspects (Zhao et al., 2021). Moreover, aquatic insects are critical providers of minerals and Vitamins (Shantibala et al., 2014). Despite their importance, information on nutritional value and the health benefits of some historically known and used insects remain scanty (Van Huis et al., 2013). Aquatic insects such as the Chironomus-larval stage of lake flies are not exceptional.

Aquatic insects are excellent sources of proteins, mineral salts, and vitamins and are known for their superior amino acids and unique fatty acids (Zhao et al., 2021). The aquatic insects occupy a critical ecological niche in the trophic flow energy (Bartrons et al., 2018). In their study, a good example is elucidated by (Prasniski & Leal-Zanchet, 2009), which revealed that the insects form food for other predatory insects, Platyhelminthes, spiders, Pisces, amphibians, and saves. In addition, studies also showed that aquatic insects had essential mineral ion components and vitamins B and amino acids, which were rare in other animal proteins. These components are significant for preventing deficiency diseases (Van Huis, 2013). Other studies by (Shantibala *et al*., 2014) documented the presence of proteins in Ephemeroptera (66.3%), Odonata (40-65%), Hemipterans (42-73%), Coleoptera (23-66%) concerning their body weights, (Xiaoming et

al.,2010). In addition, studies on stoneflies, beetles, chironomids, corixid, notonectids bugs, and mayflies confirmed high levels of lipids in insect proteins (Xiaoming et al., 2010). Further analysis of lipids revealed the presence of long-chain polyunsaturated fatty acids and omega-6 fatty acids, which are essential for growth, development, and brain function (Zárate et al., 2017). Consequently, Ayieko and Oriaro, (2008) observed that lake flies had vitamins E and A and minerals like Selenium, which were observed to supplement the animal feed. This will go a long way in promoting the blue economy strategy.

However, edible aquatic insects are susceptible to variability in environmental conditions, especially climate change, which affects water quality and biodiversity. Some species are sensitive to changes in water chemistry, water pollution, nutrient loading, and changing levels in chemical hazards, heavy metals, and persistent organic pollutants (Zhang et al.,2023). The insects' response may involve composition, distribution, richness, evenness, and dominance shifting. Besides, some may respond by changing their character traits and physiology. The species tend to take in hazardous compounds, which can be assessed from the body, the food, and water taken. The compound's properties of persistence, toxicity, bioaccumulation, and biomagnification up the food chains (Liu et al.,2022). Health of the noble source of proteins for future generations. However, the species' ecological characterization can be used to define the health of the biodiversity and the ecosystem.

2.3.1. Chironomid Larvae as Food and Feed

Malnutrition and health attributed to food insecurity remain issues of concern globally (Tscharntke *et al*., 2012). This has resulted in pressure from the growing population (U.N.D.E.S.A. & E.C.E., 2017). Hence, there is a need for alternative sources of proteins other than livestock, fish, and soya plants, which are diminishing (Van Huis, 2013). Insects for food and feeds are a potential resource that can be exploited due to their high conversion efficiency and adaptability to changing climatic conditions (Van Huis *et al*., 2013)—particularly chironomids, the edible aquatic insects that influence the ecological balance of freshwater ecosystems.

Chironomids have a long history of use as food for fish, shrimps, and amphibians and as baits (Nath et al., 2021; Shahidi & Vatandoost, 2021; Fard et al., 2014; Okedi, 1992). For instance, Okedi (1992) documented the use of Lake fly biomass as fish feed and food for the riparian

communities along Lake Victoria. Farias et al., (2012), also observed that chironomids were benthic organisms that inhabited and fed sediment and debris and were critical as fish food. Though the study observed that the larvae were nutritious for fish food. Nath et al., (2021) observed that the amount of protein varied with the change in physico-chemical parameters. A study by Sahandi, (2011) the showed that cultured fish fed on chironomid live feed produced nutritionally high-quality fish with increased growth parameters and iron.

Further, chironomids were observed as key in aquatic food webs. The larvae were linking producers and secondary consumers, which was crucial in trophic dynamics due to the numbers, abundance, and role in nutrient cycling (Kolbenschlag et al., 2023; Herren et al., 2017; Prata et al., 2023). The changes in response to environmental variability were fundamental in indicating pollution. The larvae were used in frozen form and live as feed. On the other hand, according to Rawal,2018, the larvae acted as earthworms at aquatic shorelines. Due to the red coloration, they released ammonia, nitrogen, and phosphates during culturing, as well as fish food and baits (Xu et al., 2007).

Further, it was observed that chironomids had both ecological and economic importance, particularly in nutrient cycling. Besides, high diversity was observed and associated with adaptive survival capabilities in extreme environmental conditions. Deformities were attributed to genetic variability and evolutionary adaptability, indicative of heavy metal and pesticide pollution (Cranston et al., 1995) also, the cultivation of chironomids as food for amphibians and food has gained interest. The Lake Victoria communities have also been known to consume lake flies as food, used in making flour for baking bread and cakes, and for medicinal and cultural beliefs (Ayieko & Ariaro, 2008; Ayieko et al., 2010). Further studies also observed that chironomids were previously fed to malnourished children(Ayieko & Oriaro, 2008).

Chironomids grow in sediment substrate and can potentially accumulate and bio-magnify hazardous chemicals such as heavy metals, persistent organic pollutants-POPs, organochlorine pesticides, etc. ((Lidman et al.,2020). The chemical properties of persistence, bioaccumulation, and toxicity pose a challenge to their safety and healthy use as alternative nutritious food sources (Shantibala et al.,2014) and are associated with human-induced environmental pollution(Neff & Dharmarajan,2020); E.F.S.A.,2015. Consequently, the growth of environment-sediment, happens to be a source and sink of pollutants(Chiaia-Hernandez et al.,2022) and hefty metals,

which are absorbed and transported in the food chains and food webs in aquatic ecosystems(Lidman et al.,2020).

2.4. Chironomids

Chironomus is referred to as the nonbiting midge/larval stage of the lake flies that falls under the family of Chironomidae; there has been research, and 647 species have been recorded worldwide (Arnett, 2000). Chironomids, nonbiting midges are elongated and cylindrically segmented bloodworms and possess a sclerotized head capsule and non-jointed pro-legs. The most conspicuous feature is the red coloration of the hemoglobin protein in the body; thus, the name bloodworm is given; it is a biological advantage because organisms in low-oxygen environments can survive after adapting to the habitat. However, within the class of the Insecta, the genus Chironomus is among the largest, and within macro-invertebrates, morphological analysis of only the identified larvae of the shown organism has been limited by its concealed features to enable further study of the genus; thus, only identifiable with the giant chromosomes using D.N.A. barcoding (Karima, 2021).

Cytochrome I oxidase subunit one gene (CO1) is an ideal gene marker in the differentiation between immature and highly similar organisms with similar morphology (Vuataz et al., 2024). In the inheritance of genetic material in the lineage, the gene is usually passed on and cannot be easily altered. According to Karima (2021), the previous studies on the Chironomus were done in the adult stage rather than in the larval stage, which is important and provided the morphological features used in identification that only apply to the adult stage. At the same time, the nonbiting midge is deemed necessary in the aquatic food chains and food webs, as filter feeders and environment cleaners have been given little focus on the larval stage to which the commitment to research has been devoted. Other features include physiological adaptability to harsh environmental cues, particularly resistance to desiccations, low oxygen levels, survival in nutrients waters, polluted waters, or clean waters regardless of the type: freshwater, brackish, or marine (Podder et al.,2022; Arnett,2000). Chironomus is adapted to organic and inorganic pollutants concerning industrialization, sewerage, and stormwater flow. This was observed mainly in urban water and was feared to have been occasioned by the areas of selection in the study.

The Chironomids, freshwater benthic organisms, are found in water and semi-aquatic environments. The microorganisms in the flying island are filter feeders and detritus feeders; some are herbivores and can work in very dynamic conditions, including harsh ones, therefore suitable for recording changes. For instance, the chironomids endure dynamics in temperature and low oxygen systems, and in fact, they can inhabit a conceivable freshwater habitat (Szczerkowska-Majchrzak et al.,2020; Eggermont & Heiri, 2012; Rasmussen, 1996). As such, the genus creates the link between the trophic levels within the detritus food chain as filter feeders and primary consumers in the ecosystem. Thus, the genus Chironomus is generally considered a perfect ecological indicator of change due to the changes contributed to by human influences such as urbanization and industrialization and climatic change due to natural influences. The conspecific or heterospecific in question has remained unclear because the key characteristics that set the two apart have not been visible besides whether the differentiated nature of revolving variations and unification that emerges from the shifting aquatic environment remains unclear. The purpose of this study was to find out the impact of pollution on the Chironomus species in terms of diversity using the cytochrome I oxidase subunit one gene and recording any possibilities of the change and the nutritional aspects—the consequent output on Chironomus spp. Diversity is posted on the NCBI Website-Gen bank, nutrition data is posted to publications, and a policy is formulated for biomonitoring /Environmental Health and the formulation of measures for its solution.

2.4.1. Adaptive Mechanisms for Metal Tolerance in *Chironomus*:

Chironomus species, particularly midges, are known for their ability to thrive in polluted environments, including those with high levels of heavy metals. Several adaptive mechanisms contribute to their metal tolerance (Nell et al., 2024; Shaikhutdinov & Gusev, 2022; Laviad-Shitrit et al., 2021; Sela & Halpern, 2022; Groenendijk et al., 2002). Metallothioneins are low-molecular-weight, cysteine-rich proteins that bind heavy metals such as Cd, Zn, Cu, and Hg, thus reducing their toxicity. By chelating metals, metallothioneins prevent metals from interacting with cellular macromolecules. Increased production of metallothioneins is a well-documented response in *Chironomus* to heavy metal exposure. This binding prevents free metal ions from causing oxidative damage and disrupting cellular processes. Heat Shock Proteins (HSPs) are a group of proteins that help maintain cellular protein homeostasis. They are up-regulated in response to various environmental stresses, including heavy metal exposure. In *Chironomus*,

certain HSPs, such as HSP70, are known to be up-regulated in the presence of metals like cadmium and copper, helping to refold denatured proteins and prevent cellular damage (Szebesczyk & Slowik, 2023). Heavy metals like Cu, Fe, and Hg can generate reactive oxygen species, leading to oxidative stress. An effective antioxidant defense system involving enzymes such as superoxide dismutase, catalase, and glutathione peroxidase is crucial (Anwar et al., 2024; Ighodaro & Akinloye, 2018). Elevated levels of these antioxidants in *Chironomus* help mitigate oxidative damage by neutralizing R.O.S. This is particularly important for protecting vital cellular structures such as membranes, proteins, and D.N.A. Sequestration in cellular compartments occurs where metals can be compartmentalized in specific cellular organelles or bound to insoluble granules, reducing their bioavailability (Blaby-Haas & Merchant, 2014). *Chironomus* adaptation provides a mechanism to tolerate high metal concentrations by effectively isolating toxic ions from critical metabolic processes (Tchounwou et al., 2012). Heavy metals altered the Membrane Transport and Efflux Pump. Metal ion transporters and efflux pumps can regulate the internal metal concentration by reducing uptake or actively pumping out excess metals. Specific transporters such as ATP-binding cassette transporters in *Chironomus* may contribute to the export of toxic metal ions, maintaining cellular homeostasis. Long-term exposure to metal-rich environments may lead to genetic adaptations, such as mutations that confer resistance (Garmory & Titball, 2004; Groenendijk et al., 2002). Populations of *Chironomus* exposed to polluted environments may show selection for genes that enhance metal tolerance, resulting in evolutionary adaptations that support survival under such stressors.

2.5. Genetic Diversity

Molecular phylogenetics is the field of biology that deals with identifying and understanding the evolutionary relationships between closely related organisms using molecular data from D.N.A., R.N.A., or protein sequences (Wink, 2007). The main objective of phylogenetic analyses is to find evolutionary relationships of the studied organisms, which is generally represented as a phylogenetic tree. A phylogenetic tree is a diagram of nodes and branches consisting of taxonomic units, with the nodes connecting adjacent branches. Branches represent taxonomic units that could be species, populations, or individuals. Branches define relationships between taxonomic units in terms of descent and ancestry, and such a branching pattern is known as the tree topology (Graur, 2000). Molecular data is much more reliable for evolutionary studies than

morphological and physiological data since it is strictly heritable and less prone to convergence (Wink, 2007; Brown, 2002).

Molecular data for phylogenetic analysis is obtained by sequencing selected genes, DNA fragments, or whole genomes using a sequencing machine such as the 3730xl DNA. Analyzer. High-quality sequences are necessary for accurate phylogenetic inference. During phylogenetic analysis, the quality of the alignment is also crucial because phylogenetic reconstruction and conclusions depend heavily on correct sequence alignment (Yarza et al., 2010). Alignment is essential to identify homologous positions in sequences by assigning each sequence to a separate row and arranging homologous positions in columns. There are four different methods for reconstructing phylogenetic trees: Distance matrix, Maximum parsimony, Maximum likelihood, and Bayesian Inference (Zou et al., 2024; Liu *et al*., 2010). This study will use the Neighbor-Joining Maximum likelihood method to infer evolutionary relationships (Tamura et al., 2004; Saitou & Imanishi, 1989).

After the reconstruction of a phylogenetic tree, the reliability and quality of the tree have to be assessed to ensure that the tree accurately represents the actual relationships among OUTs (*operational taxonomic units*). The most common method is the bootstrap analysis, which can be implemented in different tree reconstruction algorithms first introduced by (Felsenstein, 1985). The bootstrapping algorithm resamples the columns of a sequence alignment and creates many new alignments by random sampling, replacing the original data set. Multiple trees are then generated from the latest sets of alignments, and the statistical confidence of each branch is calculated to give the bootstrap value (Raes *et al.*, 2003). Generally, 200 – 2000 samples were used for bootstrapping, but 1000 resamples will be used in this study. Bootstrap support of 70% or higher indicates a reliable grouping or clustering in a phylogenetic tree. Without directly observable relationships between ancestors and descendants, the direction of the changes must be inferred by rooting the tree, although this is not strictly necessary (Nei, 2000*).* There are two methods for molecular tree rooting: out-group rooting and duplicate gene rooting. Out-group rooting compares the character states of the group of interest (in-group) with a closely related but sufficiently distant group.

2.5.1 Substitution Rates

Substitution rates derived from models such as the Tamura–Nei model (1993) have many uses in molecular biology, evolution, bioinformatics, and ecology. These rates are utilized for comparing genetic differences, evolutionary mechanisms, and affinities between species or genes (Tamura et al., 2007). Substitution rates are essential in building trees that represent the evolutionary relationships of species or genes. Researchers can then use these rates to calculate genetic distances between sequences and produce tree topologies and branch lengths. This matrix of substitution rates is incorporated into phylogenetic tree-constructing algorithms such as Maximum Likelihood (ML), Bayesian Inference, and neighbor-joining (N.J). These trees assist in comprehending the pattern and divergence of species or genes in the evolutionary process.

They provide the basis for assessing divergence times in species or populations to some extent (Yi, 2013). Molecular clock models use these rates to predict the time elapsed since the two sequences split from a common stock. Scientists can apply the substitution rates for the calibration of molecular clocks and the estimates of when the speciation or some evolutionary events occurred. This is particularly relevant in paleobiology, evolutionary biology, and comparative genomics (Hipsley & Müller, 2014). This can help to establish whether the area of the DNA is under positive selection, where new advantageous mutations are favored, or adverse selection, where deleterious mutations are eliminated.

Scientists can study substitution rates to identify the adaptive evolution of genes and genomes (Eyre-Walker,2006)—for instance, a region with many synonymous changes relative to non-synonymous changes commonly experiences purifying selection. Substitution rates aid in defining evolutionary constraints in the genetic sequences. In particular, lower rates in some areas indicate functional constraints since some residues function as active sites in enzymes or are conserved regulatory elements (Echave et al., 2016). Relative to substitution rates, comparative genomics can identify relatively protected areas in different species, which could correlate with key biological roles or structural integrity.

By comparing the rates of substitution in different genomic locations, one can identify other functional features like protein-coding genes, non-coding RNAs, and regulatory sequences (Mu et al., 2011; Johnsson et al., 2014). These rates can be employed in functional genomics investigations, as they help prioritize some regions for further investigation, determining gene

function, or analyzing the regulatory connections. Substitution rates can also assist in distinguishing between orthologous (comparatively closely related genes from different species) and analogous (duplicates within the same species) genes. Knowledge of these relationships is necessary for the functional annotation and study of macroevolution. Comparative genomics employs these rates to analyze gene family dynamics, species diversifications, and other over-genomic patterns of evolution.

Substitution rates are employed in problems dealing with genetic differentiation, population organization, and gene migration in and between populations (Habel et al., 2015). These rates can suggest high mutation rates or evolutionary pressures that could be picked up through high substitution rates. Biologists utilize these rates in determining the overall genetic health of species, defining E.S.U.s, and designing conservation and recovery strategies for endangered species (Casacci et al., 2014).

Substitution rates prove helpful in determining population structure, gene movement, population mobility, and selective forces (Shen et al., 2019). These models are essential in understanding how populations may behave based on the effects of environmental change, fragmentation, or climate change. These models help population geneticists analyze how various factors influence allele frequencies within a defined population over time for the sustainable conservation of species and ecosystems. Supervising Pathogen Evolution for viruses and bacteria, the nucleotide substitution rate is essential to observe shifts in genetic drift, the appearance of new strains of the pathogen, and the development of drug resistance (Wilson et al., 2016). This is very important in public health to control the spread of infectious illnesses. Scientists apply substitution rates to track changes in such viruses as H.I.V., influenza, and coronaviruses and anticipate future mutations that will inform the creation of vaccines.

Categorizing Horizontal Gene Transfer Event In microbial genomics, substitution rates could be used to identify HGT, a process through which genetic material is transferred between organisms (Nguyen et al.,2022; Adato et al., 2015). Sites with variable rates of amino acid substitution may indicate HGT. This is crucial in understanding the dynamics of emergence and the development of antibiotic resistance and pathogenicity in bacteria. Substitution rates allow for determining regions that are conserved enough to design primers, probes, and gene editing targets (Rodríguez-López et al.,2017). They also assist in the detection of variable regions for diagnostic

markers. Ideally, in molecular diagnostics, the conserved region (low substitution rates) has to be identified for PCR primers that could work across the strains or species (Viljoen et al., 2005). On the other hand, the variable sequence or high substitution rates are utilized in strain-specific diagnostics or to monitor the evolutionary changes in the pathogens.

Substitution rates are relevant for specifying and optimizing Tamura-Nei, Kimura 2-parameter, GTR.and other models (Posada et al., 2001; Banerjee, 2021). These models form the core of bioinformatics software for sequence analysis, such as MEGA, BEAST, RAxML, and MrBayes. Substitution rates are utilized to emulate evolutionary situations in prospective studies, enhancing the reliability and validity of phylogenetic and population genetics applications.

Thus, in conclusion, Substitution rates based on the models as Tamura-Nei is in more ways than one crucial for rendering solutions in various strands of biological research embracing phylogenetics, molecular evolution, genomics, population genetics, and conservation biology. Criticizing these rates allows us to better understand processes, genetic variation, and adaptive capacity with the knowledge to aid in decision-making across disciplines as far-reaching as theoretical biology and tangible outcomes in conservation to public health.

2.5.2 Evolutionary divergences on aquatic insects

Evolutionary divergence plays a critical role in shaping biodiversity by contributing to the formation of new species, genetic diversity within populations, and the development of unique ecological niches (Chantepie et al., 2024; Correia & Lopes,2023; Zamudio et al., 2016). It represents the process by which populations of organisms accumulate genetic differences over time, often leading to speciation and greater ecological complexity. Here is how evolutionary divergence affects biodiversity:

Evolutionary divergence is a crucial mechanism of speciation, the process by which new species arise (Schluter et al., 2022). When populations of a species become isolated—either geographically, ecologically, or behaviorally—they accumulate genetic differences due to natural selection, genetic drift, mutation, and recombination. Over time, these genetic differences can become significant enough to result in reproductive isolation, where the diverged populations can no longer interbreed successfully (Palumbi, 1994). This leads to the emergence of new species, thereby increasing species diversity. Example: In aquatic ecosystems like Winam Gulf,

the divergence between isolated populations of aquatic insects or fish due to environmental differences or physical barriers can lead to new species adapted to specific conditions.

Divergence can lead to adaptive radiation, where a single ancestral species rapidly evolves into multiple new species that occupy different ecological niches (Chaparro-Pedraza et al., 2022; Ackerly et al., 2006). This often occurs in environments with unoccupied niches or after mass extinctions. Example: The divergence of cichlid fishes in African lakes is a classic example of adaptive radiation driven by different ecological opportunities and selective pressures. This leads to a high diversity of species with specialized feeding strategies.

Divergence within populations leads to genetic diversity, which is the variation of genetic material within a population (Saastamoinen et al., 2018). This genetic variation is essential for the adaptability and resilience of populations to environmental changes, such as climate change, habitat fragmentation, or new diseases (Pauls et al., 2013). Populations with high genetic diversity are more likely to survive and adapt to changing conditions, maintaining ecological stability and contributing to overall biodiversity.

Evolutionary divergence allows for local adaptations, where populations develop unique traits suited to their specific environments (Wadgymar et al., 2022; Chantepie et al.,2024; Meek et al.,2023). These adaptations can enhance the survival and reproduction of individuals in different ecological contexts, contributing to the genetic mosaic of a species across its range. Example: In Winam Gulf, genetic divergence among Chironomidae populations due to varying levels of heavy metal contamination or water quality can lead to local adaptations, enhancing the overall genetic diversity of the insect community (Misiko et al., 2023).

Divergence can lead to niche differentiation, where populations adapt to occupy different ecological roles or niches within an ecosystem (Cooper, 2024; Wang et al., 2017). This reduces resource competition and allows multiple species to coexist, increasing ecological diversity. Example: In freshwater ecosystems, divergence among insect taxa, fish species, or aquatic plants can lead to specialized feeding, breeding, or habitat preferences, contributing to the complexity and stability of the ecosystem. Diverse species and genetic variations within those species ensure that multiple ecological functions are maintained, such as nutrient cycling, pollination, decomposition, and food web dynamics (Riva et al., 2023; Santamaría & Méndez, 2012). Divergence that leads to species formation with unique ecological roles can enhance ecosystem

resilience and productivity (Grime & Pierce, 2012). The divergent evolution of species with different ecological roles ensures that ecosystem services are sustained, even under environmental stress or perturbations.

Evolutionary divergence can lead to distinct biogeographic patterns, where different regions host unique assemblages of species that have evolved in isolation (Williams et al., 2023; Warren et al., 2014). This results in regional endemism, where certain species are found only in specific locations. For example, the divergence of insect taxa in geographically isolated areas of Winam Gulf may result in unique species or genetic lineages that contribute to regional biodiversity.

Regions with high rates of divergence, such as islands, mountain ranges, or isolated lakes, often become biodiversity hotspots—areas with high levels of species richness and endemism (Sonne & Rahbek, 2024; Spehn et al., 2011; N.R.C.C., 1999). Due to their unique genetic and species diversity, these areas are critical for conservation efforts. Understanding divergence patterns helps identify areas that require protection to maintain global biodiversity levels.

Divergence often leads to the evolution of novel traits or innovations that allow organisms to exploit new resources or environments (Chaparro-Pedraza et al., 2022). These novel traits can result in evolutionary success and the proliferation of species that possess them. Divergence in feeding structures, reproductive strategies, or defensive mechanisms can lead to the development of new traits that give species a competitive advantage in specific environments.

Populations with high divergence represent a genetic reservoir that can be drawn upon for future evolutionary adaptation (Olson-Manning et al., 2012; Estrada-Peña et al., 2018). This reservoir is crucial for species' long-term survival in the face of rapid environmental changes or catastrophic events. Conserving genetically diverse populations ensures the availability of genetic material for future adaptation, contributing to biodiversity sustainability.

Understanding evolutionary divergence helps identify evolutionarily significant units which are genetically distinct populations that warrant separate conservation management. Preserving these units helps maintain the evolutionary processes that generate biodiversity. Conservation strategies can focus on maintaining or enhancing gene flow between divergent populations or protecting isolated populations representing unique genetic lineages.

Divergence data can help monitor populations' evolutionary health, indicating whether they are experiencing sufficient gene flow or at risk of inbreeding or genetic drift (Willi et al., 2022). In regions like Winam Gulf, where anthropogenic impacts may lead to habitat fragmentation or pollution, monitoring evolutionary divergence can help inform management strategies to maintain or restore connectivity and genetic diversity.

Evolutionary divergence is a fundamental process that drives the generation and maintenance of biodiversity (Santamaría & Méndez, 2012). Divergence ensures the adaptability and resilience of life on Earth by promoting speciation, enhancing genetic diversity, facilitating niche differentiation, and contributing to biogeographic patterns. Understanding and preserving these processes is critical for effective conservation, sustainable management of natural resources, and maintaining our planet's ecological integrity.

2.5.3. Genetic Diversity in Chironomids

Diversity of Chironomidae (non-biting midges) is an important aspect for ecological, evolutionary, as well as for evaluation and management of environments. Chironomidae in in great variety present in many habitats of the fresh water ranging from the clean waters of clear lakes and rapid flowing streams to the heavily contaminated water in cities. Genetic variations among populations within the Chironomidae family influence their response to stressors, robustness, and viability as biomonitoring organisms for environmental status.

Firstly, there are the Chironomids that have enhanced adaptability to environmental changes. For instance, Broader Range of Environmental Tolerance. Such tolerance has enabled Chironomidae to assimilate various stress factors, including fluctuating temperatures, pH, dissolved oxygen, and pollution standards in terms of heavy metals and organic pollutants (Czechowski et al., 2020). Individuals with a trait that makes them tolerate certain stressors will be present in populations with high genetic diversity. This is because high genetic variability makes Chironomidae to adapt to various environments ranging from clean flowing streams with ample supply of oxygen to polluted water bodies in urban areas. It enables them to continue living in areas that are experiencing shifts in their conditions as a result of human activities.

Secondly, their potential for Rapid Evolution and local adaptation. Genetic variation ensures that the raw material is available in natural selection so that it can produce quick adaptive changes in response to environmental shifts. For instance, if a population is introduced to a new toxin such

as a pesticide or a heavy metal, then a proportion of the population who is resistant to the toxin due to genetic difference would be favored. These minor genetic differences that were revealed in chironomids could be as a result of local adaptation of the populations to varying levels of stress factors like heavy metals. This could result in different genetic populations of the species being exhibited within different locations in the Gulf.

Thirdly, Chironomids have the ability to enhance the resilience of aquatic ecosystems. Thus, chironomids are able to: Maintenance of Ecosystem Functions (Nicacio & Juen, 2015). Chironomidae contribute in nutrient dynamics, breaking down of organic matter, and as food for higher nutrient classes such as fish, amphibians, and birds. This genetic variation allows Chironomidae to sustain these functions, no matter the disturbance of the environment they have adapted to. It recommends that a population with a rich gene pool is capable of resisting and resilive adverse shocks to the environment including pollution occurrences, floods or droughts. It allows sustaining stability and the services of these aquatic ecosystems.

Fourthly, it is noted that 'chironomids have Buffer Against Local Extinction.' Therefore, genetic diversity can be seen as a hedge against the local extinction because high genetic variability of the population means that at least several of the members will possess traits that will help them survive the new conditions (Pedrosa et al., 2017). This is especially so for Chironomidae which inhabit unstable and polluted ecosystems that may alter dramatically in short time. In areas with very high levels of pollution or habitat degradation, Chironomidae populations will have higher genetic variability to enable them to survive and repopulate the region and continue playing their role in the ecosystem.

Fifth, their use in Bioindication and Environmental Monitoring. Chironomidae is commonly employed in studies as an indicator species of the water quality and the health of the environment since the species occur in different environments with different tolerance levels to pollution **(**Lencioni et al., 2012). This is because genetic variation within a population of Chironomidae can lead to a better understanding of the kinds of stressors affecting the populations in a more quantitative manner. Such genetics can further categorize populations, which merely persist in the polluted environment and those that evolve to specific toxicities. This may result in better environmental assessment and the corresponding control measures adopted by organizations.

Molecular techniques may reveal just unique allele related to contamination acceptance including heavy metals, pesticides or other toxins. The tracking and analysis of these markers is helpful in determining the concentrations and kinds of pollution that is actually getting into a particular water body (Arambourou et al., 2020). The isolates from Winam Gulf that demonstrated slight genotypic differences could be used to determine the genes for heavy metal tolerance, which would become a very informative tool for evaluating the levels of contamination and effects in different regions of the Winam Gulf.

Thus, knowledge of the genetic polymorphism of Chironomidae populations will allow recommendations for the further conservation and use of aquatic environments." Measures that should be taken within conservation programmes include the retention or improvement of genetic variation which is especially important in fluctuating environments. Where the environment is comparatively polluted or degraded, conservation can center on preserving or enhancing the gene pool of Chironomidae species in order to sustain their contributions to ecosystems and their adaptability.

Dispersion data could be useful in remediation and restoration activities by determining which populations would be the most appropriate to introduce or add in restored environments (Montalvo et al., 1997). Indirectly, Gene flow of diversity is likely higher in populations that may prove fit for colonization and evolution in newly restored or remediated habitats. When Chironomidae populations in areas under restoration are chosen for rehabilitation, those with higher genetic variability could improve the prospects of restoration.

Chironomids have a possibility of genetic variance in small or contaminated populace. — In small or isolated populations, genetic drift (random changes in allele frequencies) means a Continue to lose genetic diversity over time (Nowak, 2008). This can make the population more fragile and less elastic in the face of future changes in the environment. Reducing isolation between populations or establishing an environment that can sustain large populations aids in counteracting the occurrences of genetic drift and the elongation of gene pools.

Genetic variation also implies that Chironomidae populations can always accommodate for future changes and evolution. This means that populations that undergo genetic erosion may be unable to combat new or increasing pressure and may consequently be driven to local extinction

(Gagliardi, 2017). Preservation and promotion of genetic variability in Chironomidae is important to ensure the future sustainability of the aquatic habitats.

In Conclusion, Genetic variation in Chironomidae is immensely significant to their susceptibility, robustness potential and application as biomonitor in freshwater ecosystems. This harks back to the fact that high genetic variation allows them to withstand several forms of environmental pressures, stabilize ecosystems and guide conservation and management systems. Optimization of genetic variation within Chironomidae population is important in sustaining aquatic ecosystems especially in areas such as Winam Gulf, Lake Victoria where lighted human interference influences water quality and conditions of the habitats.

2.6. Challenges of Water Pollution on the Nutritional Status of Edible Aquatic Insects

Terrestrial ecosystems are generally unstable. This is accelerated by the changing climatic conditions which are attributed to anthropogenic pressures, particularly in the developing world. Africa is no exception. Yet the capacity to cope with the changes is inhibited by technological advances. Besides, though insects are successful in erratic conditions, the diminishing systems are likely to influence the productivity of insects as food and feed. This may influence the security of the noble resource and the health of the people nutritionally. Hence, the need to make use of aquatic resources.

Africa is endowed with stable aquatic ecosystems which harbor alternative protein sources: the edible aquatic insects, are essential for energy flux between aquatic and terrestrial systems. Besides, insects are biological indicators of environmental variability, particularly pollution. The insects are highly sensitive to changes in biotic and abiotic components, nutrients, and chemical toxicity. Toxicity occurs at very minute, undetectable levels which pose a risk to future generations.

2.6.1. Impact of Pollution on Aquatic Edible Insects.

Diversity and abundance of many insect species is on a declining trend, and is associated with pollution such as heavy metals, and pesticides, from agricultural wastes (Feldhaar & Otti, 2020). Contamination especially with chemical pollutants increases susceptibility to diseases either directly through direct consumption or indirectly through contaminated food. In addition, results to susceptibility to disorders and also lowers the level of tolerance to varied stressors. Pollution particularly from industries, agricultural wastes and sewage modify ecosystems and affect the

population densities and diversities. Pollutants also affect the taxa richness, and can lead to extinction of the most sensitive and pollution intolerant species, (Alstad et al., 1982).

Pollutants affect the water quality. Some are persistent chemicals with long time influence on organisms while some are acute in their impact (Malaj et al.,2014). The effects may be significant and lead to reduction in diversity and abundance and affect populations of sensitive species. Change in physical parameters like rise in temperature also affects other processes like evaporation. For instance, evaporation also increases, raising the concentrations of pollutants which in turn affects the vulnerable species (Carpenter et al., 1992).

Pollution due to chemical hazards which include acidification and metal poisoning affect the insect taxa richness. For instance, sensitive and restricted species which are pH specific tend to drift in response to low pH reduction in densities, diversity and richness. This kind of pollution is associated with agricultural wastes, industrial wastes, urbanization.

Nutrient loading resulting to eutrophication also affects the water quality both the physical and chemical parameters resulting to habitat change and lead to trophic dynamics. This results to extinction of the vulnerable species, reduced populations in some species and only isolated groups survive. Nevertheless, some species thrive in eutrophic waters and the populations increase.

Pollutants quantity and varieties also affect insects differently. Some pollutants impair the immune-system, some are neurotoxic, affect hemocytes', promote disease propagation and also increase mortality. Metal toxicity can also impair the insect physiology and sensitivity to stress depending on the kind and quantity of contaminant (Jaishankar et al., 2014). For example, scholars observed that pollutants affect growth, larval survival and developmental period, reproduction and affect enzymatic activities, (Pannetier et al., 2020).

Chironomids, well known diverse order survives in diverse environmental conditions and is pollution tolerant. However, the order is threatened by loss of habitat and the populations may be shaken by industrial pollution, dumping and rotting of organic matter. Chironomids larvae is a benthic macroinvertebrate dwelling in the bottom substrate and commonly found in eutrophic waters, significant fish diet, accounting for about 80%, (Gerstle et al., 2023). This implies that any effect on chironomids due to environmental changes will destabilize the system. Especially in sediments. Hence, the use in sediment toxicity bioassay.

In general, insects react physiologically to abiotic factors through immune reactions-death, fertility, growth rates, and mutations. Mutations lead to malformations from generation to generations and also cause mortality in larval stages. Besides, insects also display immune genetic responses through immune suppressive modifications leading to phenotypic flexibility, and also behavioral adaptability (De Roode & Lefèvre, 2012).

2.6.2. Trophic Dynamics in Aquatic Ecosystem.

Pollution has led to trophic dynamics among insects. Herbivorous insects ingest about 80% of aquatic plant materials which may include phytoplankton's, macrophytes and return back to the system via decomposition process and also through fecal material, (Bakker et al., 2016). The vascular plants take in water and nutrients via root system where translocations to other parts occur. The plants response to contaminants includes genetic variability, plant aging and health depending to quantity of pollution. The predators on the other hand prey on contaminated insects and in turn are decomposed back to the system and mineralization of accumulated materials occurs. The contaminants ingested by insects are also passed on to fish through feeding. The contaminated fish is fed on by man and the contaminants are passed on to man via ingestion. In some cases, the aquatic plants and insects are directly fed on by man and contaminants directly enter the system. Man is omnivorous. When death occurs, the insects, plants and man die and the products are mineralized back to the system. Some species adsorb organic pollutants through their bodies.

2.6.3 Effects of Pollution on Chironomids as Food

According to Gokul et al.,2023 pollutants harm aquatic life physically, physiologically, molecularly resulting to developmental problems and multiorgan toxicity (Gokul et al., 2023). For instance, acidification affects aquatic insects through changes in physiology, increases in trace metal concentrations that are toxic to some organisms, and indirectly through changing food availability by altering either photosynthetic or decomposition pathways (Mitra et al., 2022). Pollutants ingested in food or diffused through the body, travel in blood stream impact on the organs. The toxic pollutants accumulate in food chains leading to rise in toxicity in humans who consume the contaminated food (Guo et al., 2019). The toxic pollutants build up in tissues of aquatic insects. The pollutants particularly heavy metals change the antioxidant enzymes, gene suppression patterns, and interfere with endocrine system. In addition, the pollutants change the biochemical processes and the metabolic reactions.

2.7 Theoretical Framework

2.7.1 Introduction

The present research applied the use of systems theory, ecological systems theory, ecological theory and evolution theory to explain the impact of pollution on diversity and nutritional composition of aquatic edible insects. The systems theory, defined as the transdisciplinary study of systems, i.e. cohesive groups of interrelated, interdependent components that can be natural or artificial was used in understanding the inter-relationship between the biotic and abiotic factors in a lake ecosystem. Further an Ecological systems theory which describes a scientific approach to studying lifespan development that emphasizes the interrelationship of different developmental biological processes, differences in variability and specific -targeted effects was applied in understanding the spatial differences in the lake ecosystem. Ecological Theory which refers to the diverse set of principles and models used in ecology to understand patterns in nature, population dynamics, and the balance of ecosystems used to understand diversification of aquatic edible insects. The evolutionary aspects were explained using the evolutionary perspective which drew attention to the interconnection between an individual's life history and the long-range history of the selected edible insect-non-biting midge also called *Chironomus* spp. species. The theory was used to account for change in *Chironomus* spp. amongst the sampling stations species over time.

2.7.2 Pollution

Pollution is defined as the presence of substances and/or heat in environmental media (air, water, land) whose nature, location, or quantity produces undesirable environmental effects. Pollution, is also defined as the addition of any substance (solid, liquid, or gas) or any form of energy (such as heat, sound, or radioactivity) to the environment at a rate faster than it can be dispersed, diluted, decomposed, recycled, or stored in some harmless form. Environmental contamination and pollution can occur naturally such as wildfires and volcanic eruptions but are largely caused by anthropogenic activities including industrialization, transportation, and agriculture. Water pollution, also referred to as aquatic pollution is defined as the contamination of water bodies, with a negative impact on their uses. Water pollution is said to occur when toxic pollutants and particulate matter are introduced into water bodies such as lakes, rivers and seas. Bhateria & Jain, (2016) observed that Lake water gets contaminated by natural processes like weathering of rocks, leaching of soils and mining processing while (Akhtar et al., 2021) addressed natural

sources of pollution as climate change, natural disasters, geological factors, soil/matrix, and hyporheic exchange in the aquatic environment. Further, sources of degradation of water were observed to be associated with human activities (agriculture practices and urban waste), as well as the presence of considerable chemical compounds since the industrial revolution. According to (Lin et al., 2022), industrialization, agricultural activities, natural factors, and insufficient water supply and sewage treatment facilities are the main sources of water pollution. In addition, Tiwari & Pal, (2022), noted that natural processes such as eutrophication due to nutrient enrichment and human activities like improper sewage treatment and oil spills are among the contaminants of concern. Other significant causes of water pollution include: dumping solid wastes in water bodies; disposing untreated industrial sewage into water bodies; human and animal wastes; agricultural runoff containing pesticides and fertilizers. According to Mishra et al., (2023), chemical hazards also known as emerging pollutants were highly complex contaminants (EPs) of potential risks to human health and the environment's ecological balance particularly in large water bodies. Salthammer, 2020 defined Emerging pollutants (EPs) as chemicals that are not currently (or have been only recently) regulated and about which there exist concerns regarding their impact on human or ecological health but find their way into water bodies (Mishra et al., 2023). Emerging pollutants (EPs) are a group of different contaminants, such as hormones, pesticides, heavy metals (Ar, Hg, Cd, Pb), and drugs, usually found in concentrations between the order of ng and µg per liter. The global population's daily city and agro-industrial activities release EPs into the environment. Most reported EC groups include: (1) pharmaceuticals, e.g., human and veterinary antibiotics, analgesics, anti-inflammatory drugs, and β-Blockers, (2) PCPs, e.g., fragrances and insect repellents, (3) hormones and steroids, (4) disinfectants, (5) flame retardants, (6) herbicides and pesticides, (7) industrial additives. Pollution, even in minuscule amounts, impacts the ecological balance. The pollutant makes their way up the aquatic food chain and food webs and eventually find their way inside the human body. The effects of water pollution are very pronounced in our environment particularly the toxic chemicals which bioaccumulate, persist and bio magnify up trophic system, ultimately reaching humans beings. Water pollution has severe consequences to biodiversity, to humans and ecosystem; threats to marine life; Increased risk of water-borne diseases; Increases toxic chemicals (such as mercury) in water bodies; and eutrophication.

2.7.3 Lake as a sink of pollutant

Lake pollution has been a growing environmental concern worldwide due to anthropogenic activities which include: urbanization, inadequate municipal facilities make urban lakes the sink for pollutants (Akhtar et al., 2021), Indiscriminate solid waste dumping, domestic activities, sewage, stormwater runoff, industrial effluent, and soil erosion and degrade water quality of lakes from agricultural activities, industrial wastes. Pollution hampers nourishment of biodiversity, diminishes the usefulness of ecosystem services (Hossain, 2019). Aquatic pollution is determined by assessment of water quality which is evaluated by physical parameters (color, taste, odor, temperature, turbidity, solids, and electrical conductivity), chemical, parameters (pH, acidity, alkalinity, hardness, chlorine, and dissolved oxygen.) and biological parameters (nutrients- TN (Nitrate + nitrite mg/L),Nitrite- nitrogen (NO_2 -N), Nitrate- nitrogen (NO_3 -N) Inorganic forms of nitrogen, and total phosphorus (TP) and ortho-phosphate (ortho-P; soluble inorganic phosphate-,bacteria, algae and viruses.), and values compared to acceptable limits (Omer, 2019). The primary indicators of water quality predict the biological health of the lake e.g. DO, salinity, pH etc. Besides, water quality can be evaluated based on fauna, flora and human species requirements. Based on water quality, ecological niches are created which influences the composition, species richness, the distribution, abundance and diversity of biota. The lake ecosystem is therefore divided into four major zones namely: 1) Littoral zone -shallow, nutrient-rich waters near the shore, contain rooted aquatic plants and an abundance of other forms of aquatic life. 2)Limnetic zone -open-water surface layer receives sufficient sunlight for photosynthesis and contains varying amounts of floating phytoplankton, plant-eating zooplankton and fish, depending on the availability of plant nutrients.3). Profundal zone -zone of deep water not penetrated by sunlight is inhabited mostly by fish, such as bass and trout that are adapted to its cooler, darker water and lower levels of dissolved oxygen.4) Benthic zone is deepest and located at the bottom of the lake is inhabited primarily by large numbers of bacteria, fungi, bloodworms and other. Perturbations in the lake ecosystem results to hydrological modifications through nutrient loading, addition of organic contaminants (dye, humic substances, phenolic compounds, petroleum, surfactants, pesticides, and pharmaceuticals are important pollutants in wastewaters) (Catalan et al., 2024), and inorganic pollutants (non-biodegradable substances, often stemming from industrial, agricultural, and residential sources ranging from heavy metals such as lead, mercury, and arsenic to salts like nitrates, phosphates, and sulphates, enter water bodies) from point and non- point sources creating an ecological

gradient which interferes with the water balance. The effects are accelerated by increasing population growth, encroachment, and climate change.

2.7.4 Pollution and Aquatic Insects Diversity

Research has shown that about 86000 species of insects, falling under 12 orders,150 families are known to inhabit diverse freshwater ecosystems, (Elango et al., 2021). The 12 orders of aquatic insects include Collembola, Ephemeroptera, Odonata, Plecoptera, Hemiptera, Coleoptera, Diptera, Trichoptera, Melagoptera, Lepidoptera, Neuroptera and Hymenoptera. The insects are also known as model organisms for analyzing structures and function of freshwater ecosystem due to their unique characteristics: high abundance, high birth rates with short generation time, large biomass, sensitivity to pollutants and role in nutrient cycling. Despite that, aquatic insects face a challenge of hydrologic modifications in the systems (Elango et al.,2021), associated with climate change and human induced activities. For instance the changing physical environments, creating variations in habitat like erosional habitat, depositional habitats, changing substrates affect the insect communities composition, species richness, abundance, distribution and diversity.Consequently, the changes affect the stability of the physical environment due to dynamics in water quality (Whitehead et al., 2009). Anthropogenic disturbances (Husseini et al., 2019) also affect the insect species assemblages-taxa richness, abundance, densities and diversities influenced by their response due to ecosystem changes (Elango et al., 2021). Aquatic insects vary in their sensitivity to change in water quality, evaluated by the presence or absence of some species, their, abundance, diversity and distribution indices (Jumaat & Hamid, 2021). For instance, order Ephemeroptera are good indicators poor water quality and only present in highly oxygenated and moderately productive waters, Order Hemiptera survive in highly polluted areas and used to gauge toxins (Wollmann, 2000), high abundance in order Diptera particularly *Chironomus spp*.is indicative of eutrophic waters with low oxygen concentrations and high nutrient levels. Dipterans are pollution tolerant species and therefore founding in all zones and are ecologically important in food chains and food webs, and useful in biomonitoring. Orders Ephemeroptera and plecopteran are always absent in polluted and degraded waters respectively while Megaloptera are commonly found in heavy organic areas. The success in survival of aquatic insects is attributed life cycle adaptation, developed morphological, physiological and behavioral adaptations and resource partitioning strategies.

2.7.5 Pollution Versus Nutritional Composition of Aquatic Insects

The increasing population growth calls for the need for alternative nutritious food sources for food security, for socioeconomic benefits, and environmental friendly in utilization (FAO, 2021).The global consumption of aquatic insects include six out of the twelve existing orders namely: Coleoptera (31%), Lepidoptera(18%), Hymenoptera (14%) Orthoptera, Hemiptera and Isoptera. Aquatic insects are endowed with an averagely high proteins at 59.55%, amino acids ranging at 45.93 -to 62.01% of essential amino acids, EAA-balanced and of different kinds, fatty acids for growth and development dominated by palmitic and stearic-SFA, oleic-MUFA, mineral salts-Zn, Fe Ca and vitamins (Zhao et al., 2021). The nutritional composition depends on the species, the sex and the feeding strategies. However, aquatic insects ingest and also absorb some non-essential metallic elements (Ar, Hg, Cd, Pb) through their body from the substrate. Chironomus spp., also known as nonbiting midges is not an exception. The insect species larvae have a long history in use as a fishing bait, fish food, for medicinal and cultural use (Ayieko & Oriaro, 2008). And ecological for nutrient cycling. Cultivation of the *Chironomus* spp., a pollution tolerant species can grow in different environments, be cultivated in controlled environment for sustainability and industrial production and has a short life cycled. Despite the socio-economic benefits, detailed analyses of the nutritional components have received minimal attention. In addition, the effect of pollutants on the nutritional composition has not been documented. Hence, the need for proper assessment, allow for cultivation to ensure safe utilization and sustainability.

2.7.6 Aquatic Insects' Diversification

Freshwater habitats are highly susceptible to environmental changes and exhibit marked ecological gradients (Dijkstra et al.,2014). This result to ecological variations which influence diversification. The insect's amphibiotic life style correlates with habitat dependence in response to change via dispersal. Consequently, the complex lifecycle allows for adaptability to disperse or access or evade death. The diversification process is accelerated by change in geographical area, ecological roles, chemical gradients like oxygen concentration and change in salinity. The insect's morphology, physiology and behaviours change due to new ecological opportunities ad climate niche evolution leading to speciation. For instance, changing water chemistry due to geological factors coupled with biotic interactions affect the species based on level of pollution tolerance. Though the species are closely related in taxa, their level of tolerance to harsh

conditions -pH, salinity, mineral loads- may vary and be conserved over time resulting to adaptations and retention. Behavioral factors like isolation can result to shifting or migrations to new niches causing diversification. Feeding preferences correlate to ecological and geographical factors resulting to resource partitioning (Dijkstra et al.2014). Dietary specialization and species interactions like predation and parasitism may influence diversification through divergent or convergent selection resulting to diversification (Dijkstra et al.2014). In addition, hydrological regimes influence formation of habitats and their characteristics, lead to habitat stability, dispersal, reconfiguration and isolation. This influences the nature of stability or unstability affecting the sedentary and good dispersers respectively. Hence, speciation occurs i.e. their species turnover and even loss. For instance, Dipterans are good dispersers, pollution tolerant and the turn over influences the taxa richness, structure, exploitations and interactions (Adler & Courtney, 2019). Hence, are good bio monitors of ecosystem health and integrity.

2.7.7 Pollution and Genetic Diversity

Pollution in a lake ecosystem result to dynamics in abiotic and biotic components and influences natural selection. The changes affect the environment which in turn influences the primary productivity, the trophic dynamics, the populations, the taxa richness, abundance, and diversity indices. The changes in the lake system may drive evolutionary dynamics affecting biodiversity and ecosystem services (Alexander et al.,2017). Anthropogenic changes may be the course of evolution, reverse evolution generating processes resulting to homogenization in populations. The biodiversity response through behavioral, ecological, morphological and physiological adaptations (Perry et a., 2024). The changes may result to speciation reversal, reproductive isolation, natural dynamics leading to hybridization, divergence or convergence of species, homogenization of genetic and phenotypic diversity. Hence new species formed with genetic variations or result to erosion of phenotypic characteristics. Climate change also led to bursts in diversification through geographical isolation, speciation rates, taxonomic disparities associated with intrinsic and extrinsic factors. The end results are mutations in populations, change in patterns and processes of morphological evolution, and molecular evolution resulting to phylogenetic diversity, (Salzburger et al., 2014).

2.8 Conceptual Framework

The Driver-Pressure-State-Impact Response (DPSIR) framework was adopted in describing the interactions in Lake Victoria ecosystem, (Frederiksen & Kristensen, 2008). The main driver-

pollution was identified among other drivers which include biological factors (human settlement and infrastructure developments), invasion species, habitat change, and climate change. The main stressors were water pollution from both point and non-point sources. The sources were associated with human activities which included industrial activities, agricultural activities, waste disposal from domestic and food industries. In addition, environmental pollution was accelerated by habitat locations within the urban centers on the shorelines. The urban centers are well known for trade and transportation with results to increased populations, increased wastes, sewerages and also high densities of settlements particularly the slums. The effects are clearly observed in changing water quality which influences the biodiversity: aquatic plants and animals. The effects are either direct or indirect. The direct effects include change in physico-chemical parameters eg. salinity, pH, temperature, DO., high nutrient loads particularly TP and TN and increased levels in Heavy metals like which are detrimental to biota and the entire ecosystem. However, some metals like Zn and Cu are functional only at low concentrations and increasing levels beyond the maximum requirements renders the metals toxic. Indirect effects include the influence of other process in the lake ecosystem, for example the DO affects redox reactions, pH affects bioavailability of other pollutants. The impact of the stressors affects the species composition, a abundance, diversity indices and distribution. Some stressors affect the ecological integrity of the systems by toxicity. The unique characteristics of the pollutants like bioaccumulation, persistence, biomagnification, and toxicity affects the trophic dynamics by flow of energy through food webs and food chain. The effects are more serious up the food chains like heavy metals (Ar, Hg, Cd, Pb) and the organic pollutants. Besides, some pollutants cause genetic variability, change in biochemistry and physiology in life forms and this affects their ecological health and service as use as of aquatic biota as food and feed. Benthic organisms like chironomids tend to ingest contaminated particles from sediments, are also exposed to the contaminants in the environment. Therefore, the organisms are affected by change in physical, chemical and biological characteristics in lake ecosystem. The most vulnerable are the life stages. The stressors are synchronized into multistressors and have either additive effects, synergetic (multiplied) or antagonistic effects (reduced). Hence, the need to understand the interactions and the effects in a lake ecosystem.

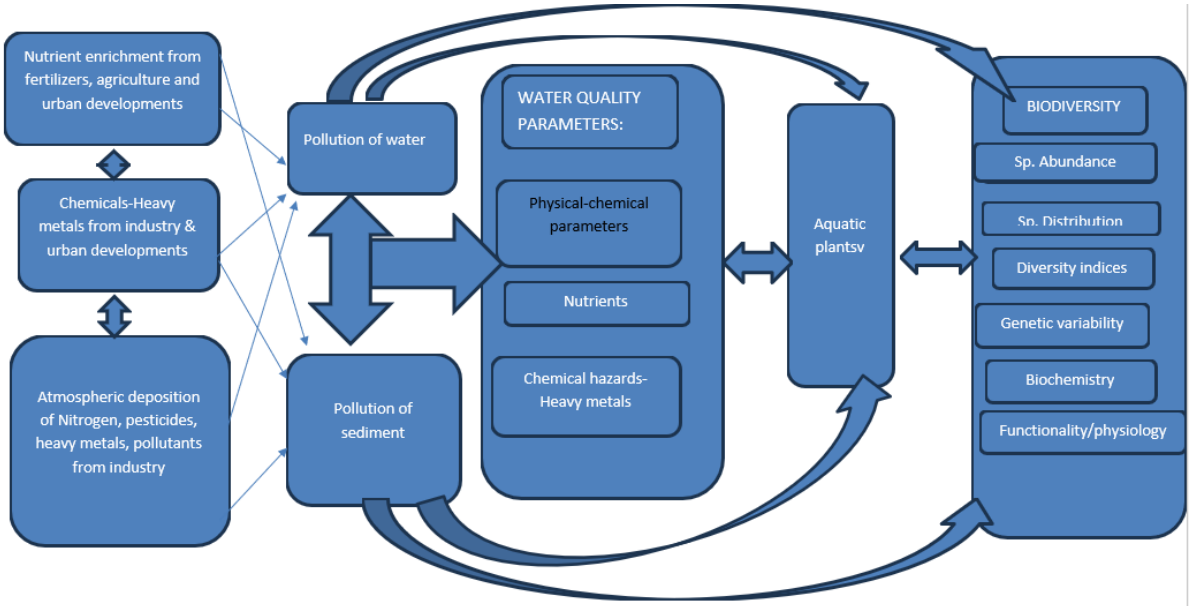

Figure 1. A schematic diagram of the conceptual framework giving a description of the interactions in a Lake ecosystem using the DPSIR model framework.

CHAPTER THREE
MATERIALS AND METHODS

3.0 Introduction

The current study was undertaken in September 2020 on the Kenyan side of Lake Victoria Kenya. The study sought to document the effect of urban pollution emanating from both point and nonpoint sources on water quality in the gulf. Further, the study was to investigate if pollution had impacts on the composition; distribution, diversity and nutritional status of aquatic edible insects in the lake.

3.1 Study Area

3.1.1 Description of the Study Area

Lake Victoria is the second largest freshwater lake in the world, with a surface area of 68,860 Km, a catchment area of 193,000 Km, an altitude of 1134 m above sea level, and approximately 400 Km long by 250 Km wide. The three countries share the lake, with Tanzania having 49%, Uganda at 45%, and Kenya at 6% (4,128 km^2). The main rivers flowing into the lake include Sio, Nzoia, Yala, Nyando, Sondu Miriu, North Awach, South Awach, and Kuja from the Kenyan side, rivers Mara, Grumeli, Eastern shore streams, Kagera, and Bukora from Tanzania. In Uganda, the main rivers are the Katonga and the Northern Shore streams. The Kagera, the most significant of these rivers, contributes 33% of the total inflow. The lake has only one major outlet- the River Nile, the longest river in the world (Figure 1a). The current study focuses on the Kenyan portion of Lake Victoria, known as Kavirondo Gulf (Rabour et al., 2023). The Winam Gulf (also known as the Nyanza or Kavirondo Gulf) is a semi-enclosed sheltered bay with an area of 1400 km^2 on the Kenyan side of the lake. It is located at latitudes 00014’14.40” and longitudes 34’34034’28.79’’. The Gulf connects to the main lake via the Rusinga channel South of Uyoma point and extends as a shallow (2-4 m) indented bay Eastwards to Kisumu.The shoreline is approximately 346 km long with flat sandy and muddy areas, predominant in the sheltered bays. The climate is tropical in the gulf and is marked by four climatic seasons: short rains, prolonged rains, short dry, and the long dry season, experienced annually. The gulf has an annual temperature range of 18.6-25^oC and average annual rainfall of 886-2609 mm, as captured by morphometric data of Lake Victoria between 1950-2000. The high rains in the region are associated with floods and storm waters, which wash pollutants from non-point sources into the gulf. The catchment area of the gulf has five (5) significant rivers, Nzoia, Yala, Kuja, Nyando,

and Sondu, flowing from the highlands through the agricultural areas and contributing 30% of the riverine inflow into the lake (Ombogo, 2016). Other vital rivers include Kisat, a highly sewage-polluted river; Kisian, which flows from agricultural areas; and Nyamasaria, which flows from suburban areas. The principal rivers, such as Sondu Miriu, are used for hydropower generation. Others flow through the sugarcane agricultural and industrial zones, such as rice paddies, where growing rice is integrated with aquaculture on a small scale. Other crops grown include cotton, horticultural crops, maize, coffee, and tea. Poor agricultural practices are associated with sedimentation, erosion, and nutrient loading in the lake. In addition, the fishery and food processing industry, a livelihood to the people, remains a significant socioeconomic activity in the form of food, employment, and even a foreign exchange earner. However, the growth of industries such as breweries, tanning, fish processing, agro-processing, and abattoirs contributes to volumes of pollutants that find their way into the lake. Besides, the region is known for livestock farming. Other activities include gold mining, sand harvesting, and oil deports.

The catchment area in Nyanza Gulf is densely populated, with a density of about 440 people per km^2 (Kenya National Bureau of Statistics, 2010), which puts pressure on available land, resulting in deforestation and increasing levels of pollutants in the gulf. The major towns along the lakeshores, including Kisumu City, Homa Bay, and Kendu Bay, are under more pressure regarding sewerage facilities and refuse deposition, which translates into non-point sources of pollution in the lake. The population around the lake uses the water for domestic, agricultural, and industrial purposes. The effluents find their way to the lake through surface runoff, compromising the water quality.

The pollutants impact the Gulf ecosystem, resulting in changing habitats for aquatic flora and fauna. For instance, the increased trends in the growth of emergent and sub-emergent macrophytes in the littoral zone and the water column create insect habitats, which form part of the aquatic food chains and food web, thus allowing the flow of energy up the trophic levels.

Table 1. The GPS, substrate type, anthropogenic and ecological activities of the sampling stations

Site	Gps: Southings	Gps: Eastings	Substrate	Human Activities and ecological description
Kisumu Bay	00º05.686.097″	034º44.086'	Silt	Sewage disposal, Littoral/sublittoral vegetation, phytoplankton blooms-water hyacinth/hippo grass
	00º05.212″	034º44.969'	Sandy	Golf club, Phytoplankton blooms Littoral and sublittoral vegetation-water hyacinth/hippo grass
	00º07.474″	034º44.630'	Clay	River Auji, waste dumping site, hippo point, next to KIWASCO
Fish landing Beach	00º08.751″	034º44.152'	Rocky	Settlements, Osienalla, Fish landing beaches beach, hotels, tourists, boat riding, Phytoplankton blooms
	00º01.506″	034º00.809'	Sandy	Beach-seining, fishing, sand harvesting, boat-riding, fishing, town, many bird's species, macrophytes, no floating vegetation
	00º03.651″	034º02.571'	Sandy	River Yala through Lake Kanyaboli enters the main lake through papyrus reeds, fishing, beach seining, sand harvesting, domestic cleaning, phytoplankton blooms
	00º04.360″	034º03.693'	Muddy	Hotels, fish landing, cage farming, motor boast transport across from Uganda
	00º05.822″	034º00.705'	Silt/rocky	Elmolo hotel, settlements, fish landing beaches – fingerlings, crocodile farming, bamboo forest
Kendu Bay	00º20.971″	034º39.321'	Silty	Hotels, Fish landing beaches, littoral, sublittoral, floating vegetation
	00º20.968″	034º39.095'	Silty	Open waters
	00º20.968″	034º39.264'	Silty	Minimal floating vegetation, a lot of hippo grass and papyrus
Homa Bay	00º31.34″	034º27.206'	Silty	Waste disposal, town settlements, town sewage treatment plant entry point, beach activities, fishing,
	00º30.784″	034º26.735'	Silty	Open waters
	00º30.337″	034º26.203'	Rocky	Undisturbed area, patchy thickets of papyrus, and floating water hyacinth next to Sikri Island provided a shedding effect
Maboko Island	00º09.569″	034º37.003'	Rocky	Settlements, hotels, fish landing beaches, floods, slightly disturbed
	00º10.238″	034º36.358'		
	00º10.097″	034º36.537'		
Ndere Island	00º10.999″	034º31.142'	Muddy	Human activities prohibited, protected area, home to wild animals including hippos, crocodiles, birds, fish
	00º11.662″	034º31.187'		Human activities prohibited, home to wild animals including hippos, crocodiles, birds, fish
	00º11.450″	034º31.168'		Human activities prohibited, home to protected wild animals

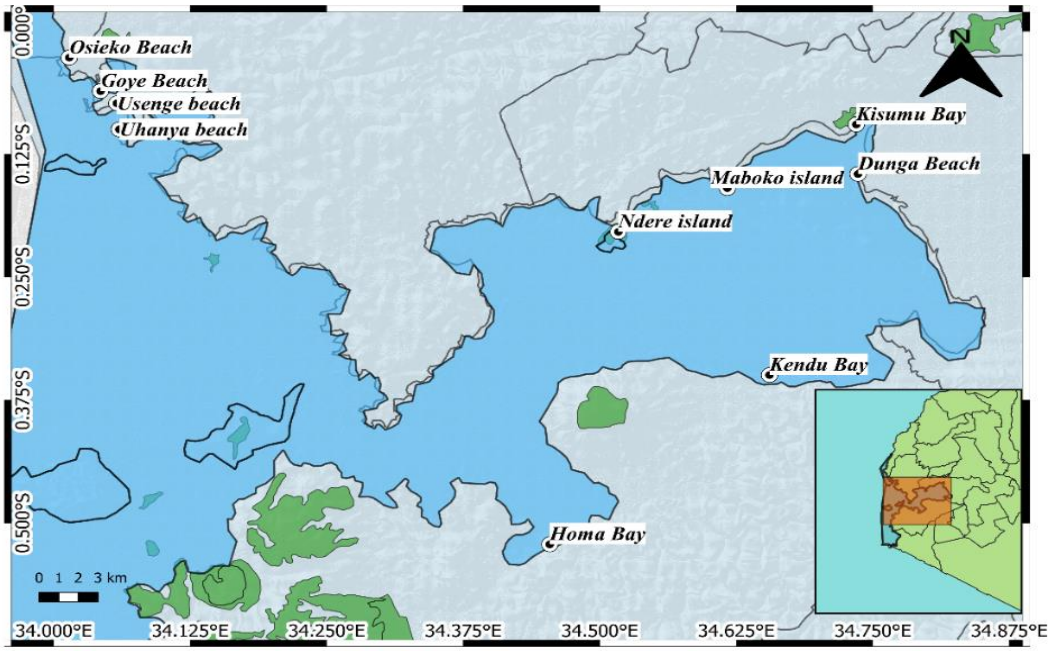

Figure 2. A map of the Winam Gulf (also known as Nyanza Gulf), the Kenyan Side of Lake Victoria showing the exact sampling stations **.**

3.1.2 Description of Sampling Stations

The description of the sampling stations for the current study was as outlined in Table 1.

3.1.2.1 Maboko Island

Maboko Island is a tiny island lying in Kisumu Bay, Nyanza Gulf, Lake Victoria, Kenya (Plate 1). The island is about 1.8 km long and about 1 km in width located 12 km from Ndere National Park. The island is historically known as a paleontological site with fossiliferous deposits discovered in 1930 (Feibel & Brown, 1991). The island is offshore at the furthest end of Kisumu Bay, endowed with hotels like Maboko Island resort and settlements earmarked for comparison purposes between inshore and offshore stations. The island represented a disturbed environment due to unlimited human activities, particularly the hotelier and hospitality industry among others. Three sampling sites were identified within the Maboko island sampling station including Maboko 1 (00º09.569″S, 034º37.003' E), Maboko 2 (00º10.238″S, 034º36.358'E), and Maboko 3 (00º10.097″S, 034º36.537'E).

Plate 1: Maboko Island Sampling Station in Winam Gulf, Lake Victoria, Kenya.

3.1.2.2 Kisumu *Bay*

Kisumu Bay is located in the capital city of Kisumu County with an approximate urban and rural population of about 397,957 and 714,668 respectively, totaling 1,155,574 as per the (Kenyan Population and Housing Census, 2019) as outlined in Plate 2.

Besides being a chief terminus for the food processing industry, the Millennium City is also a trade and transportation hub. The region experiences tropical rainforest climatic conditions with an average of 22.9 ^{o}C and two rainy seasons. Increased human population and the city's growth have pressured the sewerage and sanitation facilities. The frequent rains experienced in the low-lying areas also contribute to stormwater pollution. Kisumu Bay receives input from the rivers Auji and Nyamasaria, which converge and flow via the Dunga village slums through the Hippo Point, located southwest of the city. The streams flow via the papyrus swamps into the lake. River Kisat is smaller and originates from the swamp and flows via Kondele, the Car wash, and into the lake. The river is adjacent to a fish processing plant called Peche Food Factory and the Nyanza Golf Club. Kisian River flows into the bay through agricultural settlements and, therefore, is affected by storm waters from both point and non-point sources, hence the input of fertilizers and pesticides. Kisumu Bay was a representative of a heavily polluted sampling station. Three sites were sampled: Kisumu 1 identified as Coca-Cola (00º05.686.097″S, 034º44.086'), Kisumu 2 identified as Kisat River mouth (00º05.212″S, 034º44.969'E), and Kisumu 3 in Hippo point (00º07.474″S, 034º44.630').

Plate 2. Kisumu Bay Sampling Station in Winam Gulf, Lake Victoria, Kenya

3.1.2.3 Fish Landing Beaches

Five sampling sites were identified to purposely represent the fish landing beaches due to geology, vegetation characteristics, and economic activities as Dunga beach (00^{o}08.751'S, 034^{o}44.152'E Osieko beaches (00o01.506'S, 034^{o}00.809'E), Usenge beach (00o03.360'S, 034^{o}03.693'E), Uhanya beaches (00^{o}05.822'S, 034.00^{o}705'E) and Goye (00^{o}03.651'S, 034^{o}02.571'E).

Dunga Beach and Wetland is a resource center with a unique eco-cultural attraction due to its biodiversity and culturally rich papyrus wetland ecosystem for the local community. It's critical in eco-cultural tourism and conservation. In addition, it's a fish landing beach site with a wide range of activities, including the hospitality industry, boat riding, and trading.

Osieko Beach is about 1.8 km from Usenge and 95 km from Sio-port. It has two fish-landing beaches situated a few meters apart. The beach is a catchment area for the rivers Yala and Nzoia. It is known for fishing and inter-county trade since it lies on the peripheries of Bundalangi and Busia, Kenya, and Uganda. Sand harvesting is an alternative income-generating activity after decreased trends in fisheries. Motor-boat transport is predominantly used; hence, water pollution remains a challenge.

Usage is the largest fish landing beach with about 1000 traders. The beach is sandy, with wind and cool breezes, hence a tourist attraction with a significant population of locals and visitors.

The hotel industry is well established. However, the iconic Usenge beach is fading. This is attributed to deteriorating sanitation facilities, poor garbage handling, and inadequate drainage. The fish auction center lacks toilets and restrooms, and sewer wastewater finds its way into the lake, resulting in water pollution.

Uhanya fish landing beaches have both fishing activities and the hotel industry. The beach has an El'molo crocodile park and lodge, among other attraction sites. It is known for inter-border business and is marred by insecurity across borders.

Goye Beach is a causeway with sandy shores that are lucrative for sun and sand bathing. The beach is in the neighborhood of Olowe beaches, which have papyrus reed stands with a rich bird life of up to 34 species, including kingfishers, plovers, ducks, geese, and cormorant egrets. The habitat is suitable for mud and lungfish.

3.1.2.4 Ndere Island

Ndere Island, located 80 Km from Kisumu City off the Kisumu-Bondo highway and 1 Km from Asat Beach (Plate 3). It was gazetted in November 1986 by the Kenyan Government as a National Reserve. The uninhabited area displays a tranquil environment in comparison to the mainland. The island is a haven for birds and attracts fauna. Ndere is a grassland. Three representative stations were randomly identified as Ndere 1 ($00^{o}10.999$'S, $034^{o}31.142$'E), Ndere 2 ($00^{o}11.662$'S, $034^{o}31.187$'E) and Ndere 3 ($00^{o}11.450$'S, $034^{o}31.168$'E).

Plate 3. Ndere island sampling station in Winam Gulf, Lake Victoria, Kenya

3.1.2.5 Kendu Bay

Kendu Bay (Plate 4) is the headquarters of the North Karachuonyo sub-county in Homa Bay County along Katito-Homabay Road also doubles up as a town. It is located 30 Km southwest of Homabay and 40 km of Kisumu City. The town has a population of about 29,638 (Kenya National Bureau of Statistics, 2010) mainly known to embrace fishing activities for their livelihood. Besides, the bay is also known as a hive of activities in the hospitality industry. Human-induced activities lead to a change in the natural environment and impact the water quality negatively affecting the flora and the fauna. Three sampling sites were determined as Kendu Bay 1 (00º21.060'S, 034º39.321'E), Kendu Bay 2 (00º20.968'S, 034º095'E), and Kendu Bay 3 (00º20.971'S, 034º39.264'E to ensure proper representation of the bay.

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Plate 4. Kendu Bay Sampling Station in Winam Gulf, Lake Victoria, Kenya.

3.1.2.6 Homa Bay

Homa Bay a capital town, centrally located on the southern shore of Winam Gulf (Plate 5) is the largest in the county with an approximate population of 45,000 (Kenya Population and Housing Census, 2019). The town hosts the municipality, open-air market, waste dumping site, pier, landing site, and slaughter house. In addition, Homa bay town has fish processing and hotel industries. Consequently, motorboat transport plays a key role in the trade between the mainland and the islands. The growing human population in the town, attributed to the increase in residential areas has impacted pressure on water supply and sanitation facilities. Furthermore, the town lacks effective drainage and waste disposal facilities resulting in pollutant run-off s which degrades the water quality of Lake Victoria (Opio, 2021). The bay is therefore experiencing degradation associated with the hive of activities as sources of contamination. Hence, results in to change in the water quality which influences the flora and fauna. The changes are exacerbated by industrial activities, agriculture, and the tourism growth. Three sampling sites were determined as a representation of the station: Homa Bay 1 (00º31.334'S, 034º27.206'E), Homa Bay 2 (00º30.784'S, 034º26.735'E), and Homa Bay 3 (00º30.337'S, 034 º26.203'E).

Plate 5. Homabay Sampling Station in Winam Gulf, Lake Victoria, Kenya

3.2 Sampling Design

3.2.1 Sampling Sites

A total of six samplings Stations-Kisumu Bay, Kendu Bay, Homa Bay, Fish Landing Beaches sites, Maboko Island, and Ndere Island (Figure 1 and Table 1) were identified based on the pollution gradient. Three sites per sampling station were identified to enhance the sample size and reduce the experimental error and marked using a GPS. Three Fish Landing Beaches, FLB, with similarities in geology, vegetation characteristics, and economic activities, were also sampled: Osieko, Usenge, and Uhanya.

Sampling was done on September 8th-11th, 2020. Three sampling stations, Kisumu Bay, Homabay, and Kendu Bay, located in urban environs with approximately 3.5m maximum depth, were identified to capture the effects of urban pollution. This was to include the impact of domestic effluents, raw sewage, industrial wastes, and nonpoint sources like stormwater from the towns. Suspended and dissolved solids like oils, detergents from the carwash, wasted batteries, etc., were included. The bay is also influenced by a highly polluted river inlet, Kisat, which has sewage effluents.

Two islands, Maboko and Ndere, were identified as the coastal stations in the gulf. Ndere Island is relatively clean and has limited human activities due to the presence of Ndere Island National Reserve Park under the management of Kenya Wildlife Service, KWS. Maboko Island is the

furthest end of Kisumu Bay into the lake, representing a coastal station and a disturbed environment due to unlimited human activities, particularly the hotelier and hospitality industry. The two islands are completely isolated from the mainland and have different microclimates. Hence, they are unique microhabitats. Osieko, Usenge, and Uhanya are Fish Landing Beaches sampled to capture the effects of fisheries, processing industries, and the related human activities on aquatic insects.

3.2.2 Experimental Design

Six sampling stations were identified for the study. Mapping of each station is done by measuring50m belt inward from the shoreline to form a belt transect within an approximate depth of <3.5m. At each station, three sampling sites, S2 and S3- were identified, and *in-situ parameters* measured at each site (temperature, pH, electrical conductivity-E.C., dissolved Oxygen- DO, and turbidity, turbidity, TDS, TH, TA, ORP, Salinity). At each site, triplicate water and sediment samples were collected and pooled to form a composite, homogeneous, representative sample per site to reduce errors and increase accuracy. Three values were recorded and analyzed per station. Water samples were used to analyze nutrients (N_4-N, NO_3-N, NO_2-N, TN, TP, PO^{4-}) and Heavy metals (AR, Hg, Pb, Cd).

Similarly, sediment samples were used to analyze heavy metals—Chironomus spp. Larvae samples were collected from the three sampling sites per station and pooled to form a representative sample for the six stations. Each of the six-insect samples was analyzed for nutritional profiles-amino acids, vitamins, fatty acids, macro and micronutrients, and heavy metals.

3.3 Sampling Procedure

In-situ parameters were measured and recorded on site. Water samples for nutrient analyses were collected using Van Dorn sampler into amber bottles pre-washed in distilled walk and treatment done by adding 1g $HgCl_2$, samples stored in a refrigerated cooler box at 4°C. For heavy metal analysis, the water sample was collected using a polycarbonate sampling bottles pre-washed in acid followed by distilled water. Preservation was done by adding liquid HNO_3 at a pH before storing in cooler boxes at 4°C. The (U.S. EPA, 2001) standard operating procedure (ID: LSASDROC-200-R4 (U. S. EPA, 2001) and EPA, (SFS

3536,1835) was employed in the collection of sediment samples. A 400 cm^2 (20 x 20 cm) size AISI 316 stainless steel fabricated Eckman bottom core grab was used. The spring-tensioned, scoop jaw-like part was mounted on pivot points and set with a trigger assembly activated from a surface by a messenger. A total of three grabs dumped on a tray, and pooled to form a composite sample. The sample was then placed in 1000 ml PVC bottles and covered with aluminum foil paper, placed in a cooler box at 4ºC and transported to the laboratory for heavy metal analysis.

A profundal lake sampling procedure was employed as outlined in the standard SFS 36-1835-(ESFS 36,1835). Birge-Eckman grab sampler was used to make random triplicate grabs of submerged insect larvae sample placed into the plastic bucket through a bucket sieve with um mesh and pooled to obtain a composite sample. The contents were emptied for sorting aided by a washing bottle to flash the remaining content using alcohol, then placed in paper slips and labeled (location, date, time, collector, sampling method, habitat, habitat description, weather, and photographs of every site taken, sample number). Filled with 80% alcohol as in (ISO-EN 4253-3), closed and packed in readiness for transportation in cooler boxes at 20ºC. Sampling was carried out in the morning hours between 7 am-11.30.

3.3.2. Samples

3.3.2.1 Water Samples

Water samples for nutrient analyses were collected using a 2.2-liter vertical water bottle and a van-dorm sampler into 2.5 L amber bottles, which were pre-washed with distilled water and dried. Each sample was treated with 1 g $HgCl_2$, and mixed for 5 minutes to kill microorganisms that could lead to degradation. The sterilized samples were kept in an icebox containing ice blocks and later stored in a refrigerator at 4° C before extraction.

Water samples for heavy metal analyses were collected using a polycarbonate sampling bar of polytetrafluoroethylene container which was thoroughly pre-washed with acid and deionized water. The samples were preserved in liquid HNO_3 at a pH of < 2. The samples were placed in cooler boxes at 4ºC and transported to the laboratory for analysis.

3.3.2.2. Sediment Samples

The U.S. EPA (2020) standard operating procedure (ID: LSASDROC-200-R4) and *(*Ohio EPA, 2001) was employed in the collection of sediment samples. A 400 cm^2 (20 x 20 cm) size AISI

316 stainless steel fabricated Eckman bottom core grab was used. The spring-tensioned, scoop jaw-like part was mounted on pivot points and set with a trigger assembly activated from a surface by a messenger. A total of three grabs were done per sample, dumped on a tray, and pooled to form a composite sample. The sample was then placed in the wide bottom 1000 ml Polyvinyl chloride, PVC bottles and covered with aluminum foil paper, placed in a cooler box at 4ºC and transported to the laboratory for heavy metal analysis.

3.3.2.3. Insect Samples

Eckman grab–Birge dredge sampler was used to make random triplicate grabs of submerged insect larvae emptied into bucket through a sieve. The samples pooled to obtain a composite sample followed by sorting. then placed in paper slips and labeled (location, date, time, collector, sampling method, habitat, habitat description, weather, and photographs of every site taken, sample number). Samples preserved using 80% alcohol as in ISO-EN 5667-3, closed, packed and, transported in cooler boxes at 20ºC.

3.4. Sample Processing and Analyses

3.4.1 *In-situ* Parameters

The *in-situ* water quality sampling, also referred to as measurement of physical and chemical parameters in a water body. The measured variables included temperature, pH, electrical conductivity, dissolved oxygen- DO, and turbidity using a water quality multi-parameter instrument – (the YSI Pro DSS (Digital Sampling System). Secchi depth was measured using a standard Secchi disk of 20 cm diameter, with quadrants painted in black and white. Turbidity was measured using a 2100Q Hach Turbidimeter while pH was measured using (model 8685 AZ IP65) pH meter. Depth, temperature, electrical conductivity-E.C., and phytoplankton biomass (chlorophyll a) were measured using a submersible Conductivity-Temperature-Depth profiling system (CTD, Sea-bird Electronics") programmed to take measurements at 5 seconds intervals. Total suspended solids and total dissolved solids-TDS were determined using a gravimetric method, (Wetzel,2001). Total alkalinity-TA was measured based on titration of water sample to designated pH using 0.1N or H_2S0_4 equivalent to 5 mg of $CaCO_3$, and 1 ml of 0.02N H_2S04 equivalent to 1.00 $MgCaCO_3$. Then measured by phenolphthalein by titration to pH 8.3 using a digital titrator. Total hardness-TH was determined by titrating the standard solution of ethylene diamine tetra acetic acid (EDTA-Na_2) in form of Disodium Salt of EDTA-

Na_2 titration as outlined in (Wetzel,1991). Oxygen reduction potential- ORP measured using ORP meter while Salinity measured using a salinometer. Nutrients defined as chemical elements and compounds found in the environment that plants and animals grow and survive. For water investigations, various forms of nitrogen and phosphorus are nutrients of concern. Most common are nitrogen in form of nitrates and phosphorus in form of phosphates. Dissolved inorganic forms of phosphorus includes soluble reactive phosphorus also known as orthophosphate, PO^{-3} and dissolved inorganic nitrogen in form of nitrate ion- NO^{-}, ammonium ion-NH^{+}, nitrite ion-NO^{-} and others which include: total nitrogen-TN, total phosphorus-TP, silicates-SiO_2 and chlorophyll.

3.4.2 Nutrient Analyses

Nutrient analyses done as outlined in (U.S.EPA,1840b; U.S.EPA,1840c). Samples were pretreated and analysis done using spectrophotometric techniques. Each analysis was done in triplicate and the average value was recorded. Ammonia (NH_4-N) content was analyzed using the London phenol method/phenate method involving oxidation with sodium hypochlorite and phenol solution-4500-NH_3-F (AWWA,1995; Solorzano,1969) while nitrates (NO_3-N) and nitrites (NO_2-N) were analyzed using the cadmium–reduction method-US EPA-NERL:353.3, Methods 353.4. Total nitrogen (TN) and total phosphorous (TP) were determined on unfiltered water samples. Digestion of water samples with potassium per sulfate and the autoclaving process was carried out to convert organic nitrogen to nitrate nitrogen, Standard Methods 4500-N E while water samples oxidized using hot 5% potassium per sulfate, autoclaved then further cooled at room temperature to liberate organic phosphorus as inorganic phosphate-Standard Methods:4500-P Soluble reactive phosphorous (PO_4 – P) was analyzed using the Ascorbic acid method- 365.3, (UN EPA,1978). Silicates were analyzed using the heteropoly blue technique-IS.3025.35.1988 according to (Wetzel, 1991; APHA,1985).

3.4.3 Heavy Metal Analyses

Pre-treatment of the water samples for heavy metals analyses were carried out by adding a mixture of HNO_3: H_2O_2, in a ratio of 1:3 for microwave acid digestion-Standard Methods:3030-D. The sample was placed in a hollow solid fiber micro-extractant with a graphene oxide silica coating. The suspension raised to the 100 ml for analysis by Inductively

Coupled Plasma-Mass Spectrometry, ICP- MS technique EPA Methods (U.S.EPA,1840C). Mercury analysis was done using Cold Vapor Atomic Fluorescence Spectroscopy, CV-AFS, EPA Methods, 245.1 (IDLSDROC-200-4) as outlined in (US EPA, 1994).

The sediment samples for heavy metals analyses were dried at room temperature, oven dried at 30°C-110°C or freeze dried, ground, and sieved using a 2 mm sieve. 2.00 g obtained from the dried sample was placed in a 100 ml beaker, and 15 ml HNO_3 was added and heated at 130 ºC till boiling point for 5 hours (Forstner &Wittmann,1979). The suspension filtered, washed in 0.1M HNO_3, made up to a volume of 100 ml using distilled water and 3ml obtained for analysis using Graphite Furnace Atomic Absorption Spectrophotometer GF-AAS -EPA Methods, 200.9(ISO 4253-3,1840) for metals except for Mercury and Arsenic, analyzed by Cold Vapor Absorption technique-CV-AFS, (EPA methods, 245.1(IDLSDROC-200- 4) as outlined in (Forstner &Wittmann,1979).

3.4.4 Insect Community Composition Analyses

The three insect samples from each station were pooled and emptied into a bucket, and the content was sieved into white enamel trays through kitchen sieves. Separation of the aquatic invertebrates was done using forceps. Sorting was done to obtain rough morph- types as per the orders. Further sorting involved morphological identification, which was done by observation of external features using magnifying lenses (×10) and (×15) and a Nikon SM Z660 Zoom stereo binocular microscope (with a zoom range of ×0.8-5 with eyepiece lens of ×10 and working at a distance of 115 mm with a zoom ratio of 6.3:1). The insect larvae sample body parts were observed. The parts included the head, head capsule, thorax, abdomen, and legs for identification at the order, genus, and species levels. The sorted insects were then transferred into the vials (screw-capped vials containing 70% ethanol with inner seals or neoprene/rubber to avoid evaporation of alcohol. A well-labeled vial (including specimen identity, date of collection, name of the collector, and collection site) containing insects was stored in cool and dark cabinets. Taxonomic works followed, and identification guides were used as outlined by (Eaton & Kaufman, 2007). Non-biting midge- Chironomidae, a bioindicator, was used as a representative sample isolated across all stations for heavy metal analysis, nutritional status, and molecular analysis. Preliminary Laboratory work was done within 15 days in preparation for analytical work. The outcome was used to deduce the potential indicators of various

pollution levels– biomonitors: a bioindicator species, *Chironomus* spp. Found in nearly all stations were identified and defined for further analysis for heavy metal analyses, nutritional profiling, and phylogenetic analyses.

3.4.5 Molecular Diversity Analyses of Chironomids

Genomic DNA was extracted from insect isolates using the Deasy Blood and Tissue Kit QiagenTMkit according to the manufacturer's specifications. The concentration and purity of DNA was estimated using a NanodropTM Lite Spectrophotometer (Thermo Scientific Inc., USA) at 260-280 nm and by horizontal gel electrophoresis (Thistle Scientific Ltd, USA) on a 0.8% (w/v) agarose gel at 100 V for 30 min and visualized under UV after staining with GelRedTM (Thermo Scientific, USA).

The PCR primers targeting the cytochrome c oxidase gene and the reaction schedules were designed using Bio Edit software. Bioneer AccuPower®PCR Premix (Bioneer Inc., USA) performed PCR. To each 20 µl Bioneer tube, 1 µl of 50 ng template DNA, 0.5 µl of 10 pico-moles of each primer, and 18µl of nuclease-free water were added and mixed. Amplification was performed in a programmable Master cycler thermocycler (C1000-BioRad, USA) for the PCR conditions. PCR products were separated by horizontal gel electrophoresis on 1.5 % (w/v) agarose gel at 100 V for 45 mins and visualized under UV after staining with 2 µl GelRedTM (Thermo Scientific).

PCR amplicons were purified using the Thermo Scientific® Gene JET Purification Kit (EU Lithuania). A ratio of 1:1 volume of binding buffer was added to the completed PCR mixture and vortexed to mix correctly. If the color of the mixture remained orange or- violet, 10 µl of 3M Sodium acetate (pH 5.2) was added to alter the color to yellow. Eight hundred microliters of the solution were transferred to the Gene-JET purification column and centrifuged at 10,000rpm for 30 sec.; the flow-through was discarded. Seven hundred microliters of the buffer (diluted with ethanol) were added and centrifuged at 10,000 rpm on a rotary for 30 sec. The flow-through was discarded. Additional centrifugation is done to remove any residual buffer altogether. The purification column was transferred to clean 1.5 ml micro-centrifuge tubes, and 50 µl of elution buffer was added, followed by centrifugation at 10,000 rpm for 1 min to obtain pure DNA amplicons.

Further molecular analysis for the samples was done by sequencing purified PCR products at the Segovia Sequencing Unit by Sanger capillary sequencing on an ABI 3730xl DNA Analyzer (Applied Biosystems). Forward and reverse sequences were assembled on the CLC Main Workbench (CLC Bio, Version 6.8.3) in preparation for analyses. A consensus sequence was obtained for statistical analysis using MEGA version 11 (Tamura et al., 2021). This was followed by a BLAST search using the consensus sequence obtained.

3.4.6 Nutritional Analyses

Nutritional profiling of Chironomus Spp. larvae samples was done as outlined in the Official Methods of Analysis of the Association of Official Analytical Chemists, (AOAC).

3.4.6.1 Pre-treatment

The insect samples were treated by thoroughly washing with three exchanges of distilled water, rinsed with distilled water, sun-dried, mechanically ground by crushing into powder as dry matter-DM, vacuum packed, labeled, and stored at -4ºC awaiting analysis of nutritive components (amino acids, fatty acids, vitamins; micronutrients and micro- elements) was performed using analytical techniques. About 0.2 g of dried sample at about 103°C, weighed, mineralized and homogenized into platinum crucible. The sample was then placed into a cold muffle furnace for dry ashing, and progressively heated to 300^{o}C-450°C, temperature maintained for 5hours, followed by cooling. Acid digestion of the ash to completely dissolve followed in a closed device using temperature control microwave heating for the metal determination by spectroscopic methods. This was done by dissolving the ash in 1 ml of conc. HN03: 20 ml of water, followed by gentle heating to boil. The solution was transferred to calibrated flask, and made up to 2.0ml volume for measurements with distilled water to moisten walls of the crucible. About 3.0 ml of HNO_3 and 2.0 ml of HF added, and the sample evaporated slowly to dryness. Repeat twice to dissolve the residue.

3.4.6.2 Analyses of Amino Acids

Amino acids analyses were performed as prescribed in AOAC standard methods (AOAC, 2018). The sample was ground to an excellent powder and passed through a 0.25mm sieve (particle sizes 250µm). A three-step approach was employed to hydrolyze amino acids - performic acid oxidation (pre-oxidation), acid hydrolysis, and alkaline hydrolysis. Hydrolysis of arginine, valine, lysine, leucine, threonine, phenylalanine, isoleucine, and histidine involved

weighing 0.1 mg of Chironomus spp. Powder into a labeled digestion tube, add 50 ml 6M HCl-phenol solution, and stir. Upto 3 pieces of boiling chips were added to the solution. Hydrolysis was performed under reflux for 24 hours at 110oC-120oC using a heating block or water bath equilibrated temperature followed by cooling .20ml norleucine of internal standard was measured using a volumetric pipet and placed into each test tube. The hydrolysate was filtered by a sintered glass filter into a labeled 100ml round-bottomed, connected to a rotary evaporator, and evaporated to dryness at 60°C. 20ml H20 was added for washing, and evaporation was repeated after cooling. 50ml solution of sodium citrate buffer was added, hydrolysate, mixed, and transferred to a labeled 50ml polyethylene bottle.

Methionine, a growth-limiting amino acid, was hydrolyzed using performic acid hydrolysis and converted to methionine sulfone. The process involved adding magnetic stirring into each labeled tube containing 0.1mg of powder in digestion tubes placed in an ice bath (°C), cooled, and performic acid added, standing for 15 minutes. 5ml of formic acid reagent was added to each stoppered digestion tube and stirred for 15 minutes. The digestion tubes returned to an ice bath and allowed to oxidize for 16 hours. 0.84g sodium metabisulfite was added to decompose the performic acid and stirred for about 4-15 min to liberate SO2.

Tryptophan analyses were performed by alkaline hydrolysis, which involved the addition of 4.2M NaOH to hydrolyze the proteins, followed by U.V. detection at 280nm as outlined in AOAC methods 988.15 tryptophan (AOAC, 1988). There was no derivatization. The process involved the centrifugation of finely ground samples into amill filled with a 1mm screen and thoroughly mixed. 100mg of the sample was placed into a modified micro-Kjeldahl flask. N2 bubbled into 4.2M NaOH for de-aeration and added to the flask—three drops of 1-octanol, and the solution was treated in dry ice ethyl-alcohol bath followed by evacuation. The flask was sealed and placed in a beaker for 20 hours at a temperature of m to allow melting. The solution oven was dried at 110oC for 20 hours and then cooled to room temperature. The neck of the flask was rinsed with sodium citrate buffer at pH 4.25, and the rinse was collected in a container.

The hydrolysate was transferred quantitatively into the same 50ml twice. Neutralize the solution by adding 3.5 ml of HCL and stirring. Adjust P.H. to 4.25. Quantitatively transfer the solution to a 25mI volumetric flask and make the volume 20ml. Centrifuge the solution in a 40ml for 20min at 1150Q. Filter the supernatant through glass filter paper (Whatman GF/A). Centrifuge

the filtrate for 10min at 23000Q. Take the aliquot of the supernatant to U.V. (289nm) analysis without derivatization. This is followed by separating and quantifying free amino acids before or after protein hydrolysis by chromatography/high-performance liquid chromatography using a UV light source.

3.4.6.3 Analyses of fatty acids

Analysis of Fatty Acids was done as described in AOAC standard methods-996.01 AOAC, 2000). Sample preparation was done by Liquid-Liquid extraction, LLE using different solvents while derivatization process of fatty acids performed using acid and base catalysts. Fatty acids were converted to fatty acids methyl esters (FAMEs), then free fatty acids Identified, quantified and detected.

Extraction of Fatty acids was done using behrotest ® Serial extraction device for hot extraction in accordance to Twissel Mann hot method. The extracts were cleaned with the DEX Tech™ Plus (Leach GmbH) system over three columns (multilayer acid silica, alumina and carbon). Methyl esters prepared according to AOAC ,969.33 AOAC, 1997) for separation. A gas chromatography and mass spectrophotometric device (Thermo Scientific TSQ 7600 triple quadrupole GC-MS/MS) equipped with an AEI source and coupled with a Thermo Scientific™ TRACE™ 1310gas chromatograph was used. Liquidity injections of the sample extracts were performed using a Thermo Scientific™ Tri Plus™ RSH auto sampler, and chromatographic separation achieved using a Trace-GOLD TG-PBDE 15 m × 0.25 mmI.D. × 0.10 μm film capillary column (P/N 24507-0336). Data processing was done by acquisition of timed-SRM mode, processed and reported using Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software, version 7.2, which allows instrument control, method development, quantitative/qualitative analysis, and customizable reporting all within one platform. Quantification was done using a flame detector-FID. NLEA regulations used profile to separate cis and trans fatty acids and quantification of omega 3 and omega 6.

3.4.6.4 Analyses of Vitamins

Analyses of Vitamins using organic solvents. Hot saponification for Vitamin A, Vitamin Dand Vitamin E was done under alkaline conditions at ambient elevated temperature with antioxidant in inert atmosphere. Vitamin K, which rapidly degrades in alkaline conditions and high

temperature was performed by cold saponification after an overnight stay. Determination of – fat soluble Vitamins determined as outlined in AOAC Standard Methods 992: Vitamin A(retinol)-AOAC,992.06, Vitamin D-AOAC,992.26, Vitamin E-AOAC,992.03, and Vitamin K-AOAC,992.27.

The water soluble vitamins analyzed included vitamins C and Vitamins in B complex: Vitamin B1-thiamine, Vitamin B2-Riboflavin,Vitamin B3-Niacin,Vitamin B5-Pantothenic acid, Vitamin B6-pyridoxine,Vitamin B7-biotin,Vitamin B9-folic acid, Vitamin 12- cobalamins .Water soluble vitamins determined as described in AOAC Standard Methods, Vitamin-B1-AOAC,986.27,B2-AOAC,960.46,B3-AOAC,985.34,B5 AOAC,992.07, B6-AOAC,985.32,B7-AOAC, EN 15607:2009, B9-AOA,992.05, B12- AOAC,960.46 and Vitamin C-AOAC,985.33. Chironomus Spp. larvae samples were by mixing with acetonitrile, type I water (70:30V/V and stirred in a shaker for 2hours.10ml o f H_2O added into sonicator bath for 30 minutes, increasing the spiking solution (ISTD) to 500 ppb. The solution centrifuged for 15 minutes at 4000 rpm, 1 ml of the light and clear supernatant pipetted into a 1.5 m l - amber auto sampler bottle for vitamin analyses**.**

Vitamins were analyzed using HPLC technique carried out in an Ultimate 3000 HPLC System, Dionex, Germany equipped with an auto-sampler. The injection volume was 20 µL and the detector used was an Ultimate 3000 Variable Wavelength detector at 210 nm (UV range). The column used was Acclaim TM C30 (P/N 060325). The mobile phase was: Ammonium format, pH 4.0; Ammonium format, pH 3.0;90% Acetonitrile/10% NH4 OOCH pH 3.0 Buffer at 10mM in each component. An isocratic running used with a constant flow rate of 0.6mL min^{-1} at a temperature of 15°C

3.4.6.5 Analysis of Mineral Composition

Mineral analyses were performed to assess and document the bioavailability of contaminants in Chironomus spp. larvae which include: trace metals including Ca, Fe, Mg, Zn, Co, and Al and other inorganic elements which may be detrimental to health-heavy metals (Ar, Hg, Cd, Pb). Sample preparation followed microwave digestion methods outlined in AOAC Standard methods. This involved adding 25ml of 20% H_2SO_4 to 0.25g of homogenized sample placed in a clean silica dish, and mixed. The suspension heat in a steam bath /oven dried at 110°C, and then transferred to a furnace set at 250°C- 500°C for 6-8hours

till a white ash is formed, cooled. The dish washed with water and 2ml HNO_3 and dried on a hot plate. The dish then taken back to furnace at 500°C for 30min for ashing and 1ml HNO_3 till white ash is formed, followed by cooling. 1ml HNO_3 and 10ml H_2O added and content heated on a hot plate till the ash is dissolved. Then, content transferred into a 50ml volumetric flask, 10ml HCI and 10ml H_2O added and heated. Determination of Ca, Fe, Mg, Na, Zn, Cu, Mn, Al and Co was done as outlined in AOAC standard methods, SMPR ® 2014.04-ICP-OES(AOAC,2014). Preserved whole insect samples for heavy metals (Ar, Hg, Cd, Pb) were oven dried, ground followed by microwave digestion and analyzed by AOAC Official methods -2015.01(AOAC,2015) Inductively coupled plasma-mass spectrometry-ICP-MS for metals.

3.5 Data Analyses

3.5.1 Spatial Variation in Water Quality Parameters

Physical and chemical parameters, nutrients and chlorophyll, and heavy metals were analysed in Winam Gulf and documented. The results were expressed as mean±SD.ANOVA at 95% confidence level was used to establish spatial variations amongst the sampling stations. A Tukey's post hoc test employed to separate means. Past statistical tool software -version 4.03 used for analyses.

3.5.2 Pollution on Genetic Diversity of Aquatic Insect Species

The composition of aquatic insects was analyzed independently based on morphological approach and molecular approaches and expressed as a percentage (%). The species richness and relative abundance of the insect taxa were evaluated. The Simpson's index (D), (Simpson, 1949), Simpson diversity index (1-D), and Shannon –Weiner diversity indices (H) were calculated, following, Pielou's evenness index (J) and Shannon Equitability index (E) were determined.

The existing relationships amongst the insect communities (species vs species) were determined using Pearsons correlation coefficient(r). Principal Conical correspondence, CCA was also used to elucidate the relationships between insect abundance and the water quality parameters, (Legendre & Legendre, 1998).

The nonbiting midge, Chironomids a species representative sample was identified amongst the

stations for genetic analysis. Molecular analysis, assembled sequences were transferred to MEGA Version 11 software, and pairwise sequence alignment of the nucleotides using CLUSTAL W according to Tamura et al., (2011). Sequences were submitted to the NCBI BLAST po2tal for a sequence similarity search, and sequences with greater than 97% similarity were retrieved for phylogenetic analysis. Evolutionary histories were inferred using the Neighbor-Joining method and distances were computed using the Maximum Composite Likelihood (Tamura & Kumar, 2004). Bootstrap tests (1000 replicates) were used to cluster associated taxa and replicate trees with above 50% likelihoods indicated on the branches. The effects of physical and chemical parameters and metallic components on aquatic insect taxa were established using cluster analysis.

3.5.2.1 Diversity Indices

The current research calculated both Simpson's index and Shannon-Weiner diversity index for analyses of diversity indices as outlined in (Simpson,1949; Shannon & Weaver, 1949). A diversity index, defined as quantitative measure of the number of different types of species that were present in Winam gulf community representing different aspects which included richness, evenness, and dominance of the genera per sampling station. Abundance was expressed as the number of individuals per sampling station. Species Richness noted as 'S 'was employed to quantify how many species made the aquatic insects' community in the gulf and corresponding species list. Simpson's Index of diversity was calculated according to Simpson, 1949 as follows:

D=∑I =1S (ni N)2(22.5.1) (22.5.1) D=∑i=1S (ni N)2

where n_i is the number of individuals in species *i,*

N = total number of individuals of all species, and

$n_i/N = p_i$ (proportion of individuals of species i), and

S = species richness.

Simpson's *D* ranges from 0 to 1, with 0 representing infinite diversity and 1 representing no diversity, so the larger the value of *D*, the lower the diversity.

Simpson's index is a complement of (1-*D*).

Simpson's Dominance Index, the inverse of the Simpson's Index (1/*D*).

Shannon-Weiner Diversity Index, also known as the Shannon's diversity index, denoted as H and put into consideration the species richness ad evenness, calculated as follows:

H=−∑i=1Spi∗lnpi (22.5.2) (22.5.2) H=−∑i=1Spi∗ln⁡pi

where p_i = proportion of individuals of species *i*, and

ln is the natural logarithm, and

S = species richness.

H ranges from 0 to $H_{max.}$ H_{max} is different for each community and depends on species richness.

Evenness Index represented by Pielou's evenness index (Pielou, 1966) refers to how close in numbers each species in an environment is and is calculated as:

J=H H_{max} (22.5.3) (22.5.3) J=H H_{max}

J ranges from 0 to 1. Higher values indicate higher levels of evenness.

At maximum evenness, *J* = 1.

Species dominance - *J* and *D* was used as a measures of species dominance (the opposite of diversity) in a community. Low J indicates that 1 or few species dominate the community.

3.5.2.1.1 Simpson's Diversity Index (D)

Simpson's Diversity Index (D) is a measure of diversity often used to quantify the biodiversity of a habitat. It considers the number of species present, as well as the abundance of each species (Simpson, 1949). Simpson's Diversity Index (D) is also defined as a measure of the relationship between the number of different species in a habitat (species richness) and the number of individuals within each species (species evenness). A highly biodiverse and stable environment will have a high D value.

Simpson's Diversity Index can be calculated using the following formula:

$$D = \frac{N(N-1)}{\sum n(n-1)}$$

D = Simpson's Diversity Index

Σ = sum of

N = total number of organisms of all species

N = total number of organisms of each species

A highly biodiverse and stable environment had a high *D* value. This also indicated that the particular environment has "good biological health". In contrast, an unstable and non-biodiverse environment had a low *D* value. This also indicated that the particular environment has "poor biological health". Simpson index, has inability to provide unbiased estimators for poorly sampled communities Augoust et al., 2021), complexity in evaluating true diversity in populations with varying species richness and equitability (Lou et al., 2012), and the challenges in comparing diversities between groups due to undesirable properties of raw indices like Simpson's index (Pallmann et al., 2012) .

3.5.2.1.2 Shannon-Wiener index

Shannon-Wiener index of diversity (information index) is used by ecologists when a system contains too many individuals for each to be identified and examined. A small sample is used; the index (*D*) is the ratio of the number of species to their importance values (e.g. biomass or productivity) within a trophic level or community. $D = -\Sigma^{s}_{i}\ p_i\ \log\ p_i$, where *s* is the total number of species in the sample, *i* is the total number of individuals in one species, p_i (a decimal fraction) is the number of individuals of one species in relation to the number of individuals in the population, and the log is to base-2 or base-*e*. Shannon's index accounts for both abundance and evenness of the species present. In excel, Shannon diversity index calculated using the equation H = -SUM (Pi * ln (Pi)) while in Microsoft Excel is calculated using the equation =-SUM (FIRST_CELL_LOCATION: LAST_CELL_LOCATION).

However, Shannon cannot be used to compare diversity distributions that have different levels

of scale. Second, it cannot be used to compare parts of diversity distributions to the whole.

3.5.3 Effects of Pollution on Nutritional Status of Aquatic Edible Insects

Analyses of the nutritive components in the selected insect species, Chironomids (the nonbiting midge) amongst the sampling stations was done, and results expressed as means (± SE). The results were displayed in tables and figures. ANOVA at 95% confidence level was done to establish spatial variations. Tukey's Pairwise test employed to separate the means. Pearsons's correlation coefficient (r) was employed to establish any existing associations between water quality parameters and the nutritive components-amino acids, fatty acids, vitamins, macronutrients, micronutrients in Chironomids.

The effect of pollution on nutritive components was determined using Pearson's correlation coefficient to determine any existing relationship between nutritive components (amino acids, fatty acids, vitamins and macro-micronutrients) and the water quality parameters (in-situ parameters, nutrients and chlorophyll, heavy metals in water and heavy metals in sediment). Then a graphical presentation of the results done. Loading plots were used to represent the correlations and nutritive components followed by Principal component analysis, PCA (Legendre & Legendre, 1998).

3.5.3 .1. Pearson's correlation coefficient

The Pearson correlation coefficient is a descriptive statistic, which summarizes the characteristics of a dataset. Specifically, it describes the strength and direction of the linear relationship between two quantitative variables. Pearson correlation coefficient used when the relationship is linear and when both variables are lastly when normally distributed. The use cases of the Correlation Coefficient are common in the environmental area and have proved to be highly effective in showing the correlation between environmental variables (Nilo et al., 2024). The Pearson correlation coefficient (r) is the most common way of measuring a linear correlation. It is a number between –1 and 1 that measures the strength and direction of the relationship between two variables. When one variable changes, the other variable changes in the same direction.

3.5.3.2. Analysis of Variance

Analysis of Variance (ANOVA) is a statistical formula used to compare variances across the

means (or average) of different groups. A range of scenarios use it to determine if there is any difference between the means of different groups. ANOVA, is also used to determine differences between research results from three or more unrelated samples or groups. ANOVA tests can be used to study the statistical significance of various environments on test scores. Most frequently ANOVA techniques used in the biological and environmental sciences to contrast a continuous dependent variable y across levels of one or more categorical independent variables x. In the present study, ANOVA was used to determine the spatial differences in variables across stations

CHAPTER FOUR
RESULTS

4.1 Spatial Variations in Water Quality Parameters

4.1.1 Physical and Chemical Characteristics

The physical and chemical characteristics of water in Winam Gulf, Lake Victoria, across six sampling stations, were analyzed and recorded in (Table 2). The air temperature ranges from around 22.1°C at Ndere Is. to 27.8°C at Kendu Bay, while Water temperatures are slightly higher and range from around 23.27°C at Maboko Is. to 26.1°C at Kendu Bay. The pH levels range from 6.34 at Maboko Is. to 8.17 at Homa Bay, indicating slightly acidic to slightly alkaline conditions across the sampling sites. Electrical conductivity ranges from 126.97 µS cm^{-1} at Fish L.B. to 201.97 µS cm^{-1} at Kendu Bay. Dissolved oxygen levels vary from 5.72 mgL^{-1} at Kendu Bay to 8.62 mgL^{-1} at Maboko Island, showing variation amongst the sampling stations, indicating variation in availability of oxygen while Oxidation-reduction potential (ORP) ranging between 211.43 mV at Homa Bay to 246.35 mV at Fish L.B. Significant variation was observed in total hardness, ranging between 126 mgL^{-1} at Ndere Is. to 379.5 mg^{-1} at Kisumu Bay. However, total alkalinity values ranging between 45.5 mg^{-1} at Fish L.B. to 167.05 mgL^{-1} at Kisumu varied significantly; in comparison, salinity values remained consistent at 0.08 mgL^{-1} across most sites, with a slight variation to 0.06 mgL^{-1} at Fish L.B.TDS levels also varied between 83.92 mg^{-1} at Fish L.B. to 117.43 mgL^{-1} at Kisumu Bay. In general, the results indicated that there were no statistically significant differences between the six study sites for all the measured parameters (Air Temp, Water Temp, pH, E.C., D.O., ORP, Hardness, Alk, Salinity, TDS) as all the p-values were more significant than 0.05 suggesting that the water quality parameters across these six sites in Winam Gulf, Lake Victoria, were relatively uniform, therefore no significant spatial variation.

Table 2. Spatial variation in physical and chemical parameters of water samples from six study sites within Winam Gulf, Lake Victoria.

Sampling Stations	*Air Temp (ºC)*	*Water Temp (ºC)*	*PH*	*E.C. (u Scm^{-1})*	*D.O (mgL^{-1})*	*ORP*	*Hard (mgL^{-1})*	*Alk (mgL^{-1})*	*Salinity (mgL^{-1})*	*TDS (mgL^{-1})*
Maboko Is.	23.47±0.20	23.47±0.20	6.34±1.81	175.1±2.3	8.62±0.97	242.25±8.03	268.00±16.00	57.20±16.89	0.08±0.00	108.55±0.65
Kisumu	23.70±0.35	27.9±0.62	7.46±0.31	183.97±8.48	6.75±1.17	218.88±6.16	379.50±40.84	167.05±18.75	0.08±0.01	117.43±3.00
Fish LB	24.07±1.53	26.07±0.41	7.74±0.08	126.97±1.15	7.02±0.80	246.35±13.44	155.00±15.10	45.50±4.43	0.06±0.00	83.92±2.90
Ndere Is.	22.13±0.38	25.73±0.43	7.90±0.16	169.43±1.70	6.65±1.09	227.10±0.00	126.00±0.00	60.00±0.00	0.08±0.00	108.25±0.00
Kendu bay	27.77±3.40	26.13±2.33	7.58±0.03	201.97±34.57	5.72±0.06	236.90±7.62	181.33±55.58	58.67±1.15	0.08±0.00	107.25±1.72
Homa bay	25.23±1.13	27.27±0.81	8.17±0.06	155.90±34.57	7.13±0.26	211.43±12.36	220.67±52.62	54.67±4.62	0.08±0.00	112.02±2.63
F. statistics	0.486589	0.770872	1.21521	1.21521	0.056408	0.323605	0.230431	N1.466525	0.072464	NS0.017812
p-value	0.624093	0.490891	0.324247	0.324247	0.945353	0.728463	0.796951	0.261986	0.930423	0.982366
Significance	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

NOTE: E.C – Electrical conductivity, D.O – Dissolved oxygen, ORP –Oxygen Reduction Potential, Alk – Alkalinity, TDS –Total Dissolved Solids

4.1.2. Nutrients and Chlorophyll

Analyses of the water samples from Winam Gulf revealed significant spatial variations in nutrient concentrations and influenced the growth of aquatic plants and the health of aquatic insects (Table 3). For instance, NO_3^- (Nitrates, µg L-1) concentrations ranged between 5.54 µg L-1at Fish LB to 32.21 µg L-1at Kendu Bay, with a p-value = 0.0127, < 0.05, indicating a significant difference in concentration among the study sites. This suggested that different sampling sites experienced varying nitrate inputs, potentially from agricultural runoff, sewage, or other anthropogenic sources. Highly significant variations($p=0.0027$) in NO_2^- (Nitrites, µg L-1) concentrations were in a range of 1.67 µg L-1 (Fish LB) to 11.13 µg L-1 (Kendu Bay), indicating differences in concentrations among the study sites. Nitrites are often present in water bodies as an intermediate in the nitrogen cycle and can indicate recent pollution or microbial activity. Therefore, high concentrations at specific sites could suggest localized pollution events or ineffective nitrogen removal processes.TN (Total Nitrogen, µg L-1), on the other hand, was in a range of 29.95 µg L-1(Fish LB) to 122.75 µg L-1 (Kisumu Bay) with $p>0.05$, showing insignificant differences in concentrations among the sampling stations. This suggests that, despite some variability, TN levels were relatively uniform across stations, potentially due to widespread sources or consistent nitrogen inputs. Highly significant differences($p=0.0018$) were also observed in concentrations of SRP (Soluble Reactive Phosphorus, µg L-1) ranging between 22.95 µg L-1(Fish LB) and 102.00 µg L-1 (Kisumu Bay) among the sampling stations. SRP, a readily available form of phosphorus, increases algal growth. High levels at specific sites, like Kisumu, suggested the presence of potential sources of phosphorus pollution, such as agricultural runoff or sewage discharge. Contrary, the TP (Total Phosphorus, µg L-1) concentrations ranging between 43.52 µg L-1 (Fish LB) and 436.86 µg L-1 (Kisumu Bay), at p-value= 0.1445, were statistically insignificant amongst the study sites. This could have suggested that phosphorus inputs were widespread or consistently distributed across the sites. NH_4^+ (Ammonium ions, µg L-1) concentrations were in a range of 17.51 µg L-1 (Fish LB) to 126.23 µg L-1 (Homa Bay) with significant differences (at $p<0.05$) amongst the sampling stations. Ammonium is a form of nitrogen readily available for plant uptake but can be toxic at high concentrations. High levels at Homa Bay could suggest localized pollution sources or natural processes contributing to ammonium enrichment. The findings also revealed highly

significant variations in SiO_2 (Silica, mgL-1) concentration in a range of 3.60 mgL-1 (Fish LB) to 26.08 mgL-1 (Maboko), at p-value <0.001, among the study sites. Silica is an essential nutrient for diatom growth, and its variability can affect primary productivity. Differences in silica levels could have suggested variable inputs or consumption rates across the sites. CHLO (Chlorophyll, mgm-^{3}) concentrations, however, showed insignificant differences in a concentration ranging between 237.24 mgm-^{3} (Kisumu Bay) and 1021.8 mgm-^{3} (Kendu Bay) at a p-value >0.05. The lack of differences could have been attributed to relatively consistent algal growth across the sites despite some variability in nutrient concentrations: chlorophyll, an indicator of algal biomass and primary productivity. In general, Nitrate (NO_3^-) ammonium (NH_4^+) concentrations show significant differences among sites, indicating localized variations in nitrogen inputs and dynamics. In contrast, Nitrite (NO_2^-), soluble reactive phosphorus (SRP), and silica (SiO_2) concentrations exhibited highly significant differences, suggesting substantial spatial variability in these nutrients' sources or processing rates. Total nitrogen (TN), total phosphorus (TP), and chlorophyll (CHLO) show no significant differences across sites, indicating relatively uniform levels of these parameters throughout the study area.

Table 3. Spatial variation in nutrient concentrations of water samples from six study sites within Winam Gulf, Lake Victoria.

Sampling Stations	**NO_3 (μgL^{-1})**	**NO_2 (μgL^{-1})**	**TN ($\mu g\bar{L}^{1}$)**	**SRP (μgL^{-1})**	**TP ($\mu g\bar{L}^{1}$)**	**NH_4 (μgL^{-1})**	**SiO_2 (mgL^{-1})**	**CHLO (mgm^{-3})**
Maboko Is	13.45±0.09d	8.96±0.18b	39.16±4.61	56.29±6.23c	97.81±7.39	28.28±4.89d	26.08±0.55a	244.47±20.58
Kisumu	24.00±8.15b	9.95±2.12ab	122.75±43.46	102.00±16.10a	436.86±204.11	84.18±36.58b	25.87±0.42a	237.24±57.62
Fish LB	5.54±1.62e	1.67±1.13c	29.95±2.84	22.95±6.67d	43.52±6.67	17.51±5.80f	3.60±1.43d	353.52±141.70
Ndere Is.	22.66±0.71b	10.77±1.18a	50.56±19.01	74.38±8.26b	180.67±82.46	60.33±4.38c	24.18±0.44ab	492.44±91.16
Kendu bay	32.21±2.39a	11.13±0.65a	46.96±0.86	58.67±4.54c	110.67±16.67	31.62±9.11e	20.44±0.49c	1021.8±739.15
Homa bay	19.41±5.43bc	9.78±1.74ab	67.67±28.60	98.19±15.61a	256.38±104.54	126.23±29.34a	19.86±0.19c	4294.05±3289.88
F. statistics	4.741	7.040	2.1639943	7.74777326	2.0406757	4.3574634	144.52418	1.32720.4
p-value	0.0127132	0.0027409	0.1268606	0.0018277	0.1445171	0.0170981	<0.001	0.3170887
Significance	S	HS	NS	HS	NS	S	HS	NS

NOTE: Means followed by the same letter along a column are not significant at $p>0.05$. NO_3 – Nitrates, NO_2 – Nitrites, TN – Total nitrogen, SRP –Soluble Reactive Phosphorous, T. P. – Total Phosphorous, NH_4 – Ammonium ions, SiO_2-, CHLO - Chlorophyll

4.1.3 Heavy Metal Concentrations in Water, Sediment, and Insect Samples

Analyses of heavy metals in water samples, in sediment samples and insect samples was done and documented as in Table 4a,4bi,4bii,4biii).

4.1.3.1 Arsenic (As)

The concentration of Ar in Insect Samples ranged between 0.018 mgL-1(Kendu Bay) and 1.33 mgL-1 (Kisumu Bay) at a p-value< 0.001, indicating a highly significant difference (HS) across sampling stations. The concentration of Insect samples from Kisumu Bay was notably higher than that of other stations, which may have been associated with local sources of arsenic contamination.

While the concentration of Ar in Sediment Samples was observed to vary differently, at $p<0.05$, amongst the sampling stations, the concentrations exceeded the KEBs/WHO standards (0.02). They ranged between 0.011 mgL^{-1}(Kendu Bay) and 2.37 mgL^{-1} (Kisumu Bay). Kisumu recorded the highest concentrations in sediments. This could have suggested the accumulation of arsenic in the sediment, which could lead to biomagnification in benthic organisms.

However, the concentration of Ar in water Samples indicated insignificant variations (NS) amongst sampling stations ranging between 0.013 mgL-1 (Kendu Bay) and 1.62 mgL-1 (Kisumu Bay) concentrations. Arsenic levels in the water were generally below the KEBS standard of 0.02 mg/L except for Kisumu Bay and Fish LB, suggesting localized contamination.

4.1.3.2 Mercury (Hg)

The concentration of Hg depicted highly significant differences ($p<0.001$, HS) in Insect Samples, which were in the range of 0.003 mgL^{-1} (Kendu Bay) and 0.087 mgL-1 (Ndere Island) in concentrations across sampling stations. Ndere Island recorded the highest mercury concentration in insect samples, which could pose a risk of mercury exposure to higher trophic levels. However, the concentration of Hg in water Samples in a range of 0.001 mgL^{-1} (Maboko Island) and 0.006 mgL^{-1} (Homa Bay) revealed no significant difference (NS) among the sites. Mercury levels in water are generally below the KEBS standard of 0.05 mg/L, indicating low direct contamination in water. In addition, the concentration of Hg in Sediment Samples varied significantly across the sampling stations and was in a range of 0.001 mgL^{-1} (Kendu Bay) and 0.005 mgL-1 (Kisumu Bay) at a p-value <0.05. Higher concentrations were recorded in Kisumu Bay, suggesting the potential for mercury accumulation and its effects on benthic fauna.

4.1.3.3 Lead (Pb)

The concentration of Pb in Insect Samples was in a range of 0.087 mgL^{-1} (Ndere Island) and 4.38 mgL^{-1} (Kisumu Bay), at p>.05, indicating insignificant differences(NS) across sites in insect samples. However, concentrations at Kisumu Bay are notably higher than the KEBS standard of 0.01 mgL^{-1}, suggesting significant contamination. Contrarily, the concentration of Pb in Water Samples revealed substantial variations, ranging from 0.192 mg/L (Maboko Island) and 1.877 mgL-1 (Homa Bay) at p<0.05 among the sampling stations. Elevated lead levels, especially at Homa Bay and Kisumu Bay, suggest localized pollution, possibly from industrial or urban runoff. The concentration of Pb in Sediment Samples ranges from 0.137 mgL-1 at Ndere Island to 2.177 mgL-1 at Homa Bay at a p-value of less than 0.001, indicating a highly significant difference (HS) in lead concentrations across sites. High lead levels in sediments suggest potential long-term contamination and risk to benthic organisms and food webs.

4.1.3.4 Cadmium (Cd)

The concentration of Cd in Insect Samples and water samples was statistically insignificant amongst the sampling stations, ranging between 0.314 mgL-1(Kendu Bay) and 1.124 mgL^{-1} (Kisumu Bay) and from 0.010 mgL-1(Maboko Island) and 0.730 mgL-1 (Kisumu Bay), at a p>0.05 value of 0.9998, respectively. Cadmium levels in some sites exceeded the KEBS standard of 0.01 mgL^{-1}, posing a potential risk to aquatic life and higher trophic levels. Elevated levels at Kisumu Bay could suggest localized sources of cadmium pollution. However, the concentration of Cd in Sediment Samples was elevated and varied significantly amongst the sampling sites and in a range of 0.397 mgL^{-1} (Fish LB) and 0.763 mgL-1 mgL^{-1} (Homa Bay), at a p-<0.05. The higher levels in sediments could have indicated potential for bioaccumulation in benthic organisms. In general, arsenic, mercury, and lead concentrations showed significant to highly significant differences in various sample types across sites, suggesting localized sources of pollution. Insect samples, particularly at Kisumu Bay and Homa Bay, showed high contamination levels, indicating potential risks of biomagnification. Some heavy metal concentrations (e.g., lead and cadmium in insects) showed no significant differences, suggesting that contamination levels may have been uniformly high or low across the sites. The elevated concentrations of heavy metals in some sites, especially Kisumu Bay and Homa Bay, indicated a potential risk to aquatic life and human health if the insects were being consumed as part of the food chain.

Table 4(a). Heavy Metal Concentrations in Water, Sediment, and Insect Samples expressed as mean (±SE) in milligrams/liter

	Sample type	Maboko Island	Kisumu Bay	Fish LB	Ndere Island	Kendu Bay	Homa Bay	KEBS STDS	F Stat	p-value	Signf.
Arsenic (Ar)	Insect	0.035±0.00^{c}	1.33±0.06^{a}	0.387±.03^{b}	0.350 ±0.03^{b}	0.018±0.00^{c}	0.343 ±0.01^{b}	0.02	2016.70	<0.001	HS
	Water	0.021±0.00	1.62±0.04	0.22±0.01	0.34±0.03	0.013±0.00	0.147±0.01	0.02	1.55369	0.2459	NS
	Sediment	0.031±0.00^{c}	2.37±0.06^{a}	0.307±0.02^{b}	0.58±0.03^{b}	0.011±0.00^{c}	0.253±0.03^{b}	0.02	3.78681	0.0273	S
Mercury (Hg)	Insect	0.035±0.00^{a}	0.08 ±0.01^{a}	0.007±0.00^{b}	0.087 ±0.00^{a}	0.003 ±0.00^{b}	0.006 ±0.00^{b}	0.05	543.452	<0.001	HS
	Water	0.001±0.00	0.001±0.00	0.00±0.00	0.003±0.00	0.001±0.00	0.002±0.00	0.05	1.57884	0.2391	NS
	Sediment	0.002±0.00^{b}	0.005±0.0^{a}	0.002±0.00^{b}	0.001±0.00^{b}	0.003±0.00^{b}	0.0017±0.0^{b}	0.05	4.72680	0.0128	S
Lead (Pb)	Insect	0.257 ±0.00	4.38 ±0.03	0.740±0.03	0.087 ±0.01	0.314±.020	2.253 ±0.01	0.01	0.00019	0.9998	NS
	Water	0.192±0.00^{c}	2.43±0.01^{a}	0.443±0.01^{c}	0.0867±0.01^{d}	0.254±0.00^{c}	1.877±0.01^{b}	0.01	5.70992	0.0063	S
	Sediment	0.250±0.00^{b}	2.78±0.08^{a}	0.503±0.04^{b}	0.137±0.00^{b}	0.336±0.02^{b}	2.177±0.01^{a}	0.01	896.344	<0.001	HS
Cadmium, (Cd)	Insect	0.512±0.02	1.12±0.04	0.573±0.01	0.639 ±0.01	0.497 ±0.00	0.953 ±0.01	0.01	0.00019	0.9998	NS
	Water	0.01±0.01	0.73±0.002	0.320±0.00	0.587±0.01	0.520±0.00	0.573±0.00	0.01	1.07426	0.4216	NS
	Sediment	0.507±0.02	0.701±.025	0.397±.007	0.763±0.04	0.481±0.00	0.763±0.04	0.01	3.21848	0.0450	S

NOTE: Means followed by the same letter along a row are not significant at p>0.0

Table 4(b i). Comparison of Heavy Metals in Sediment Versus the KEBS STDS

	Arsenic (Ar)	**Mercury (Hg)**	**Lead (Pb)**	**Cadmium (Cd)**
Maboko Island	Exceeds	Within	Exceeds	Exceeds
Kisumu Bay	Exceeds	Within	Exceeds	Exceeds
Fish LB	Exceeds	Within	Exceeds	Exceeds
Ndere Island	Exceeds	Within	Exceeds	Exceeds
Kendu Bay	Within	Within	Exceeds	Exceeds
Homa Bay	Exceeds	Within	Exceeds	Exceeds

Table 4(b ii). Comparison of Heavy Metals in Water Versus the KEBS STDS

	Arsenic (Ar)	Mercury (Hg)	Lead (Pd)	Cadmium (Cd)
Maboko	Exceeds	Exceeds	Exceeds	Exceeds
Kisumu	Exceeds	Exceeds	Exceeds	Exceeds
Fish	Exceeds	Within	Exceeds	Exceeds
Ndere	Exceeds	Within	Exceeds	Exceeds
Kendu	Exceeds	Exceeds	Exceeds	Exceeds
Homa	Exceeds	Exceeds	Exceeds	Exceeds

Table 4(biii). Comparison of Heavy Metals in insect samples Versus the Kebs St

Location	**Arsenic (As)**	**Mercury (Hg)**	**Lead (Pb)**	**Cadmium (Cd)**
Kisumu Bay	Exceeds	Exceeds	Exceeds	Exceeds
Homa Bay	Exceeds	Exceeds	Exceeds	Exceeds
Ndere	Exceeds	Exceeds	Exceeds	Exceeds
Kendu Bay	Exceeds	Exceeds	Exceeds	Exceeds
Maboko	Exceeds	Exceeds	Exceeds	Exceeds

4.2 Diversity of Aquatic Edible Insects

4.2.1. Number of Insect Species per Study Site

A total of 383 insect samples representing 19 species, 19 genera, 16 families, and 6 orders were obtained from the study area (Table 5, Figure 2a, Figure 2b). Out of these 17, 164, 31, and 22 samples were obtained from the fish landing beaches, Kisumu Bay, Kendu Bay, and Homabay respectively, while 44 and 48 samples were obtained from the offshore stations (Maboko and Ndere islands) respectively. All the insect species were present in Kisumu Bay. Six species *Agrionvirgo sp.*, *Sericostomatidae* sp, *Polycentropus* sp., *Pentagenia viltigera*, *Ablebesmyia* sp., and *Ambryosus mermon* were present in Ndere island while all other species were present in Maboko island except *Agrion virgo, Psepheaus* sp., *Brauchycentridae* sp., *Sericostomatidae* sp., *Caenis moesta*, *Pentagenia viltigera*, *Gillis altilis* and *Microvelia borealis.*

Table 5. Insect fauna based on the taxa-order, family and the genus within the different sampling stations in Winam gulf, Lake Victoria

Insect taxa				**Sampling Stations**						
Order	Family	Genus	Genus Code	Maboko Island	Kisumu Bay	Fish L Beaches	Ndere Island	Kendu Bay	Homa Bay	Species Richness
Hemiptera	Veliidae	*Microvelia borealis*	Mbor	--	--	01	-	-	-	01
	Nepidae	*Ramastra represented*	Ram	--	--	01	--	--	--	01
		Nepa apculata uheri	Nap	--	--	01	04	--	01	06
	Naucoridae	*Ambryosus mermon*	Amor	27	03	12	09	19	02	72
		Gillis altilis	Galt	--	--	04	-	--	-	04
	Corixidae	*Corixini sp*	Cor	05	118	20	--	--	03	**146**
	Pleidae	*Paraplea brunni*	Pbru	01	03	--	--	--	--	04
Diptera	Chironomidae	*Ablebesmyia sp*	Able	01	17	02	23	00	01	44
		Chironomus sp	Chiro	01	01	01	00	09	12	24
Ephemeroptera	Leptophlebiidae	*Habrophlebia sp*	Hab	02	01	--	--	03	--	06
	Polymitarcyidae	*Pentagenia viltigera*	Pvil	--	--	15	01	--	--	16
	Baetidae	*Baetis calorina*	Bcal	01	--	05	--	--	03	09
	Caenidae	*Caenis moesta*	Cmoe	--	--	06	--	--	--	06
Tricoptera	Phryganeidae	*Polycentropus sp*	Poly	01	--	01	01	--	--	03
	Sericostomatidae	*Sericostomatidae sp*	Seri	--	--	01	01	--	--	02
	Brauchycentridae	*Brauchycentridae sp*	Brau	--	--	01	--	--	--	01
Coleoptera	Psephenidae	*Psepheaus sp*	Psep	--	21	01	--	--	--	22
	Hydrophilidae	*Hydrophyllus sp*	Hydr	05	--	01	--	--	--	06
Odonata	Agriidae	*Agrion virgo*	Avir	--	--	01	09	--	--	10
Species richness				44	164	74	48	31	22	383
% composition				11.5	43.3	18.8	12.5	8.1	5.7	

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4.2.2. Insect Taxa Richness and Abundance

Species richness refers to the number of species in a given area. Analyzing species richness across different sites helps understand biodiversity levels and the health of aquatic ecosystems. Table 5 shows the species richness and % composition of aquatic insects across six sampling sites in Nyanza Gulf, Lake Victoria:

The current study findings revealed that Maboko Island had a Species Richness of 44% (%) and a Composition of 11.5% (Table 5, Figure 2a), with a moderate level of species richness compared to other sites. Various taxa, such as Naucoriidae (Ambrysous mermon) and Hydrophilidae (Hydrophilus sp.) indicated a relatively balanced ecosystem with multiple habitats and resources. The moderately high richness suggested the site had a stable environment with suitable water quality and minimal pollution stressors.

Kisumu Bay, with a Species Richness of 164 and % composition of 43.3%, had the highest species richness among all sites, accounting for 43.3% of the total species richness (Table 5, Figure 2a). The high diversity was associated with favorable environmental conditions such as optimal water temperature, adequate dissolved oxygen, moderate nutrient levels, and low levels of pollutants. The diversity of families and genera (e.g., Chironomidae, Corixidae) reflected a complex and healthy aquatic ecosystem.

Fish L Beaches with a Species Richness: 74% Composition: 18.8% had a relatively high species richness, accounting for 18.8% (Table 5, Figure 2a). The presence of diverse taxa like Chironomidae (Chironomus sp.) and Corixidae (Corixini sp.) suggested a stable environment with varied habitats and food sources. The site could have benefited from moderate nutrient inputs that promoted primary productivity without causing harmful eutrophication.

Ndere Island had a Species Richness of 48 expressed as a % Composition: 12.5% had a moderate species richness, contributing 12.5% to the overall composition (Table 5, Figure 2a). The diversity of insect families like Baetidae (Baetis calorina) and Caenidae (Caenis moesta) indicated a relatively stable and oxygen-rich environment. However, the lower richness compared to Kisumu Bay and Fish L Beaches suggested possible localized environmental stressors or habitat limitations.

Kendu Bay had a Species Richness of 31, with a composition of 8.1% and relatively lower species richness values, accounting for only 8.1% of the total species richness (Table 5, Figure 2a). The low diversity could have been due to several factors, including high nutrient or pollutant levels, which could lead to eutrophication, reduced dissolved oxygen, or heavy metal contamination. These conditions would favor more tolerant species and reduce overall diversity.

Homa Bay had the lowest species richness among all sites (Table 5, Figure 2a): 22 species richness expressed as a percentage composition of 5.7%. The low diversity could have indicated poor environmental conditions such as high pollutant concentrations (e.g., heavy metals like lead and cadmium), low oxygen, or extreme pH levels. These stressors can eliminate sensitive taxa and favor a few tolerant species, reducing biodiversity.

Species Richness Differences revealed that Kisumu Bay had the highest species richness (164), suggesting a diverse and healthy ecosystem with favorable environmental conditions. At the same time, Maboko Island (44), Fish L Beaches (74), and Ndere Island (48) showed moderate species richness, indicating relatively stable ecosystems but possibly with localized stressors or habitat limitations. Kendu Bay (31) and Homa Bay (22) had the lowest species richness, potentially due to environmental degradation caused by pollution, nutrient overloading, or other stressors that reduce habitat quality and favor tolerant species over sensitive ones.

The differences in species richness across the sites suggested varying levels of environmental quality and habitat suitability. Sites with higher richness (e.g., Kisumu Bay) likely had better water quality, diverse habitats, and balanced nutrient levels. In comparison, those with lower richness (e.g., Kendu Bay, Homa Bay) could have been impacted by pollution, habitat degradation, or other stressors. The analysis highlighted the importance of managing water quality and pollution levels to maintain diverse and healthy aquatic insect communities in Nyanza Gulf, Lake Victoria.

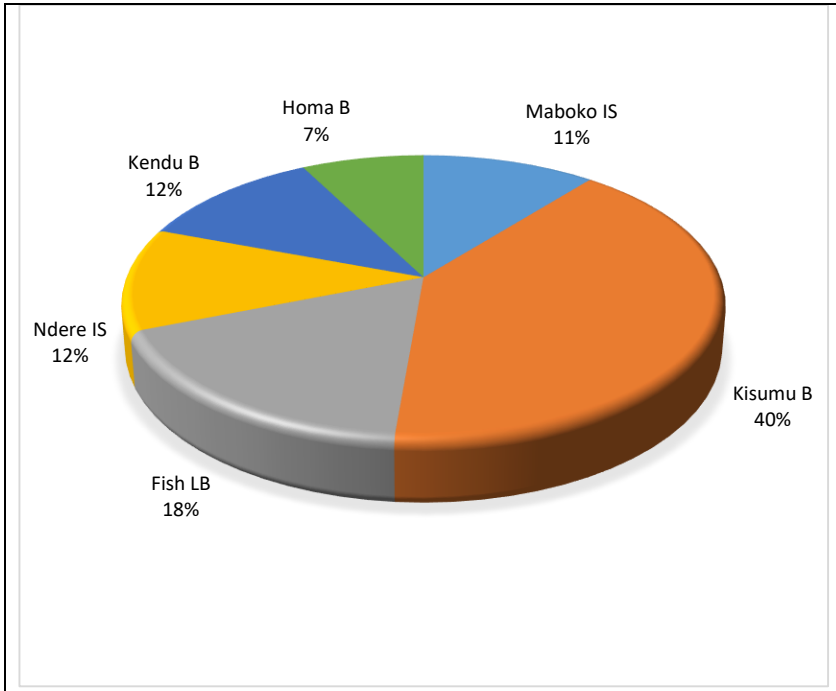

Figure 2(a). A pie-chart of aquatic insect species composition expressed as a percentage (%) sampled per study site within Winam Gulf, Lake Victoria.

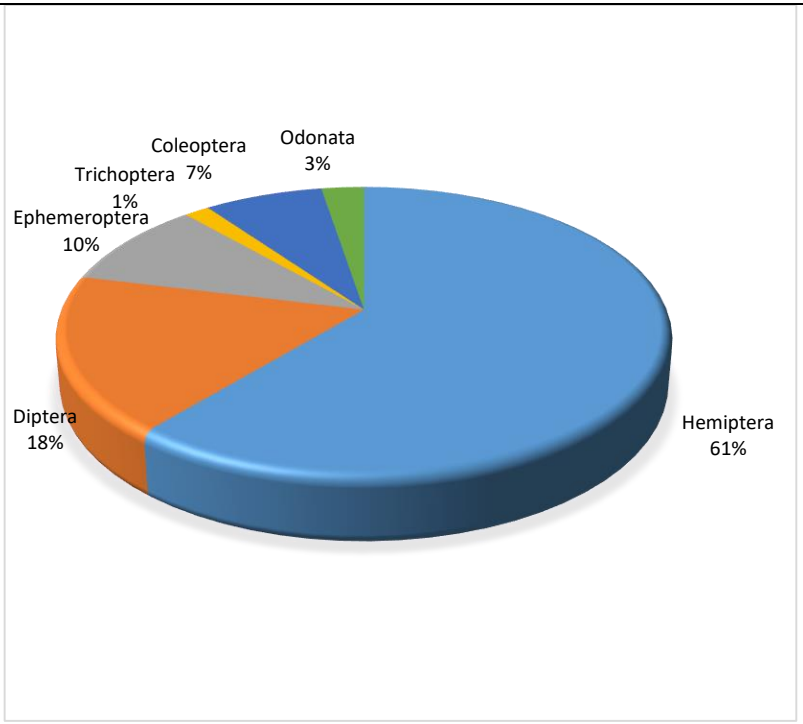

Figure 2(b). A pie chart of aquatic insect orders expressed as a percentage (%) composition sampled in Winam Gulf, Lake Victoria.

4.2.3. Diversity Indices of Aquatic Insect Species

Table 6. Diversity parameters and species richness for aquatic insects in Winam Gulf, Lake Victoria

Sampling station	*Taxa_S*	*Individuals*	*Dominance_D*	*Shannon_H*	*Evenness_e^H/S*	*Equitability_J*	*Fisher_alpha*	*Chao-1*
Maboko Is	9	44	0.407	1.364	0.4348	0.621	3.424	14
Kisumu	7	164	0.5456	0.9436	0.367	0.4849	1.485	8
Fish LB	15	72	0.1655	2.109	0.5492	0.7787	5.765	29
Ndere Is.	6	44	0.3585	1.246	0.5796	0.6956	1.877	9
Kendu bay	3	31	0.4693	0.8851	0.8078	0.8057	0.82	3
Homa bay	5	21	0.3787	1.245	0.6944	0.7734	2.076	5
Species Richness	17	376	0.2128	1.989	0.4301	0.7022	3.663	17.5

NOTE: The Diversity indices were calculated using the P.A.S.T version 4.03 statistical tool.

Table 6 shows diversity parameters and species Richness for aquatic insects in different sampling stations within Winam Gulf, Lake Victoria. The diversity indices provided included Dominance (D), Shannon Index (H), Evenness (eH/S), Equitability (J), Fisher's Alpha, and Chao-1. The indices of aquatic insects are attributed to environmental factors that influence the taxa richness, abundance, distribution, and other diversity indices.

The current study revealed that Maboko Island had a Taxa (S): 9, Individuals: 44, and a Species Richness, Moderate, with 9 taxa recorded, suggesting a reasonably diverse aquatic environment. The number of individuals (44) was relatively low, which indicated a stable but not overly productive habitat. The evenness (0.4348) and Shannon index (1.364) suggested moderate diversity with some degree of dominance among a few species. Kisumu Bay had taxa (S): 7, Individuals: 164, with a low Species Richness. Despite having many individuals (164), Kisumu Bay had low species richness, indicating a less diverse community. The high dominance index (0.5456) and low evenness (0.367) suggested that a few species dominated the site, associated with environmental stressors such as pollution or eutrophication, which could have reduced habitat suitability for sensitive taxa.

Fish L Beaches had a Taxa (S): 15, Individuals: 72, and a high Species Richness as recorded Fish L Beaches showed the highest species richness among all sites, indicating a highly diverse aquatic insect community. The number of individuals was moderate (72), and the high Shannon index (2.109) and evenness (0.5492) suggested a balanced and stable ecosystem with no single species dominating. The site likely provided favorable environmental conditions, such as good water quality, diverse habitats, and adequate food sources.

Ndere Island with a Taxa (S): 6, Individuals: 44, and low Species Richness. Ndere Island had low species richness, similar to Kisumu Bay, but with far fewer individuals (44). This could have suggested a less diverse community, possibly affected by specific local environmental conditions such as limited habitat types, poor water quality, or other stressors. The relatively moderate dominance (0.3585) and evenness (0.5796) suggested a community that could have been undergoing stress or recovering from disturbance.

Kendu Bay had a Taxa (S) of 3, Individuals of 31, and a low Species Richness. It had the lowest species richness and number of individuals. This indicated a highly stressed or degraded environment, likely impacted by factors such as high pollution levels, eutrophication, or heavy metal contamination. The dominance (0.4693) and low evenness (0.8078) indicated a community dominated by a few tolerant species.

Homa Bay had a Taxa (S): 5, Individuals: 21, and a low Species Richness. It has the lowest number of individuals and the lowest species richness. The low Shannon index (1.245) and evenness (0.6944) suggest a low-diversity community with potentially degraded habitat quality. Like Kendu Bay, this site may be heavily impacted by pollution or habitat loss, resulting in decreased sensitive species.

The highest Richness recorded at Fish L Beaches (15 taxa) exhibits the highest species richness and diversity. The site appeared to have favorable environmental conditions, such as good water quality, moderate nutrient levels, and low pollution, which supported a variety of aquatic insect species. Moderate Richness observed at Maboko Island (9 taxa) showed moderate species richness, suggesting a reasonably healthy environment but potentially with some limitations in habitat diversity or quality. Low Richness was also revealed at Kisumu Bay (7 taxa), Ndere

Island (6 taxa), Kendu Bay (3 taxa), and Homa Bay (5 taxa), showed low species richness, indicating that the sites could face significant environmental stressors such as pollution, eutrophication, or habitat degradation.

4.2.3.1. The effects of water quality parameters on Diversity indices

The diversity indices which included Dominance (D), Shannon Index (H), Evenness (e^H/S), Equitability (J), Fisher's Alpha, and Chao-1(Table 6) gave insights into the biodiversity, species distribution, and potential impacts of various environmental factors. The diversity and richness of aquatic insects were influenced by physical, chemical, nutrient, and heavy metal parameters.

4.2.3.1.1 Physical parameters

Temperature affects aquatic insects' metabolism, growth, reproduction, and distribution. Optimal temperatures support diverse communities, while extreme temperatures or rapid changes can stress or kill sensitive species. Sites like Fish L Beaches showed high diversity (Shannon Index = 2.109) and Evenness (0.5492), possibly indicating optimal temperature conditions that support a variety of insect taxa. Sites like Kisumu, with lower diversity (Shannon Index = 0.9436), might have experienced more temperature fluctuations or suboptimal conditions.

Adequate dissolved oxygen levels are crucial for most aquatic insects. Low D.O. levels, often due to eutrophication or organic pollution, could cause hypoxia, reducing the diversity of sensitive species (e.g., Ephemeroptera-Table 5, Figure 2b). Fish L Beaches had the highest Evenness (0.5492) and Shannon diversity index (2.109), suggesting good D.O. levels that could support diverse insect communities. In contrast, sites like Kendu Bay showed lower diversity (Shannon Index = 0.8851) and could have had lower D.O. levels, favoring tolerant species. Different insect taxa have specific habitat requirements. Sites with various substrates (sand, gravel, organic matter) provided diverse habitats that supported high species richness and diversity. Fish L Beaches and Maboko Island exhibit higher species richness (15 and 9 taxa, respectively) and diversity, likely to have had favorable substrate conditions that provided suitable habitats for a range of insect species.

4.2.3.1.2 Chemical Parameters

Most aquatic insects thrive in neutral to slightly alkaline pH (6.5 to 8.5). Deviations from this range could have led to physiological stress or mortality. Sites with moderate diversity, such as Ndere Island (Shannon Index = 1.246), could have had pH levels within the favorable range, supporting diverse communities. Excessively high or low pH values could explain the lower diversity observed in sites like Kisumu. Electrical conductivity indicated the concentration of ions in water. High E.C. could have affected osmoregulatory processes, impacting insect survival and diversity. The diversity and richness at sites such as Fish L Beaches suggested moderate ion concentrations, supporting diverse communities. Lower diversity in other sites could have indicated extreme ionic conditions unsuitable for many taxa.

4.2.3.1.3 Nutrient Parameters

Nitrogen and Phosphorus Compounds (NO_3^-, NO_2^-, NH_4^+, SRP, T.P.) could have influenced diversity indices. Elevated nutrient levels could cause eutrophication, leading to algal blooms and oxygen depletion. Low oxygen conditions could have reduced the diversity of sensitive taxa (e.g., Ephemeroptera-table 5, Figure 2b) while favoring more tolerant species (e.g., Chironomidae). Sites like Kendu Bay, which has low diversity (Shannon Index = 0.8851), could have been affected by high nutrient levels, causing eutrophication and reducing diversity. Conversely, with higher diversity, Fish L Beaches could have moderate nutrient levels that supported balanced ecosystems without leading to severe oxygen depletion. Silica supported diatom growth, a primary food source for many aquatic insects. Adequate silica levels could have supported diverse insect communities by maintaining a healthy diatom population. Sites with higher insect diversity, such as Fish L Beaches, could have had sufficient silica levels that supported a balanced food web, promoting higher insect diversity and richness.

4.2.3.1.4 Heavy Metals

Heavy metals can bioaccumulate in aquatic insects, causing toxic effects such as impaired growth, reduced reproduction, and increased mortality. Sensitive taxa are likely to be more affected, while more tolerant taxa could thrive in polluted conditions. Sites with lower diversity and Evenness, such as Kisumu (Dominance = 0.5456, Evenness = 0.367), could have higher levels of heavy metals selected for tolerant species. The observation could be reflected in lower

Fisher's Alpha (1.485), indicating lower species diversity due to potential heavy metal stress. Heavy metal contamination can degrade habitats, making them less suitable for many insect species.

Consequently, it results in shifts in community composition, with pollution-tolerant taxa dominating and sensitive taxa declining. Lower species richness and diversity in sites like Homa Bay (Fisher's Alpha = 2.076) suggested habitat degradation due to pollution, potentially from heavy metals or other contaminants. The diversity parameters in the data indicated how physical, chemical, nutrient, and heavy metal factors influenced aquatic insect communities. Fish L Beaches showed the highest diversity and Evenness, suggesting favorable environmental conditions with optimal physical, chemical, and nutrient parameters. Sites like Kisumu and Kendu Bay, with lower diversity and higher dominance, could have been impacted by nutrient pollution (eutrophication) or heavy metal contamination, leading to reduced diversity and a shift towards more tolerant species. Managing these environmental factors is crucial to maintaining healthy, diverse aquatic insect populations in Winam Gulf, Lake Victoria.

4.2.4. Existing relationship in water quality parameters and aquatic insects

4.2.4.1. Canonical Correspondence Analysis (CCA)

Canonical Correspondence Analysis (CCA) biplot illustrated the relationships between environmental variables (such as physical, chemical, and nutrient parameters) and the distribution of aquatic insect taxa across different sampling sites. CCA is a multivariate statistical method used to explore and visualize the relationships between biological communities and their environmental gradients.

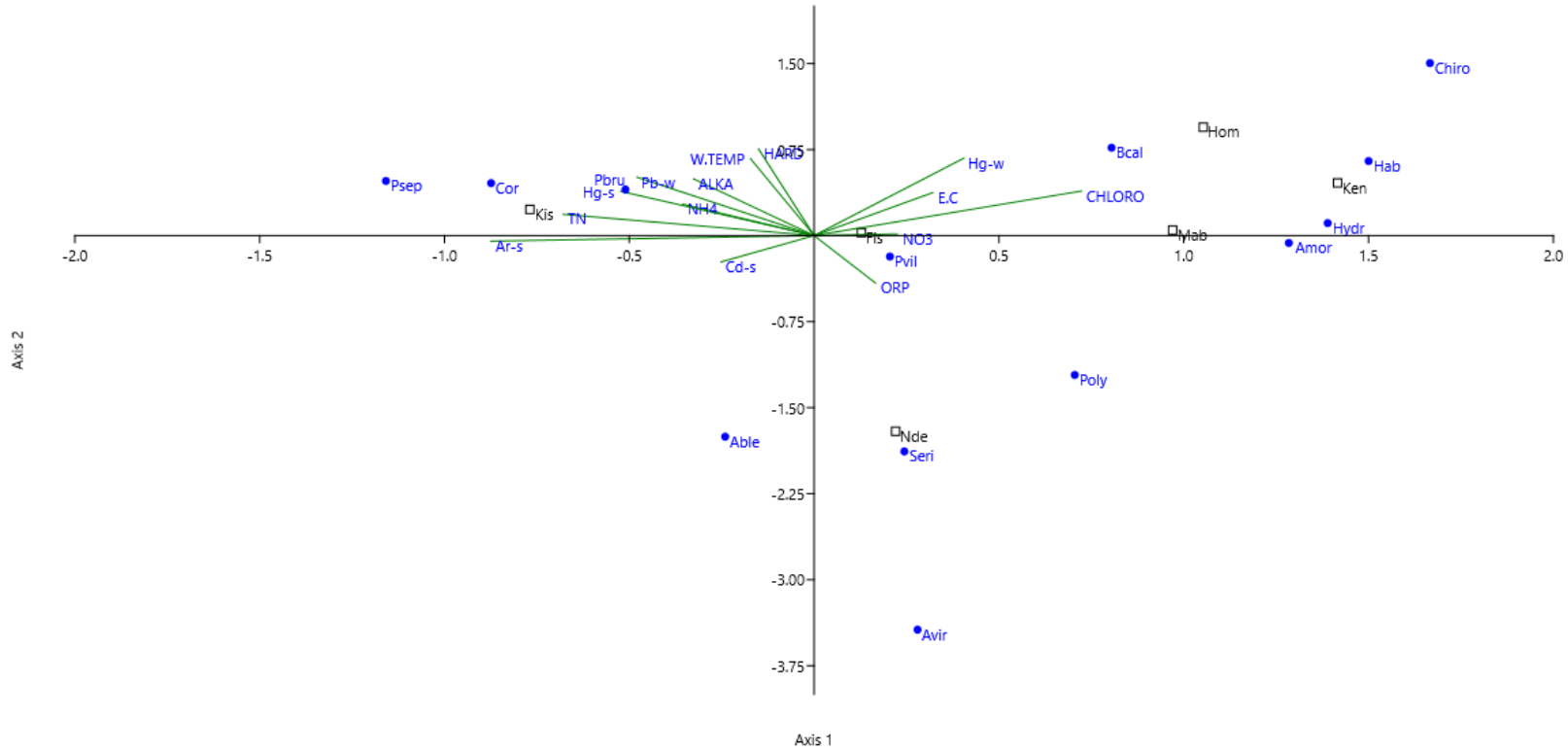

Figure 3. Conical Correspondence Analysis (CCA) of physical and chemical parameters, metallic components and Aquatic insects**. NOTE:** Aquatic insect taxa in the ordination of space of the 1st and 2nd taxa. Taxa codes correspond to Table 6; the insect taxa are blue anthethe square boxes are the sampling sites. KEY: *A-(Mbor) Microvelia borealis, B-(Galt) Gillis altilis, C-(Amor) A. merman, D-(Cor) Corixi sp., E- (Pbru)Paraplea brunni, F-(Able) Ablebesmyia sp, G-(Chiro)Chironomus sp., H-(Hab)Habrophlebia sp, I- (Pvil)Pentagenia viltigera, J-(Bcal)Baetis carolina, K –(Cmoe)C. moesta, L- (Poly)Polycentropus sp, M-(Seri) Sericostomatidae sp, N- (Brau) Brauchycentridae sp, O-(Psep) Psepheaus sp, P-(Hydro) Hydrophyllus sp, Q-(Avir) Agrion virgo.* The Diversity indices were calculad using the P.A.S.T version 4.03 statistical tool

The Canonical Correspondence Analysis (CCA) findings (as outlined in Figure 3) revealed that Environmental Variables influenced the insect taxa. For example, chlorophyll, electrical conductivity (E.C.), nitrate (NO_3), and oxygen reduction potential (ORP) appeared to have had a substantial influence on the insect community structure. Sites and taxa in the direction of these vectors were associated with higher levels of the parameters. Conversely, variables like water temperature (WTEMP), alkalinity (ALKA), ammonium (NH_4), and heavy metals such as cadmium in sediment (Cd-s) had vectors pointing in other directions, suggesting different influences on the insect community.

Species-environment relationships were observed. *Chironomus* sp. (Chiro), located on the far-right side of Axis 1, suggested a strong association with high chlorophyll and electrical conductivity—Chironomus larvae, known to thrive in nutrient-rich environments, are often tolerant of pollution. *Polycentropus* sp. *(Poly)*and *Ablabesmyia* sp. *(Able*) were positioned on the lower left, indicating they were more associated with sites that had higher concentrations of heavy metals such as arsenic in sediment (Ar-s). The species could have been more tolerant to heavy metal contamination. *Psephnaeus* sp. *(Psep)*and *Corixini* sp. (Cor) were located on the upper left, suggesting they preferred different environmental conditions, potentially linked to moderate water quality parameters.

The findings also revealed Site-Specific Insights. Homa Bay (Hom) is positioned far to the right, indicating conditions associated with high chlorophyll and electrical conductivity, which correlated with specific pollution-tolerant taxa like *Chironomus* sp. and *Baetis calorina (Bcal).* Kisumu (Kis) was situated closer to the center, indicating it could have been a range of moderate environmental conditions that support various species. Ndere Island (Nde) and *Sericostomatidae* sp. (*Seri*) were located on the lower side, indicating associations with higher levels of nutrients such as ammonium (NH_4) and cadmium in sediment (Cd-s), suggesting potential stress from these contaminants.

4.2.4.2. Pearson's Correlation Coefficients Between Individual Insect Species in Winam Gulf.

Table 7: Pearson's correlation coefficients between insect species within Winam Gulf.

	A	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*	*K*	*L*	*M*	*N*	*O*	*P*	*Q*
A		0.00	1.00	0.93	0.61	0.62	0.58	0.45	0.00	0.04	0.00	0.37	0.18	0.00	0.77	1.00	0.86
B	1.00		1.00	0.93	0.61	0.62	0.58	0.45	0.00	0.04	0.00	0.37	0.18	0.00	0.77	1.00	0.86
C	0.00	0.00		0.37	0.70	0.37	0.70	0.13	0.98	0.78	1.00	0.36	0.82	1.00	0.36	0.07	0.77
D	-0.05	-0.05	-0.45		0.01	0.40	0.51	0.91	0.90	0.68	0.93	0.46	0.65	0.93	0.00	0.68	0.61
E	-0.27	-0.27	-0.21	0.93		0.47	0.45	0.81	0.58	0.43	0.61	0.56	0.40	0.61	0.01	0.88	0.56
F	-0.26	-0.26	-0.45	0.42	0.37		0.25	0.44	0.69	0.31	0.62	0.78	0.43	0.62	0.35	0.47	0.09
G	-0.29	-0.29	-0.21	-0.34	-0.39	-0.56		0.73	0.54	0.83	0.58	0.11	0.28	0.58	0.56	0.50	0.41
H	-0.39	-0.39	0.69	-0.06	0.13	-0.39	0.18		0.41	0.28	0.45	0.58	0.20	0.45	0.97	0.54	0.39
I	1.00	1.00	-0.01	-0.06	-0.29	-0.21	-0.31	-0.42		0.05	0.00	0.33	0.14	0.00	0.75	0.98	0.96
J	0.83	0.83	-0.15	-0.22	-0.40	-0.50	0.11	-0.53	0.81		0.04	0.61	0.47	0.04	0.54	0.93	0.61
K	1.00	1.00	0.00	-0.05	-0.27	-0.26	-0.29	-0.39	1.00	0.83		0.37	0.18	0.00	0.77	1.00	0.86
L	0.45	0.45	0.45	-0.38	-0.30	0.15	-0.71	-0.29	0.48	0.26	0.45		0.12	0.37	0.40	0.26	0.31
M	0.63	0.63	-0.12	-0.24	-0.43	0.40	-0.53	-0.61	0.68	0.37	0.63	0.71		0.18	0.58	0.71	0.11
N	1.00	1.00	0.00	-0.05	-0.27	-0.26	-0.29	-0.39	1.00	0.83	1.00	0.45	0.63		0.77	1.00	0.86
O	-0.15	-0.15	-0.46	0.99	0.94	0.46	-0.30	-0.02	-0.17	-0.32	-0.15	-0.43	-0.29	-0.15		0.64	0.66
P	0.00	0.00	0.78	-0.22	0.08	-0.37	-0.35	0.32	-0.02	0.05	0.00	0.55	-0.19	0.00	-0.25		0.63
Q	-0.09	-0.09	-0.16	-0.27	-0.30	0.75	-0.42	-0.44	-0.02	-0.27	-0.09	0.51	0.71	-0.09	-0.23	-0.25	

NOTE: KEY: A-(Mbor) *Microvelia borealis, B-(Galt) Gillis altilis, C-(Amor) A. merman, D-(Cor) Corixi sp., E- (Pbru)Paraplea brunni, F-(Able) Ablebesmyia sp, G-(Chiro)Chironomus sp., H-(Hab)Habrophlebia sp, I- (Pvil) Pentagenia viltigera, J-(Bcal) Baetis carolina, K –(Cmoe)C. moesta, L- (Poly) Polycentropus sp, M-(Seri) Sericostomatidae sp, N- (Brau)Brauchycentridae sp, O-(Psep) Psepheaus sp, P-(Hydro) Hydrophyllus sp, Q-(Avir) Agrion virgo.*

The current study's findings revealed Strong Positive Correlations (Table 7). Species pairs with high positive correlation coefficients (close to +1) indicated that the species tended to increase or decrease together in abundance. For example, A (*Microvelia borealis*) and B (*Gillis altilis*) correlated by 1.00, suggesting a perfect positive relationship. I (*Pentagenia vittigera*) and J (Baetis California) correlated 1.00, indicating the two species likely co-occurred in similar environmental conditions or shared identical ecological niches. P (*Pspephaeus* sp.) and Q (*Agrion virgo*) showed a high positive correlation of 0.71, which suggested similar habitat preferences or responses to environmental conditions.

Strong negative correlations were also observed. Species with a strong negative correlation (close to -1) could have indicated competition or exclusion, whereas one species could thrive while the other did not. For example, G (*Chironomus* sp.) and F (*Ablebesmyia* sp.) had a correlation of -0.56, which indicated that when one species could be abundant, the other would be less likely to be present, M (*Sericostomatidae* sp.) and G (*Chironomus* sp.) had a correlation of -0.53, which suggested potential competition or different habitat requirements.

In some cases, no weak correlations were observed. Species pairs with correlation coefficients close to 0 indicated little to no relationship between their abundances. For instance, B (*Gillis altilis*) and K (*Caenis moesta*) correlated by 0.04, suggesting no significant relationship between these species' abundances. N (*Brauchycentridae* sp.) and P (*Hydrophyllus* sp.) showed a weak correlation of 0.00, implying independent distribution or non-related ecological factors.

Other groups revealed cpecies clusterings, where some species tended to form clusters with high inter-correlations, suggesting similar ecological or environmental preferences. For example, Species A, B, I, and J seemed to form a cluster with high correlations among them, potentially indicating shared habitat or similar ecological roles. Possible ecological interactions, which included Positive correlations, could indicate mutualistic relationships, similar environmental requirements, or similar responses to environmental stressors. In some cases, Negative correlations suggested competitive exclusion, differences in predator-prey dynamics, or differing habitat needs.

4.2.5 Morphological Identification of *Chironomus* sp.

The current study findings revealed that 68 chironomids were collected and identified using morphological approach and results recorded in Table 8 below.

Table 8. Chironomidae (Diptera) collected from sampling sites.

Order: Diptera							
Family: Chironomidae							
Genus	Maboko Island	Kisumu Bay	FLB	Ndere Island	Kendu Bay	Homa Bay	Total in Winam gulf
Ablebesmyia spp.	01	17	02	23	00	01	44
Chironomus spp.	01	01	01	00	09	12	14
Total	02	18	03	23	09	13	68

The genus *Chironomus*, belonging to the family Chironomidae, comprises of non-biting midges that are widely distributed in freshwater habitats worldwide. Due to their varying levels of tolerance to pollution, species of *Chironomus* are commonly used in biomonitoring studies as bioindicators of water quality. Morphological identification of *Chironomus* species involved examining specific characteristics of the larval head capsule, antennae, mandibles, and the setae on the thorax and abdomen. The features could vary significantly between species, allowing for their identification under a microscope. However, the presence of cryptic species—species that are morphologically similar but genetically distinct—presented a significant challenge in the accurate identification of *Chironomus* species. Two species were identified morphologically: *Chironomus* spp. and *Ablebesmyia* spp.

4.2.6 Phylogenetic Analysis

Table 8 and Figure 11 list accession numbers of insect species from Winam Gulf, Lake Victoria, focusing on different isolates of species from the Chironomidae family. The data included information on the isolate identity, species, identity percentage, accession numbers of isolates, NCBI sequences with the highest similarity, and the country of origin. The isolate Identity

column listed the unique identifiers for each isolate CH1, CH2, CH3, CH4, CH5, CH6, CH7, and CH8 that were sequenced and analyzed in the study. Each identifier represented a distinct sample collected from Winam Gulf. The identity percentage showed how similar each isolate was to known sequences in the NCBI database. A 100% identity indicated a perfect match, while values slightly less than 100% (e.g., 99.04%, 99.11%) showed very high similarity with minor variations. For example, CH1 and CH2 had 100% identity to *Chironomus pseudothummi*, meaning the isolates were identical to the known species in the database.

The species column listed the identified species based on sequence comparison. Most isolates belonged to *Chironomus pseudothummi* and *Chironomus transvaalensis,* with some labeled *Chironomus*sp. (indicating unidentified or less specific taxa). *Chironomus pseudothummi* was a common chironomid species known to inhabit freshwater environments, while *Chironomus transvaalensis* was another species within the same genus. Accession Number of Isolates provided unique NCBI accession numbers (ON455096.1, ON455097.1, ON455098.1, ON455099.1, ON4550100.1, ON4550101.1, ON4550102.1, and ON4550103.1) for each sequence submitted to the NCBI database. The numbers served as references to access the sequence data online. The accession numbers were crucial for researchers who want to verify or use the genetic information for further comparative studies. NCBI Sequences with Highest Similarity column listed the NCBI accession numbers of sequences that had the highest similarity to the isolates from the study. The sequences were the closest matches in the NCBI database. For instance, CH1 and CH2 had sequences 100% similar to NCBI entries ON455096.1 and ON455097.1, respectively, from Kenya as per the study. The country of Origin column indicates the country of origin of the sequences with the highest similarity. Most isolates matched sequences from Kenya (the current study), indicating that the sequences were identified as the same species in the present study. Other origins mentioned include Israel, the Islands of French Polynesia, and the Skadar Lake basin hotspot, suggesting that similar species or strains were also found in other geographically distant locations.

Table 9. Accession numbers of insect species from Winam Gulf

Isolate Identity	*Identity %*	*Species*	*Accession no. of isolates*	*NCBI sequences with highest similarity*	*Country of origin*
CH1	100	*Chironomus pseudothummi*	ON455096.1	ON455096.1	Kenya (this study)
CH2	100.0	*Chironomus pseudothummi*	ON45097.1	ON45097.1	Kenya (this study)
CH3	99.04	*Chironomus pseudothummi*	ON455098.1	ON455098.1	Kenya (this study)
CH4	99.11	*Chironomus pseudothummi*	ON455099.1	ON455099.1	Kenya (this study)
CH5	99.27	*Chironomus pseudothummi*	ON455100.1	ON455100.1	Kenya (this study)
CH6	99.64	*Chironomus pseudothummi*	ON455101.1	ON455101.1	Kenya (this study)
CH7	-	*Chironomus sp*	ON455102.1	ON455102.1	Kenya (this study)
CH8	98.72	*Chironomus pseudothummi*	ON455103.1	ON455103.1	Kenya (this study)
EM5	99.11	*Chironomus transvaalensis*	JQ025715.1	JQ025715.1	Israel (this study)
SC06001	90.43	*Chironomidae sp.*	SC_06001	KX051969.1	Islands of French Polynesia
LA_LSLDI_232	98.93	*Chironomus transvaalensis*	MT535109.1	MT535109.1	Skadar lake basin hotspot
LA_LSLDI_234	98.93	*Chironomus transvaalensis*	MT534930.1	MT534930.1	Skadar lake basin hotspot
LA_LSLDI_229	98.93	*Chironomus transvaalensis*	MT534886.1	MT534886.1	Skadar lake basin hotspot

NOTE: Species expressed as a % from NCBI sequences with the highest similarity and their country of origin.

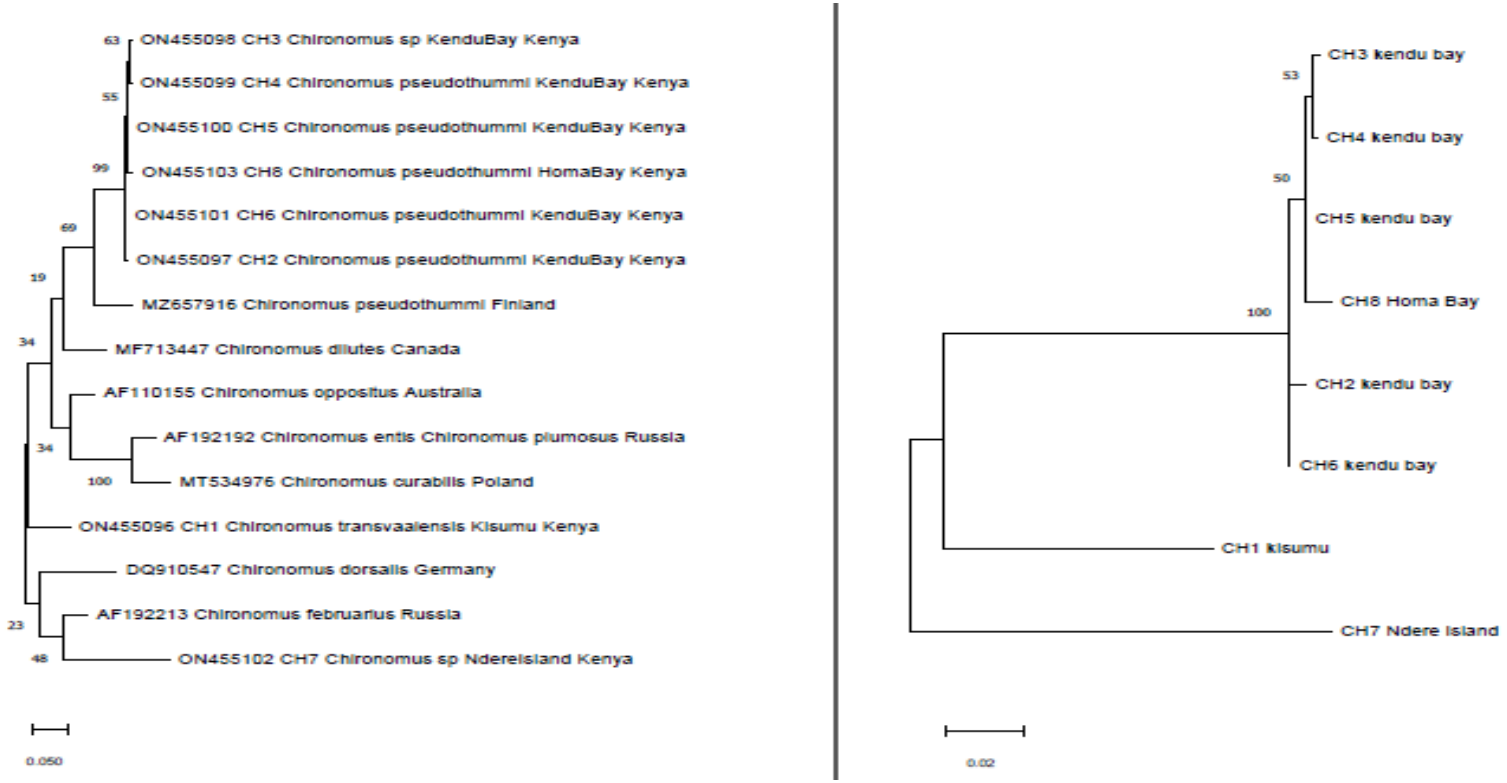

Figure 4. Evolutionary relationships among the taxonomic groups were inferred from the sing NJ method. Bootstrap probabilities are indicated on nodes as percentages while the scale represents millions of years ago

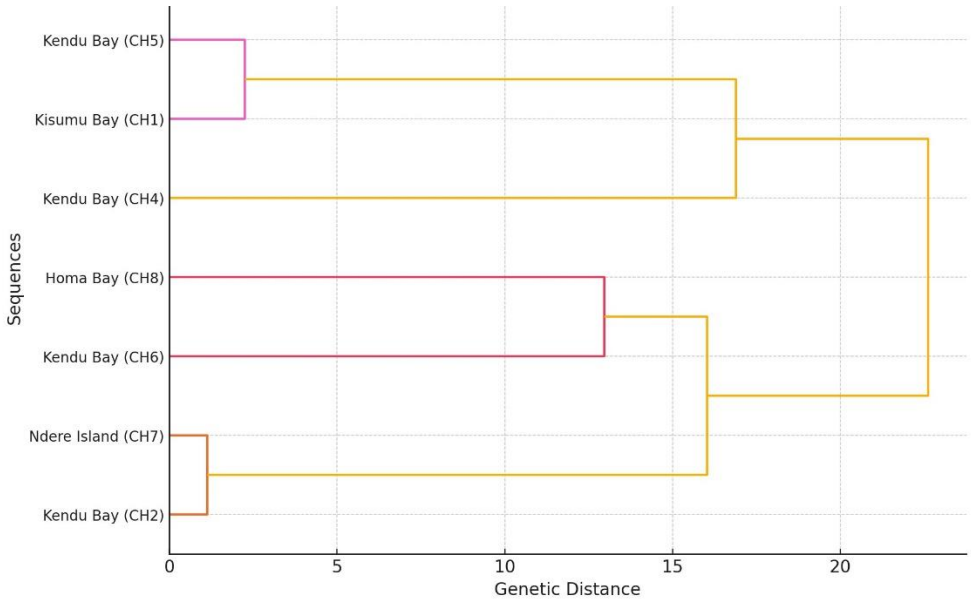

Figure 5. The phylogenetic tree above represented the evolutionary relationships between sequences from different sampling stations based on their genetic divergence

4.2.7 Evolutionary Relationships Amongst the Taxonomic Groups of Family Chironomidae

The lengths of each branch were directly related to the divergence or what may be described as the 'genetic distance' between the sequences (Figure 5). Chimeras associated with each other in the genetic content of the sequences will have shorter branches, while the ones that are distantly related will have longer branches. The genetic distances result showed that sequences that were together, like Kendu Bay (CH2) and Ndere Island (CH7), were genetically flown closer to each other. The tree showed two primary clusters: the second one included the sequences from Kendu Bay, that is CH2, CH5, CH6, and Ndere Island, is CH7; the third one included the sequences of Kisumu Bay is CH1, and Homa Bay, is CH8. There were also different types of sequences, namely Divergent Sequences. Those sequences found on distant branches, for instance, Kisumu Bay (CH1) and Homa Bay (CH8), implied that the populations had undergone ge6netic changes over a long period or along different environmental pressures. The clustering pattern was informative on the aspects of gene flow, the structure of the population, and historical isolation at the various sampling stations. For instance, the physical proximity of Kendu Bay (CH5) and Kendu Bay (CH6) may have pointed to either high levels of immigration in the two populations or recent separation in the same region. This phylogenetic tree was instrumental in presenting

the sequences' genetic similarity from the various sampling stations in Winam Gulf and the evolution of such sequences. Knowledge of these relationships would be essential if we are to make informed decisions for ecological management, putting considerations in place for the conservation of this species, and conducting evolutionary studies concerning aquatic ecology.

4.2.8 Substitution Rates

Table 10 outlines the maximum likelihood estimation (MLE) of Substitution Rates between different nucleotide bases (A, T, C, G) under the Tamura-Nei (1993) model for nucleotide substitution. The model accounted for the differences in substitution rates between transitions (changes between purines or pyrimidines, like A ↔ G or C ↔ T) and transversions (changes between a purine and a pyrimidine, like A ↔ C or G ↔ T) as well as for unequal base frequencies. The matrix shows the probability of substitution (r) from one nucleotide base (row) to another base (column). Each cell contained values representing the substitution rate from the row's nucleotide to the column's nucleotide. The row labels (A, T, C, G) represented the nucleotide bases from which the substitution occurred. The column labeled (A, T, C, G) represented the nucleotide bases to which the substitution occurred. Substitution Rates for Each entry in the matrix represented the MLE substitution rate from one base to another. Diagonal Entries that were not filled ("-") because they would represent a substitution of a nucleotide to itself were not considered. Off-diagonal entries provided the estimated rates of substitution between different nucleotides. The substitution rate from A to T was 8.0456, G to C was 4.251, and G to A was the highest at 18.8519. Substitution Patterns and Rates in the matrix provided insights into the frequency of nucleotide substitutions. High Substitution Rates (e.g., G to A = 18.8519) suggested that the substitution occurred more frequently than others and was attributed to higher mutability or selective pressure favoring the change. The moderate substitution rates were observed between T to G. The Low Substitution Rates (e.g., C to G = 5.3128) indicated substitutions that occurred were less frequent, attributed to fewer changes, or subjected to more substantial evolutionary constraints. Transitions (substitution between purines: A ↔ G or pyrimidines: C ↔ T) typically occurred more frequently than transversions (substitution between a purine and a pyrimidine: A ↔ C, A ↔ T, G ↔ C, G ↔ T). For instance, A to G (18.8519) and T to C (5.0410) were examples of transitions, while A to C (4.2519) and G to T (8.0456) were examples of transversions.

T**able 10:** Maximum Likelihood Estimation of Substitution of the Matrix 2

From\To	***A***	***T***	***C***	***G***
A	-	8.0456	4.2519	8.9391
T	11.2043	-	5.0410	5.3128
C	11.2043	9.5388	-	5.3128
G	18.8519	8.0456	4.2519	-

NOTE: The matrix had entries that were probability of substitution (r) from one base (row) to another base (column). Substitution patterns and rates were estimated under the Tamura-Neil, (1993) model (Tamura & Nei,1993).

Table 11. Estimates of Evolutionary Divergence Between Sequences

	CH1_kisumu	CH2_kendu_Bay	CH3_kendu_bay	CH4_kendu Bay	CH5_kendu Bay	CH6_kendu_Bay	CH7_Ndere_Isl	CH8_Homa_Bay
CH1_kisumu								
CH2_kendu_bay	0.144							
CH3_kendu_bay	0.142	0.011						
CH4_kendu_bay	0.140	0.011	**0.002**					
CH5_kendu_bay	0.142	0.008	**0.002**					
CH6_kendu_bay	0.140	**0.004**	0.006	0.006	0.004			
CH7_Ndere_Island	0.156	0.178	0.173	0.175	0.175	0.173		
CH8_Homa_Bay	0.142	0.015	0.008	0.008	0.006	0.011	0.173	

4.2.9 Evolutionary Divergences

Table 11 provided estimates of evolutionary divergences between DNA sequences from different sampling stations in Winam Gulf, Lake Victoria. Evolutionary divergences measure the genetic distance between sequences, reflecting how much they diverged from a common ancestor. This information is crucial for understanding the sampled populations' genetic relationships and evolutionary history.

Table 11 compares sequences from different sampling stations: Kisumu Bay (CH1), Kendu Bay (CH2, CH4, CH5, CH6), Ndere Island (CH7), and Homa Bay (CH8). A unique identifier, such as CH1, CH2, etc, represents each sequence. The sequences are from different locations within Winam Gulf, indicating that the study is examining genetic diversity across geographically distinct sites.

The entries in Table 11 represented the evolutionary divergence values between pairs of sequences. The values were numerical estimates of genetic distance and indicated how much the sequences had changed relative to each other. The diagonal of the matrix was left blank because it would represent the divergence of a sequence with itself, which is always zero. Each off-diagonal value represented the genetic distances between two sequences. For example, the evolutionary divergence between Kisumu Bay (CH1) and Kendu Bay (CH2) was 10.50969.

Understanding Evolutionary Divergence Values was significant; higher values indicated greater genetic distance between sequences, suggesting that they had evolved separately for a longer time or under different environmental pressures. For example, the divergence between Kisumu Bay (CH1) and Homa Bay (CH8) was 14.00318, which was relatively high, indicating substantial evolutionary divergence. Lower Divergence Values suggested closer genetic relatedness or recent common ancestry. For example, the divergence between Kendu Bay (CH6) and Ndere Island (CH7) was 0.00596, indicating almost no genetic difference and suggesting very recent divergence or gene flow between these sites.

The variation in divergence values among sequences from different sampling stations could be attributed to geographical distances, environmental factors, or historical isolation. Sequences from Kendu Bay (CH2, CH4, CH5, CH6) showed varied divergence values with sequences from other locations, suggesting diverse evolutionary pressures or levels of gene flow within the area. High divergence between sequences from Kisumu Bay (CH1) and Homa Bay (CH8) suggested

the sites could have been more isolated or experienced different environmental conditions influencing their evolutionary paths.

Patterns of Gene Flow and Population Structure. Low divergence values, such as between Kendu Bay (CH6) and Ndere Island (CH7), suggested possible gene flow or connectivity between the populations. This could occur due to water currents, the movement of organisms, or other factors facilitating genetic exchange. Identifying regions with low divergence was important for understanding population structure and connectivity in Winam Gulf. Management strategies can focus on these connections to preserve genetic diversity.

Potential for Adaptive Divergence. Some sequences showed intermediate divergence values (e.g., Kendu Bay (CH4) and Ndere Island (CH7) = 8.71703), indicating partial genetic differentiation. This could suggest ongoing processes of adaptive divergence, where populations were diverging in response to specific local environmental conditions. Regions with intermediate divergence values could have been critical for studying adaptive evolution and understanding how local conditions shape genetic diversity.

4.3 Nutritional Status of Aquatic Edible Insects

4.3.1 Amino Acids

Nine essential amino acids (*arginine, Valine, lysine, leucine, methionine, threonine, tryptophan, phenylalanine, Isoleucine, and histidine*) were identified from the non-biting midges (Table 12). The mean concentration of Arginine varied across the sites, with Ndere Island showing the highest concentration (~2.17 µg ml-1) and Homa Bay the lowest (~1.76 µg ml-1). The variability was relatively low, as indicated by the minor standard deviations. The significant difference in Arginine levels p-value < 0.0001 in Table (12) suggested differential physiological needs or environmental conditions influencing Arginine metabolism at the sites. Valine Concentration: Ndere Island also showed the highest concentration of Valine (~1.87 µg ml-1), while Homa Bay had the lowest (~1.44 µg ml-1). There was a noticeable variability in Valine levels, especially at Kendu Bay. Variations in Valine could be associated with protein synthesis demands or specific environmental stressors affecting Valine metabolism differently across sites.

Methionine Concentration Homa Bay had the highest mean of Methionine concentration (~2.21 µg ml-1), while Ndere Island had the lowest (~1.87 µg ml-1). The amino acid showed significant

spatial variation (p-value < 0.0001), highlighting the potential as an indicator of local environmental conditions. Methionine was involved in detoxification pathways. Higher levels in some locations could have reflected a response to environmental contaminants, such as heavy metals.

Phenylalanine Concentration was notably higher in Homa Bay (~2.58 µg ml-1) compared to other sites. The difference was statistically significant (p-value < 0.0001. High levels of Phenylalanine in Homa Bay could have been linked to specific ecological factors such as water quality, availability of precursors, or genetic differences in Chironomus populations.

Isoleucine Concentration Kendu Bay showed the highest mean concentration of Isoleucine (~2.02 µg ml-1), while Homa Bay showed the lowest (~1.62 µg ml-1). The amino acid also showed significant spatial variation (p-value < 0.0001). Isoleucine was essential for protein synthesis and energy metabolism. Variations in its levels could have reflected site-specific environmental pressures or nutritional differences.

Table 12. Mean (±STD) of amino acids concentrations in *Chironomus* species

Sampling site	**Concentration (µg/ml) per sampling site**									
	Arginine	*Valine*	*Lysine*	*Leucine*	*Methionine*	*Threonine*	*Tryptophan*	*Phenylalanine*	*Isoleucine*	*Histidine*
Kisumu	2.02±0.04	1.583±0.04	2.48±0.0	2.39±0.02	1.96±0.04	1.41±0.0	1.21±0.02	2.18±0.03	1.92±0.02	0.6±0.57
Homa bay	1.76 ±0.03	1.44±0.026	2.30±0.03	2.32±0.006	2.21±0.03	1.98±0.03	1.33±0.06	2.58±0.05	1.62±0.03	0.84±0.01
Ndere Isl.	2.17±0.04	1.877±0.03	2.20±0.01	2.6±0.01	1.87±0.006	1.69±0.02	1.45±0.00	2.22±0.01	1.74±0.01	1.62±0.02
Kendu bay	2.00±0.06	1.71±0.12	1.94±0.03	2.12±0.0	1.97±0.05	1.84±0.02	1.25±0.17	2.44±0.01	2.02±0.01	1.00±0.04
F-stat.	55.91	22.06	NS	NS	62.62	NS	NS	137.80	243.90	6.447
p-value	HS (<0.0001)	HS (0.0003183)	NS	NS	HS (<0.0001)	NS	NS	HS (<0.0001)	HS (<0.0001)	S (0.01578)

NOTE: Significant spatial differences ($p<0.05$) are depicted by different subscripts.

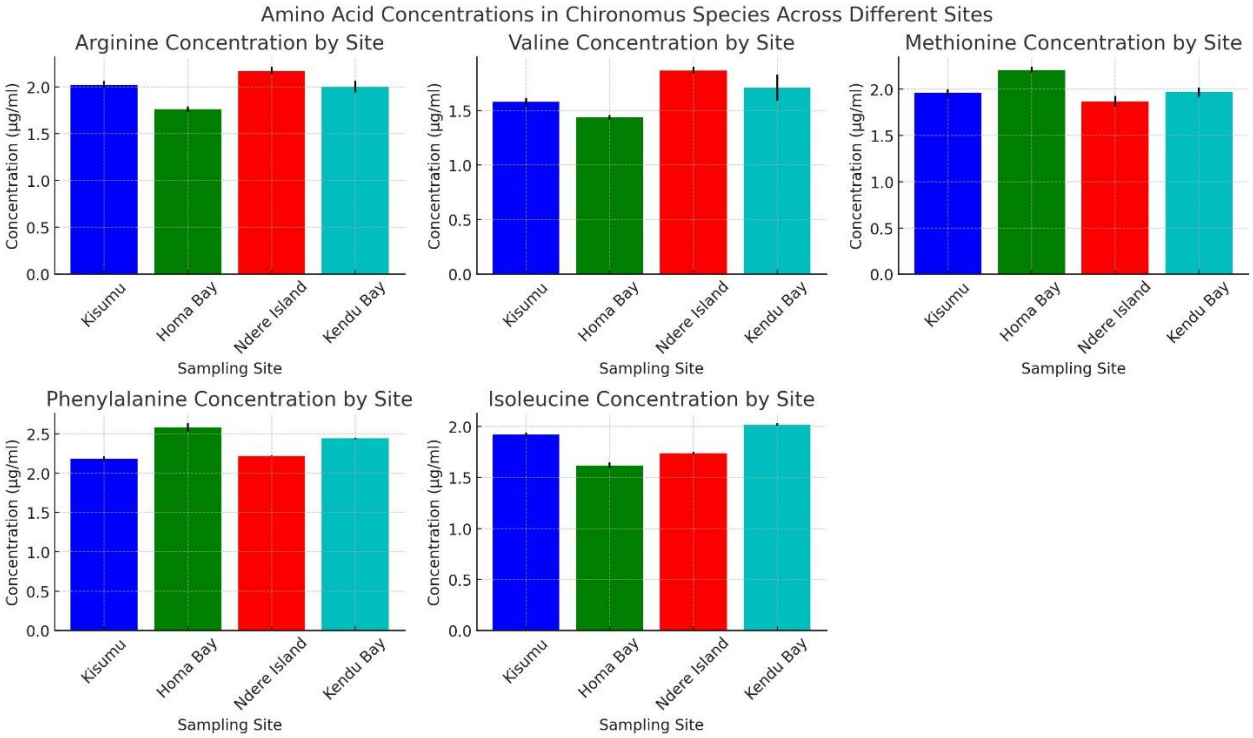

Figure 6. Bar plots showing visual representation of the mean concentrations of selected amino acids (Arginine, Valine, Methionine, Phenylalanine, and Isoleucine) in Chironomus species across different sampling sites: Kisumu, Homa Bay, Ndere Island, and Kendu Bay. The error bars represent the standard deviation (±STD), indicating variability in the measurements.

The Arginine, Valine, Methionine, Phenylalanine, and Isoleucine concentrations show highly significant (HS) differences between the sites, indicating that the levels of these amino acids vary significantly among the sampling locations (Figure 6). For example, the F-statistic for Arginine was 55.91, with a p-value < 0.0001, showing highly significant differences in concentrations across the sites. Methionine also showed considerable variation with an F-statistic of 62.62, p-value < 0.0001, suggesting different environmental conditions or genetic factors influencing the concentration in other locations. Lysine, Leucine, Threonine, and Tryptophan concentrations were labeled as NS (Not Significant), meaning there were no statistically significant differences in the mean concentrations of these amino acids between the sampling sites. This suggested that the amino acids could have been regulated similarly across different environments or that the sites had insufficient variation to detect differences. Histidine showed significant (S) differences among sites, with an F-statistic of 6.447 and a p-value of 0.01578. This could have indicated that while there was some variation in Histidine concentrations, the variation was less pronounced than in amino acids like Arginine or Methionine.

4.3.2 Vitamins

Fat and water-soluble vitamins were detected, although only the former (A, D, E, and K) showed statistically significant variation in concentration. The variations were attributed to the change in sampling locations ($p < 0.05$) and was followed by Tukey's pairwise posthoc test (Table 13a and 3b). Overall, the concentrations of the fat-soluble vitamins were higher compared to the water-soluble vitamins (B group and C).

4.3.2.1. Fat Soluble Vitamins in Chironomus spp.

Table 13a. Mean (±STD) of fat-soluble vitamins concentrations in nonbiting midge sampled in varying stations in Nyanza Gulf

Sampling Site	**Fat Soluble Vitamins (µg/ml)**			
	Vitamin A	*Vitamin D*	*Vitamin E*	*Vitamin K*
Kisumu Bay	0.16±0.01	18.46±0.02	29.48±0.01	1.25±0.01
Homa Bay	0.19±0.02	15.56±0.01	23.67±0.01	1.43±0.03
Ndere Island	0.29±0.01	11.57±0.13	31.40±0.8	1.50±0.08
Kendu Bay	0.34±0.02	20.90±0.16	23.68±0.07	2.03±0.03
F-stat.	12.09	40.30	32.84	18.78

p-value	HS(<0.0001)	HS(<0.0001)	HS(<0.0001)	HS(<0.0001)

NOTE: Significant spatial differences (p<0.05) are depicted by different subscriptsTable 12b: Mean (±STD) of water-soluble vitamins concentrations in nonbiting midge

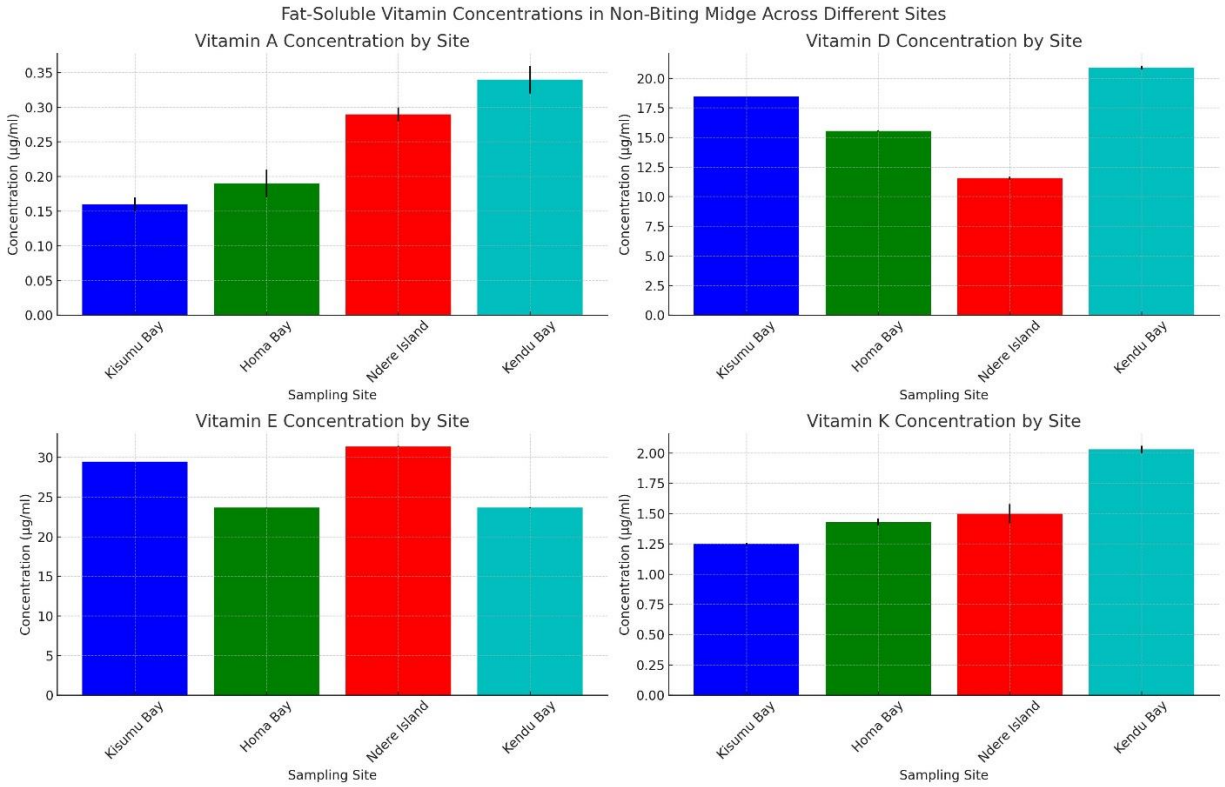

Figure 6. Fat soluble vitamins concentrations in non-biting midges acoss different sampling stations.

The findings of the current study revealed that amongst the fat-soluble vitamins (Table 13a, Figure 6), samples from Kendu Bay had the highest concentrations of vitamins A (0.34±0.02 µg ml-1), vitamin D (20.90±0.16 µg ml-1), and vitamin K (2.03±0.03 µg ml-1). In contrast, Ndere Island samples had the highest vitamin E concentrations (31.40±0.8 µg ml-1). Some water-soluble vitamins were undetected, including B1, B6, B12, and C in Kisumu Bay samples, B7 in Homa Bay samples, B3, B6, and C in Ndere Island samples, and B5, B6, B9, and C in Kendu Bay samples.

ANOVA at p<0.05 showed insignificant variations in water-soluble vitamins. The findings revealed a mean concentration of Vitamin A, highest at Kendu Bay (0.34 µg ml-1) and lowest at

Kisumu Bay (0.16 µg ml-1). The differences were highly significant, as indicated by the F-statistic (12.09) and p-value (<0.0001). The considerable variation in Vitamin A levels could have been due to differences in environmental conditions, such as water quality, food availability, or pollution levels, which influenced the accumulation and metabolism of Vitamin A in non-biting midges. Vitamin D Concentrations had the highest mean concentration at Kendu Bay (20.90 µg ml-1) and the lowest at Ndere Island (11.57 µg ml-1). The F-statistic (40.30) and p-value (<0.0001) suggested highly significant differences among sites. Vitamin D is involved in calcium metabolism and immune function. Variations in the concentration could have indicated site-specific environmental factors or adaptive responses to different levels of sunlight exposure, dietary intake, or stressors such as pollution. Vitamin E concentrations were highest at Kendu Bay (23.68 µg ml-1) and lowest at Homa Bay (23.67 µg ml-1). The F-statistic (32.84) and p-value (<0.0001) indicated significant differences. Vitamin E is a potent antioxidant that helps to protect cells from oxidative stress. The considerable variation in the levels could have reflected different levels of oxidative stress or pollution across the sampling sites, potentially due to varying levels of heavy metals or organic pollutants. The mean concentration of Vitamin K was highest at Kendu Bay (2.03 µg ml-1) and lowest at Kisumu Bay (1.25 µg ml-1). The F-statistic (18.78) and p-value (<0.0001) suggested highly significant differences among sites. Vitamin K is essential for blood coagulation and bone metabolism. Variations in the concentration could have been linked to different physiological demands or environmental conditions, such as nutrient availability and exposure to pollutants.

These significant disparities in fat-soluble vitamin levels may have indicated the availability of nutrients and dietary intake, the metabolic load, or contamination by toxic substances like heavy metals or pesticides on Chironomus species. Variations in pH, DO, temperature, and pollution levels can also affect the distribution and concentration of vitamins in organisms residing in the water. In particular, high levels of vitamins relating to antioxidant protection (for example, vitamin E) at specific locations could have signified higher oxidative stress levels due to such factors as contamination by heavy metals or organic pollutants. High-polluted sites could have had adaptive physiological acclimatization, which could have led to high vitamin concentrations. For example, higher vitamin D levels in subjects at Kendu Bay could have been due to increased

sun exposure, diet, or adaptations to the body's response to stress by the body and the local and general environmental conditions. These fat-soluble vitamins can be used as biomarkers of nutritional condition, health, and stress level in the organisms inhabiting the spatial samples. These include trophic interactions, pollution effects, and overall habitat characteristics of the body of water.

4.3.2.2. Water Soluble Vitamins

Table 13b: Mean (±STD) of Water -Soluble Vitamins concentrations in nonbiting midge

Sampling Site	Water Soluble (µg/ml)								
	Vit B1	Vit B2	Vit B3	Vit B5	Vit B6	Vit B7	Vit B9	Vit B12	Vit C
Kisumu Bay	0.05±0.0	0.00±0.0	0.087±0.01	0.05±0.01	0.00±0.00	0.08±0.0	0.13±0.01	0.00±0.00	0.00±0.0
Homa Bay	0.00±0.0	0.04±0.0	0.06±0.02	0.047±0.01	0.07±0.02	0.00±0.0	0.10±0.0	0.10±0.02	0.04±0.0
Ndere Is.	0.09±0.0	0.03±0.01	0.00±0.0	0.03±0.00	0.00±0.00	0.49±0.42	0.06±0.01	0.08±0.00	0.00±0.0
Kendu Bay	0.07±0.01	0.05±0.01	0.07±0.02	0.00±0.00	0.00±0.00	0.07±0.02	0.00±0.0	0.03±0.00	0.00±0.0
F-stat.	NS	NS	NS	NS	NS	NS	NS	NS	NS
p-value	NS	NS	NS	NS	NS	NS	NS	NS	NS

The results from Table 13 b showed that Vitamin B1 (Thiamine) mean concentrations ranged between 0.00 µgml-1 (Homa Bay) to 0.09 µgml-1 (Ndere Island), though the differences were not statistically significant (NS). The differences suggested that Vitamin B1 levels were relatively uniform across the sites, possibly due to similar environmental conditions or food sources. Vitamin B2 (Riboflavin) mean concentrations were deficient across all sites, ranging from 0.00 µgml-1 (Kisumu Bay) to 0.05 µgml-1 (Kendu Bay), with no significant differences between sites (NS). Uniform and low levels of Vitamin B2 could indicate limited availability in the environment or minimal dietary requirements for this vitamin in *Chironomus*. Vitamin B3 (Niacin) Concentrations were in the range of 0.06 µgml-1 (Homa Bay) to 0.09 µgml-1 (Ndere Island). The differences were statistically insignificant. The insignificant variations could suggest that factors influencing Vitamin B3 levels were consistent across all sites.

Similarly, trivial differences in Vitamin B5 (Pantothenic Acid) concentration ranging between 0.04 µgml-1 (Homa Bay) and 0.07 µgml-1 (Kendu Bay) were observed amongst the sampling stations. This could be attributed to uniform levels indicating similar dietary intake or metabolic

demand for Vitamin B5 across sites. All sites had low concentrations of Vitamin B6 (Pyridoxine), with the highest being 0.07 µgml-1(Homa Bay). No significant differences are observed (NS). Minimal variation suggested that the requirement or availability of Vitamin B6 was consistent across different environments. There was a noticeable variation in Vitamin B7 (Biotin) levels, with Ndere Island showing the highest concentration (0.49 µgml-1). However, the difference was still statistically insignificant. While there was some level variation, the lack of significance suggested that this was within natural variability or due to non-environmental factors.

Vitamin B9 (Folic Acid) mean concentrations ranged from 0.06 µgml-1(Ndere Island) to 0.13 µgml-1 (Kisumu Bay), with no significant differences. Consistent levels across sites implied uniform environmental conditions affecting Vitamin B9.

Vitamin B12 (Cobalamin) concentration was insignificant and in a range of 0.00 µgml-1(Kisumu Bay and Homa Bay) to 0.08 µgml-1 (Ndere Island). The observed concentration was relatively low. Low concentrations could be due to uniform microbial sources or dietary intake of the vitamin. In Vitamin C (Ascorbic Acid) Concentration, all sites showed 0.00 µgml-1, except Homa Bay (0.04 µg/ml), though the differences were statistically significant (NS). The consistent absence of Vitamin C across most sites suggested limited availability or low dietary requirement for the vitamin in non-biting midges.

The results also showed no significant spatial differences in the concentrations of water-soluble vitamins (B1, B2, B3, B5, B6, B7, B9, B12, and C) among the sampling sites in Nyanza Gulf. The results suggested that environmental conditions, food sources, or metabolic needs were related to the relatively consistent vitamins across the different sites. Unlike fat-soluble vitamins, which showed significant spatial differences, water-soluble vitamins appeared more uniformly distributed or regulated in *Chironomus* populations within the study area. The results reflected uniform dietary intake, similar environmental conditions affecting vitamin metabolism, or a lack of specific ecological stressors influencing the particular vitamins. Further studies correlating the data with additional environmental parameters could provide deeper insights.

4.3.3 Fatty Acids-phd revised

Twenty (20) saturated, monounsaturated, and polyunsaturated (omega-3 and omega-4) fatty acids were detected in the *Chironomus* spp. (Table 12a). ANOVA at $p<0.05$ revealed significant variation in SFA-C14:0, C15:0, C15, C16:0, C17:0, C18:0, C20:0 and MuFA:C16:1, C18:1n-9 and C18:1n-11 and C20:1. The means were separated by Tukey's pairwise posthoc test revealed significant differences in saturated fatty acids post hoc test).

Table 14. Mean (±STD) of saturated, monounsaturated, polyunsaturated omega-3, and polyunsaturated omega-6 fatty acids concentrations in Chironomus sp. sampled from Nyanza gulf

Type of Fatty Acid	**Chemical Formula**	**Concentration (µg/ml) per sampling site**				**F-stat.**	**p-value**
		Kisumu bay	Homa bay	Ndere island	Kendu bay		
Saturated Fatty Acids	C14:0	2.20±1.14	0.19±0.003	1.223±0.004	1.205±0.009	6.668	<0.0001
	C15:0	1.448±0.004	0.253±0.002	0.125±0.001	0.245±0.002	1.683	<0.0001
	C16:0	19.11±0.050	18.12±0.002	14.28±0.001	18.25±0.003	2.204	<0.0001
	C17:0	2.36±0.030	0.343±0.009	0.172±0.002	0.341±0.001	1.757	<0.0001
	C18:0	0.51±0.000	4.315±0.000	4.435±0.003	4.594±0.001	1.322	<0.0001
	C20:0	1.968±0.025	0.125±0.000	0.284±0.002	0.168±0.004	NS	NS
	C22:0	0.0±0.000	0.0±0.000	0.224±0.001	0.0±0.000	NS	NS
	C24:0	0.0±0.000	1.448±0.006	0.123±0.002	0.0±0.000	NS	NS
Monounsaturated Fatty Acids	C16:1	6.35±0.002	7.33±0.003	1.525±0.002	6.17±0.003	4.668	<0.0001
	C18:1n-9	12.14±0.007	32.12±0.001	23.05±0.001	23.169±0.004	1.322	<0.0001
	C18:1n-11	7.234±0.004	4.04±0.034	2.495±0.017	4.140±0.003	NS	NS
	C20:1	1.573±0.120	2.170±0.624	1.783±0.580	2.733±0.006	4.216	0.046
	C24:1	0.089±0.003	0.0 ±0.000	0.11±0.0006	0.0 ±0.000	NS	NS
Poly-unsaturated Fatty Acids (Omega-3)	C18:3n-3	3.31±0.004	2.55±0.001	3.55±0.002	2.409±0.002	NS	NS
	C20:5n-3	0.323±0.025	0.570±0.001	0.3387±0.0032	0.8087±0.004	NS	NS
	C22:5n-3	0.0±0.000	0.551±0.001	1.133±0.002	0.458±0.003	NS	NS
	C22:6n-3	0.0±0.000	0.341±0.002	1.323±0.003	2.51±0.001	NS	NS
Poly-unsaturated Fatty Acids (Omega-6)	C18:2n-6	21.26±0.009	21.882±0.003	23.16±0.002	21.78±0.010	NS	NS
	C18:3n-6	0.0±0.000	0.278±0.003	0.0±0.000	0.154±0.002	NS	NS
	C20:2n-6	0.0±0.000	0.286±0.001	0.201±0.001	0.707±0.001	NS	NS

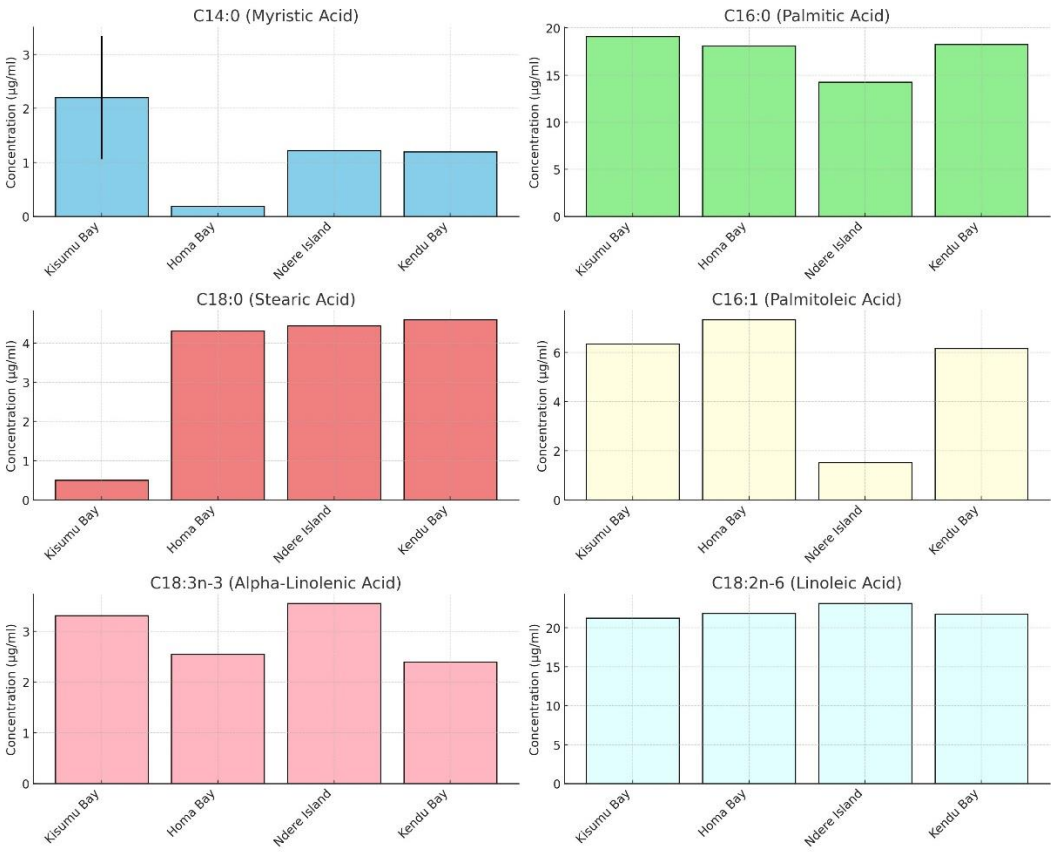

Figure 7. Bar graphs of concentrations of different fatty acids across the four sampling sites: Kisumu Bay, Homa Bay, Ndere Island, and Kendu Bay. The graphs show mean concentrations and the variability of each fatty acid.

The results from Table 14 and Figure 7-bar graphs indicated that C14:0 (Myristic Acid) at Kisumu Bay showed a significantly higher concentration of C14:0 compared to other sites, with a significant variation. Homa Bay, Ndere Island, and Kendu Bay had relatively lower concentrations, indicating site-specific variation. The concentration of C16:0 (Palmitic Acid) was high in all sites, with Kisumu Bay, Homa Bay, and Kendu Bay showing slightly higher levels than Ndere Island. The fatty acid was abundant across all sites, but there were significant differences, as indicated by the p-value (<0.0001). C18:0 (Stearic acid) at Homa Bay, Ndere Island, and Kendu Bay showed much higher concentrations of C18:0 compared to Kisumu Bay, which was attributed to site-specific factors affecting the levels of Stearic acid, potentially related to different environmental conditions or pollution levels. There was a notable drop in the

concentration of C16:1 (Palmitoleic Acid) at Ndere Island compared to the other sites, which had relatively higher levels. This could have indicated environmental stressors affecting this site's synthesis or accumulation of monounsaturated fatty acids. Ndere island had the highest concentration of C18:3n-3 (Alpha-Linolenic acid, Omega-3), indicating a potential difference in food web dynamics or environmental quality that favored the presence of the essential fatty acid. In contrast, C18:2n-6 (Linoleic Acid, Omega-6) concentrations were relatively consistent across all sites with minimal variation, suggesting a widespread presence of this fatty acid in the environment.

The significance of the variation of fatty acid across sites was determined in (C14:0, C16:0, C18:0, C16:1, C18:3n-3, C18:2n-6) at p (<0.05). The substantial variation indicated that the fatty acids were related to the local physical conditions like pollution, nutrient availability, or any ecological factors, which were more suitable for environmental analysis. Moreover, the two fatty acids identified as saturated fatty acids, namely, C16:0 (Palmitic Acid) and C18:0 (Stearic Acid), possessed ecological relevance and abundance. SFA play essential roles in energy storage and membrane structure, and some were among the most ecologically relevant and abundant in aquatic environments, which included C18:3n-3 (Alpha-Linolenic Acid, Omega-3) and C18:2n-6 (Linoleic Acid, Omega-6). The PUFAs are indispensable for membrane fluidity, cell signaling, and anti-inflammatory processes. While some had statistical significance and biological interpretation, which included: Out of the identified fatty acids, Myristic Acid C14:0, Palmitic acid C16:0, Stearic acid C18:0, and Palmitoleic acid C16:1 give relatively high F-statistics, and low p-value, which implies significant variability across the sites. These differences were, therefore, ecologically accounted for by either stress, pollutants, or nutrients/essential elements. Other fatty acids, such as C18:1n-9 (Oleic Acid), were present but less abundant or more general in their ecological distribution than the selected acids. Signs of pollutants or specific environmental situations were less conspicuous in the cases. There was sometimes variation in the relative abundance of some fatty acids compared to others, and this variation was more consistent across all four sampling sites for some fatty acids, facilitating easy comparison and interpretation. This consistency offered a sounder rationale for ecological or environmental inference. Some fatty acids had small but significant p-values, for example, C17:0

(Heptadecanoic Acid) and C15:0 (Pentadecanoic Acid). Still, their ecological roles or fluctuations in the community were generally hidden behind the more prominent and functionally critical fatty acids, such as C16:0 or C18:0.

The levels of fatty acids observed also differed significantly among the multiple sampling sites, suggesting that these reflect site-specific physical characteristics or other aspects such as food quality, nutrient availability, or pollution. Understanding the variations could help monitor environmental health and the functioning of the different fatty acids within the aquatic ecosystems.

4.3.3.1. PUFA -omega 3 and PUFA omega 6

PUFA-Omega-3 Fatty Acids, Kisumu Bay had the highest mean of Omega-3 concentration (~12.3 µgml-1), with moderate variability, while Homa Bay had the lowest mean Omega-3 concentration (~8.6 µgml-1), with relatively low variability (Table 14; Figure 8). Ndere Island showed moderate Omega-3 levels (~10.1 µgml-1) with slightly higher variability. Kendu Bay had the lowest concentration (~7.9 µgml-1) but with lower variability. PUFA-Omega-6 Fatty Acids, Homa Bay showed the highest mean Omega-6 concentration (~22.1 µgml-1), indicating a substantial fatty acid level with moderate variability (Table 14; Figure 8). Kisumu Bay also had high Omega-6 levels (~18.7 µgml-1), but slightly less than Homa Bay, while Ndere Island and Kendu Bay had relatively lower Omega-6 concentrations (~19.3 µgml-1and ~21.6 µgml-1, respectively). Significant Variations Variation was observed in Omega-6 levels, generally higher than Omega-3 levels across all sites. In contrast, the Variation in fatty acid levels across sites could have indicated differences in environmental stress, nutritional quality of the food web, or pollution levels.

In Conclusion, the significant differences in Omega-3 and Omega-6 fatty acid concentrations across sites suggested that environmental factors, such as pollution or nutrient availability, could have influenced fatty acid synthesis, metabolism, and storage in aquatic organisms. Further analysis, such as correlation studies with pollutant levels, would be needed to draw more specific conclusions about environmental health and stress indicators.

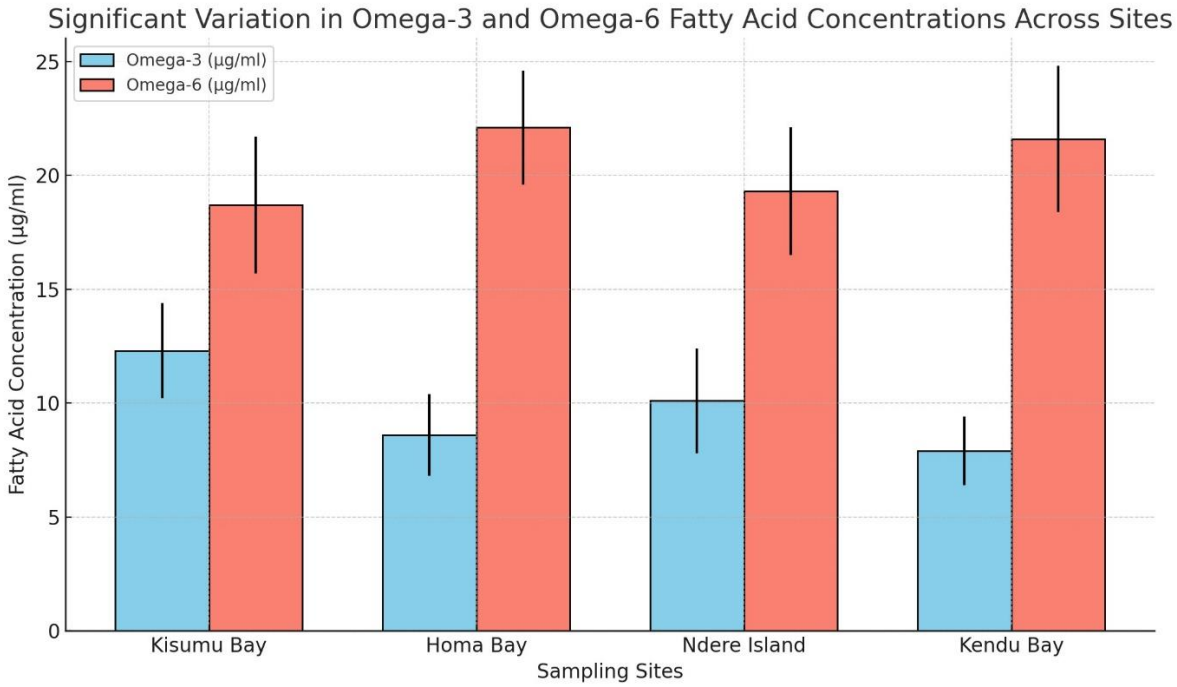

Figure 8. Bar graph of variation in Omega-3 and Omega-6 fatty acid concentrations across four sampling sites: Kisumu Bay, Homa Bay, Ndere Island, and Kendu Bay. The error bars represent the standard deviations (SD) for each site, indicating the variability in fatty acid concentrations.

4.3.4 Micronutrients and Macronutrients phd revised

4.3.4.1. Micronutrients (Zinc (Zn), Iron (Fe), and Copper (Cu))

The findings of analyses of micronutrients were recorded in (Table 15) and displayed in (Figure 9). The findings revealed that the concentrations of Zinc were highest at Kisumu Bay (124.03 mgL-1) and lowest at Ndere Island (83.67 mgL-1), with significant spatial differences (F-stat = 1026.36, $p < 0.0001$). The elevated levels in Kisumu Bay samples suggested potential sources such as industrial discharges, urban runoff, or agricultural activities, while the lower levels in Ndere Island samples could have reflected less anthropogenic influence or dilution effects (FAO, 2016; Van Huis et al., 2013). Iron concentrations were in a range of 3.40 mgL-1 (Kisumu Bay) and 2.28 mgL-1 (Kendu Bay), showing significant variation (F-stat = 322.9, $p < 0.0001$). Higher levels in Kisumu Bay and Homa Bay could have resulted from sediment resuspension or localized pollution. Lower levels in Kendu Bay could have suggested different sediment characteristics or lower pollution levels (Kourimska & Anna, 2016). The highest copper concentrations were found in Kisumu Bay (94.7 mgL-1), while Ndere Island recorded

the lowest (40.43 mgL-1). The significant differences (F-stat = 2026, $p < 0.0001$) could be attributed to mining, antifouling paints, or domestic wastewater discharges. High copper levels could be toxic to aquatic life, affecting respiratory processes and enzyme activity and potentially reducing biodiversity by eliminating sensitive species (Van Huis et al., 2013).

4.3.4.2. Macronutrients (Calcium (Ca), Magnesium (Mg), Sodium (Na), Aluminum (Al), Manganese (Mn) and Cobalt (Co)

The findings of analyses of macronutrients were recorded in Table 15 and displayed in Figure 9. The findings revealed that Calcium concentrations were highest in Kisumu Bay (272.67 mgL-1) and lowest at Ndere Island (135.67 mgL-1). The significant variations (F-stat = 984.3, $p < 0.0001$) suggested differences in geological substrates or anthropogenic activities affecting water hardness (Kourimska & Anna, 2016). High calcium levels were crucial for maintaining aquatic biodiversity as they influenced the presence of species with specific calcium requirements. Magnesium levels were highest in Kisumu Bay (172.67 mgL-1) and lowest at Ndere Island (147.67 mgL-1), with significant differences (F-stat = 61.89, $p < 0.0001$). Magnesium is vital for photosynthesis in aquatic plants and serves as a cofactor in various biochemical processes. Variations in magnesium levels could have reflected site-specific differences in water chemistry or geological factors. Sodium concentrations ranging between 200 mgL-1 (Kendu Bay) and 146.67 mgL-1(Ndere Island) showed significant spatial differences (F-stat = 356.2, $p < 0.0001$). High sodium levels could have affected water salinity, influencing osmoregulation in aquatic species and limiting the presence of freshwater species that were not salt-tolerant. The highest aluminum concentration was recorded in Kisumu Bay (7.53 mgL-1) and the lowest in Kendu Bay (4.16 mgL-1), with significant differences (F-stat = 596.7, $p < 0.0001$). Aluminum is typically toxic to aquatic organisms, especially in acidic waters, and high concentrations can cause physical and physiological stress. Manganese concentrations were relatively low but varied significantly, with the highest levels in Kisumu Bay (0.62 mgL-1) and the lowest in Homa Bay and Kendu Bay. The significant spatial differences (F-stat = 401.2, $p < 0.0001$) could have suggested localized pollution sources or natural geochemical processes, while Cobalt concentrations were highest in Kisumu Bay (0.95 mgL-1) and lowest in Kendu Bay (0.62 mgL-1). The significant variations (F-stat = 258.2, $p < 0.0001$) could have resulted from mining or other metal-related industries.

Table 15. Mean (±STD) of micronutrients and macronutrient concentrations (mgL^{-1}).

	Kisumu bay	**Homa bay**	**Kendu bay**	**Ndere Island**	**F-stat.**	**p-value**
Zn	124.033±3.0	113.367±0.28	96.367 ±0.49	83.667±1.77	1026.36	<0.0001
Fe	3.403 ±0.06	3.323 ±0.03	2.277 ±0.49	2.783±0.10	322.9	<0.0001
Cu	94.700 ±0.42	61.167±1.77	40.533 ±0.49	40.433±0.78	2026	<0.0001
Ca	272.667±3.54	178.33±3.53	253.00±3.53	135.667±4.24	984.3	<0.0001
Mg	172.667±4.24	156.667 ±1.41	166.33 ±0.71	147.667±2.12	61.89	<0.0001
Mn	0.62±0.06	0.0867±0.01	0.153±0.02	0.117±0.01	401.2	<0.0001
Al	7.533±0.14	2.433±0.14	4.167±0.07	4.143±0.02	596.7	<0.0001
Co	0.953±0.01	0.7530±.01	0.62± 0.01	0.66±0.03	258.2	<0.0001
Na	186.0±2.82	153.0±3.54	200.0±3.54	146.667±2.83	356.2	<0.0001

NOTE: Significant spatial differences ($p<0.0001$) are depicted by different subscripts.

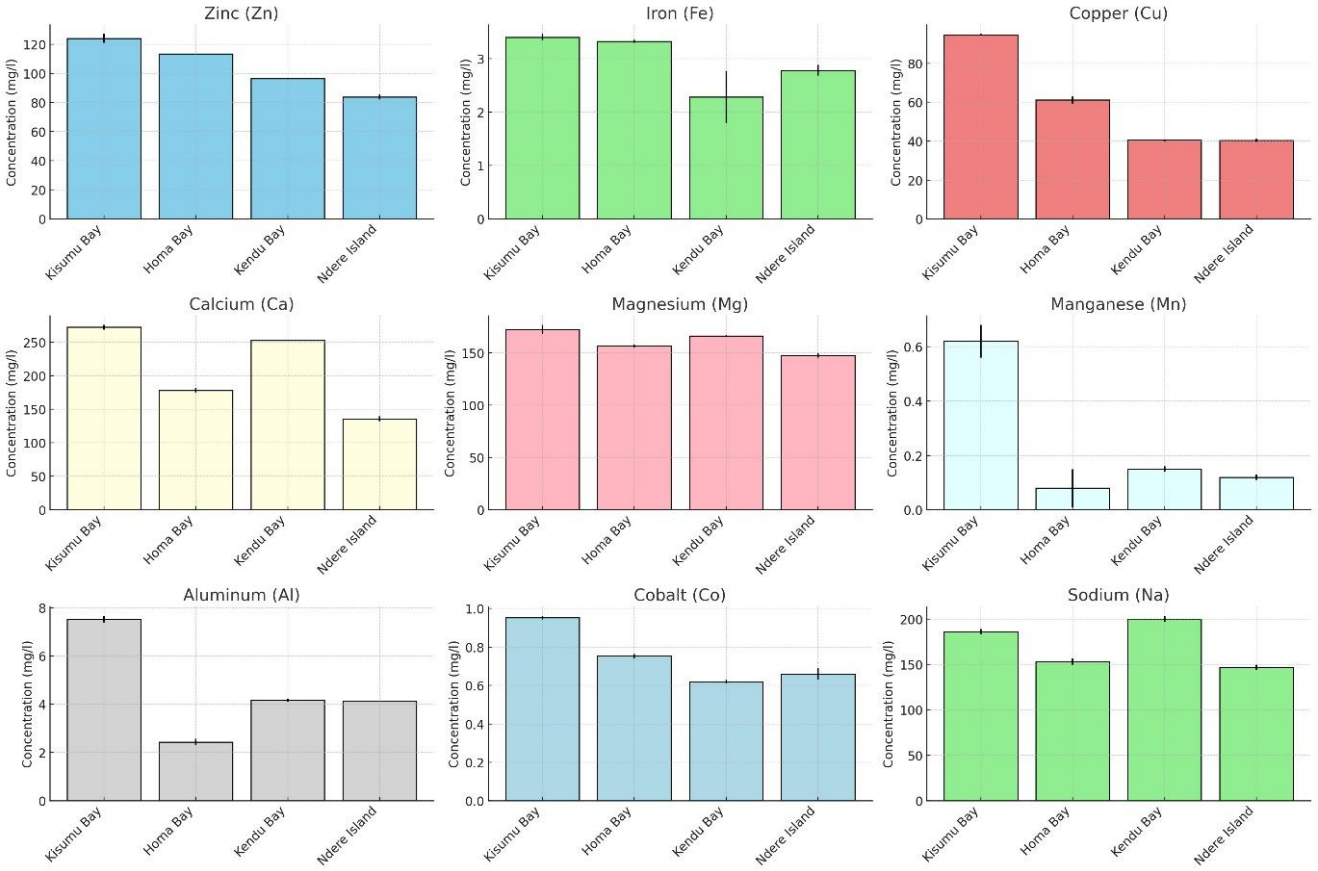

Figure 9. Bar Graphs of variation in micronutrients and macronutrients across different sampling sites. Each nutrient will be represented by a separate bar graph

4.4 Effect of Pollution on Nutritional Status of Non-biting midges

4.4.1. Effects of Pollution on Amino Acids

4.4.1.1. Effects of In-Situ Parameters on Amino Acids

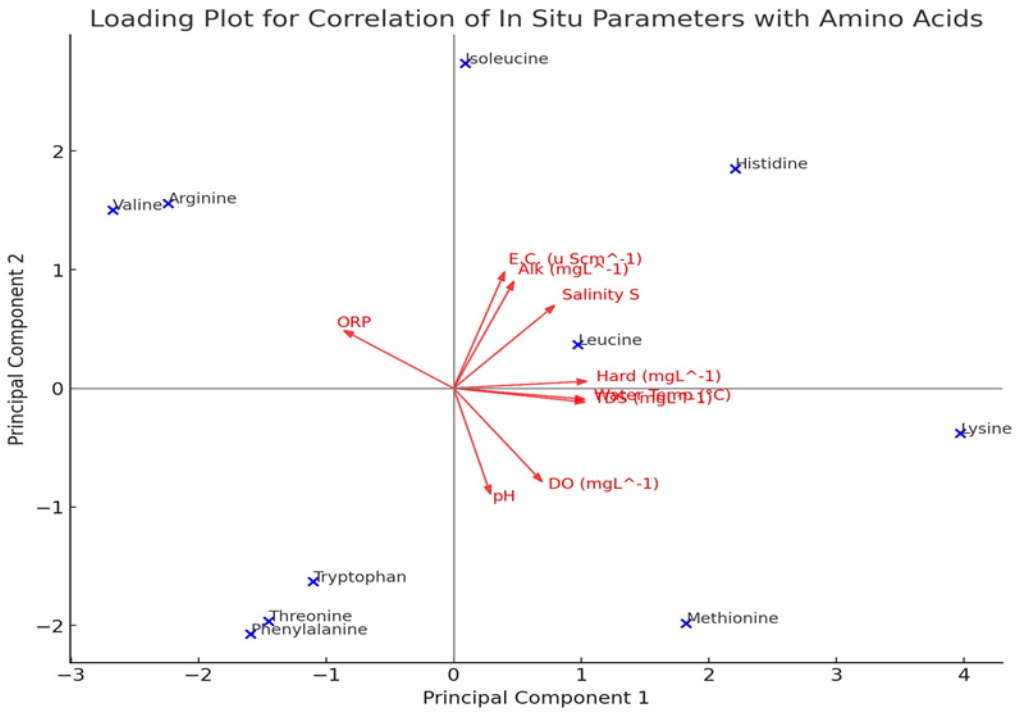

Figure 10. The loading plot above represents the correlation between in situ parameters (such as water temperature, pH, and others) and amino acids using Principal Component Analysis (PCA).

The PCA gave an example of Amino Acids (Blue) position in relation to the principal components (PC1 and PC2) signifying the extent in variance of Correlation with the in-situ parameters as depicted in fig: 10. In-situ Parameters (Red Arrows) indicated the loadings (correlation coefficients) of every parameter with the principal components. Each arrow meant the degree and direction of the parameter that affected the amino acids. Which the findings of the current study showed that while parameters like Hardness(mgL-1), TDS(mgL-1), water temperature (°C) and dissolved oxygen (mgL-1) had somewhat similar effect to the principal components, ORP and pH had a positive and negative impacts respectively to the amino acids and had varying effects.

The influence that in-situ parameters had on amino acids was also modelled by the Pearson correlation coefficient. Lysine had a strong positive direct correlation with water temperature. The same trend was observed in isoleucine, histidine and E.C and TH. On the other hand, D.O. and p H had a negative correlation in isoleucine. The dispersion between water temperature, D.O., TH and TDS was negative with valine as well.

4.4.1.2. Relationship between the Nutrients and Chlorophyll on Amino Acids.

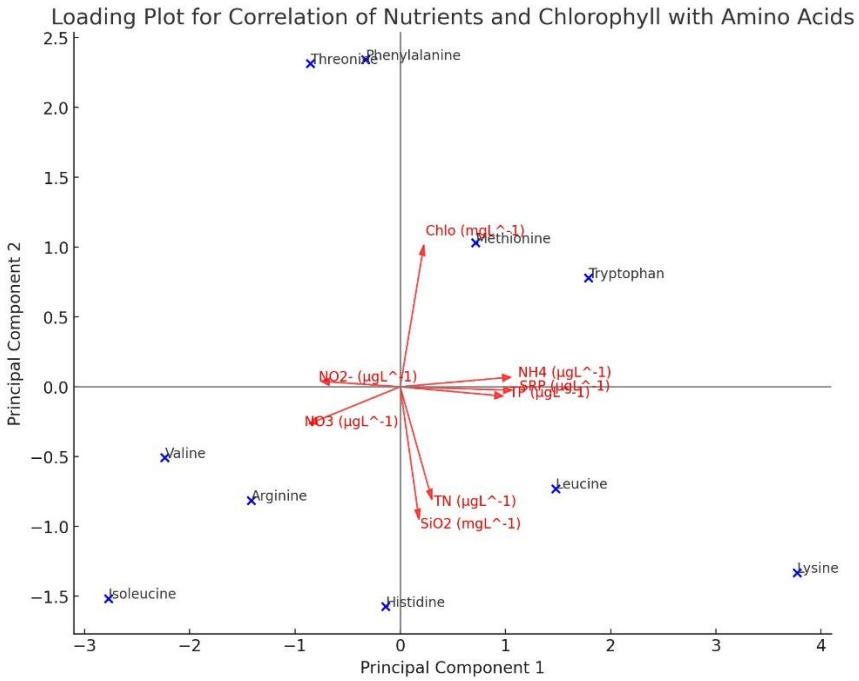

Figure 11. Nutrients and Chlorophyll (in red) with the correlation coefficients of each nutrient or chlorophyll parameter with the principal components.

Amino Acids (Blue Points) are shown as amino acids based on their first two principal components, PC 1 and PC 2, which reveal whether they are similar or different regarding their degree of association with nutrients and Chlorophyll (Figure 11). Nutrients and Chlorophyll (Red Arrows) showed the loadings (correlation coefficients) of each nutrient or chlorophyll parameter with the principal components: Whereas, the parameters like NO^{2-} (µgL-1), NO3 (µgL-1), TN (µgL-1), SiO_2 (mgL-1) and TP (µgL-1) have similar direction explaining similar influence on amino acids as whereas Chlo (mgL-1) have different direction which indicate different influence as compare to other parameters. The PCA helped to determine connections between the different nutrients and the intensity of chlorophyll depending on the various amino acids, for which the relationships that could not be easily observed from the data alone are shown.

The current study also showed that nutrients enhanced and inhibited amino acids, though the nitrites did not significantly affect the amino acids. As can be seen, nitrates impact isoleucine and TN on tryptophan. SRP impacted lysine, valine, and isoleucine, while TP and NH4 impacted lysine, valine, and isoleucine. The results reveal that silicates impacted leucine,

arginine, methionine, threonine, and phenylalanine of lactose. This influenced methionine, threonine, phenylalanine, and isoleucine, as well as mean levels of Chlorophyll. The varied levels also had an effect.

4.4.1.3. Effects of Heavy Metals in Sediment on Amino Acids

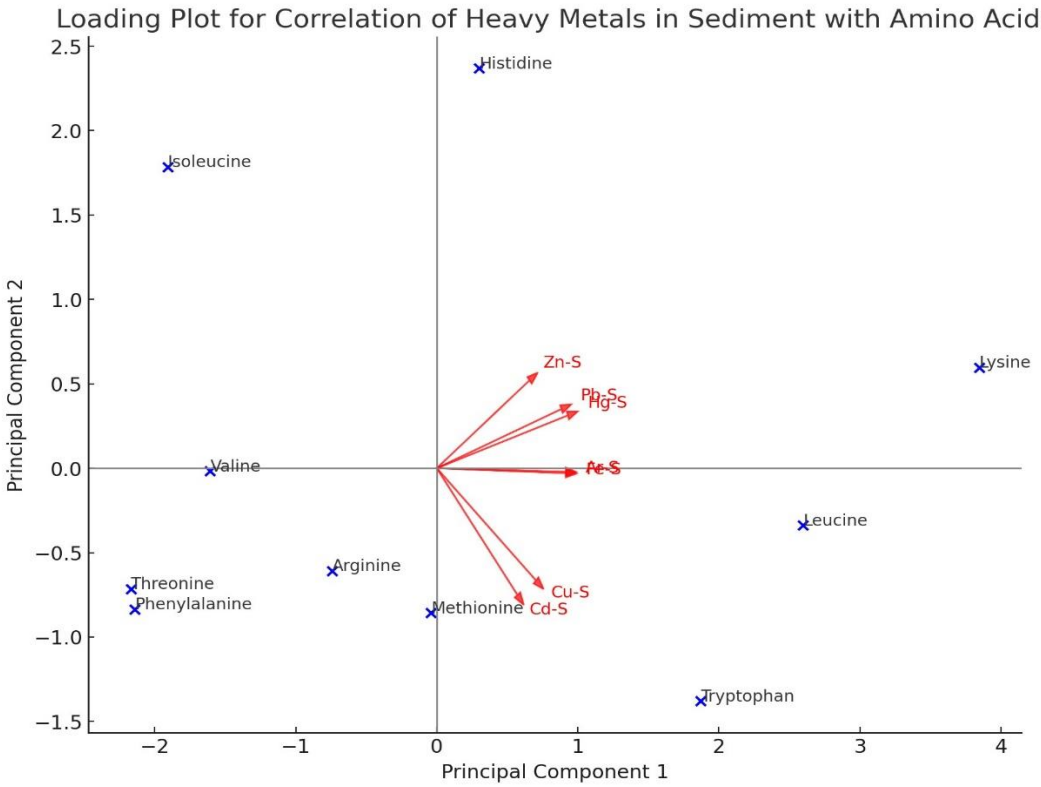

Figure 12. Heavy metals in sediment (in red) represented correlation coefficients of each heavy metal with the principal components

Amino Acids (Blue Points) were amino acids projected based on their principal components, namely PC1 and PC2, depending on their proximity or otherwise with the vector about the heavy metals, as shown in Figure 12. Heavy Metals (Red Arrows) gave the respective heavy metal loadings (correlation coefficients) with the principal components. The length of the arrows signifies the strength of the influence of each metal on each amino acid, and the direction of the arrows pointed toward the metal shows the relative influence. Elements like Ar-S, Hg-S, Pb-S, and Zn- S had very close proximity, indicating they could have had similar impacts or even had the amino acids in synergy. The Cd-S and Cu-S trends were opposite, meaning they differed in influence. The plot helped give an overall view of the relations between the various concentrations of the specific and overall heavy metals in the sediment

and the amino acids, especially in shapes that are not easily discernible from the two separate parallel quantity-quantity data series.

Regression analysis of Heavy metals from sediment samples showed positive and negative correlations with amino acids. A positive relationship with lysine, leucine, and tryptophan was revealed for heavy metals at the 0.05 significance level. Still, arginine, valine, threonine, phenylalanine, isoleucine, and histidine had an adverse effect. However, the model had a statistically significant impact on arsenic (Ar) related to lysine, leucine, and threonine. Lead (Pb) and Cadmium influenced the valine, isoleucine, histidine, and Isoleucine; Pb influenced the lysine, and Cd influenced the tryptophan.

4.4.1.4. Effects of Heavy Metals in Water on Amino Acids

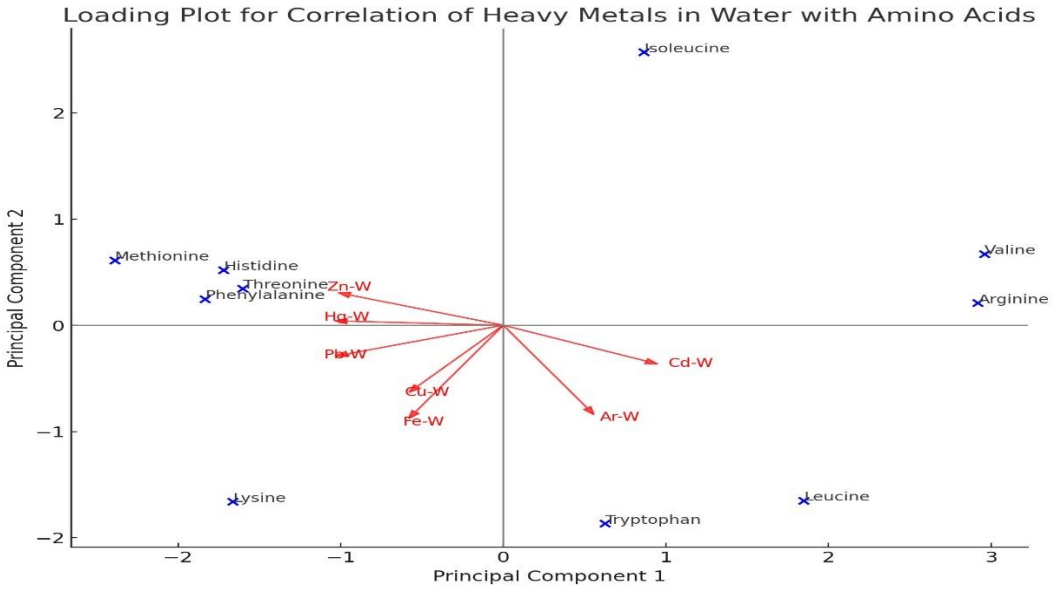

Figure 13. Heavy metals (in red) represented the correlation coefficients of each heavy metal-in water with the principal components.

The position of amino acids (blue points) in terms of their first two principal components (PC1 and PC2), which represent their resemblance or dissimilarity to the heavy metals in water, is illustrated in Figure 13. Heavy Metals (Red Arrows) depicted each heavy metal's loading or correlation coefficient with the principal components. The length and direction of each arrow represented the nature and extent to which each metal affected the amino acids. It again indicated that the Zn-W, Hg-W, Pb-W, and Fe-W may have had similar effects or relationships with the amino acid as the points are clustered, and Cd-W and Ar-W had opposite directions, showing different impacts than the other metals. The plot of this task offered a clear map of

how various heavy metals in water relate to amino acids and what kind of patterns or relations cannot be understood from the densely distributed data sets.

Heavy metals in water both enhanced and inhibited the synthesis of amino acids. Hg and Pb significantly influenced the synthesis of arginine and valine. Nonetheless, Ar was found to have an approximate statistically significant positive impact on arginine, valine, and tryptophan, while for Cd, there was no clear distinction in its impact.

4.4.2. Effects of pollution on Vitamins

4.4.2.1. Effects of In-situ Parameters on Vitamins

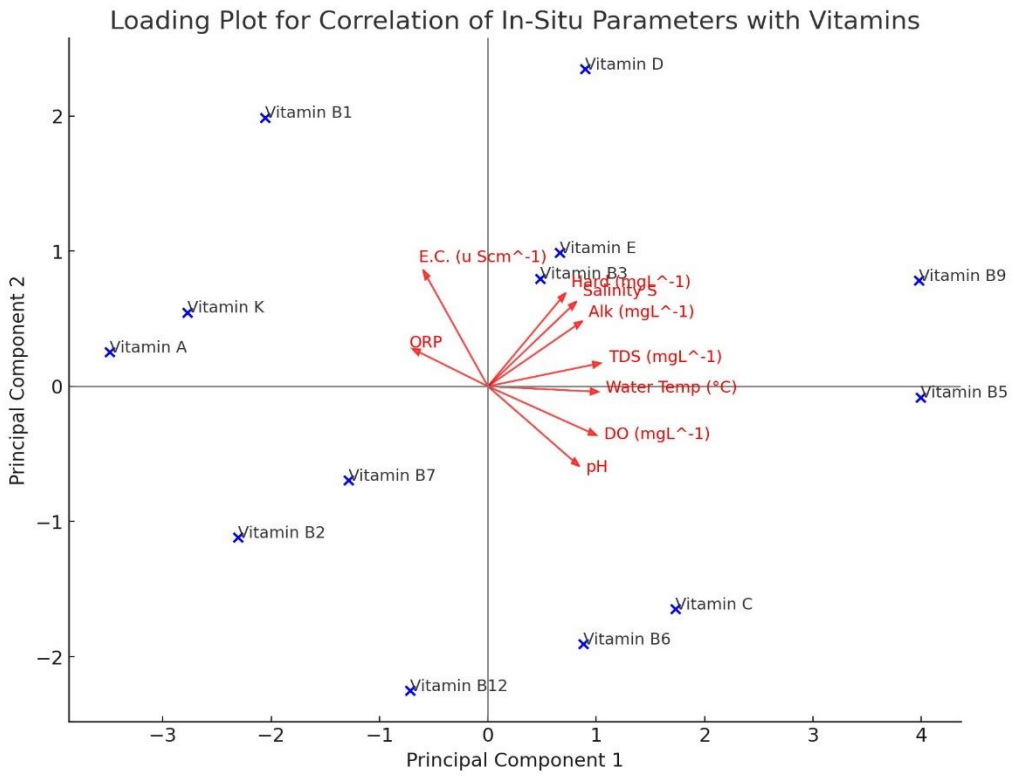

Figure 14. *In-Situ* parameters (in red) represented the correlation coefficients of each parameter with the principal components**.**

Vitamins (Blue Points) are located as per their principal components (PC1 & PC2) (Figure 14). The points' position showed their similarity or dissimilarity about the in situ parameters. In-situ parameters (Red Arrows) indicated the factor loadings (correlation coefficients) of all the parameters with the components. The length of an arrow pointing from a specific in-situ parameter to a vitamin indicated the relative level of influence exerted on that vitamin by the

in-situ parameter. In contrast, the direction of the arrow pointed to a vitamin represented the direction of the effect that the in-situ parameter had on the vitamins. It was also observed that the distribution of vitamins was significantly affected by some chemical and physical factors such as E.C. (u Scm-1), ORP, TDS (mgL-1), Water Temp (°C), and pH. Compared with other vitamins, there were differences in the positioning of Vitamin B5 and Vitamin B9, suggesting different correlations with in-situ data.

The current study also showed that T.H. and pH only significantly affected water-soluble vitamins. Usually, the result of Vitamin B5 changes with the increase of pH, while the result of B2 changes with the rise of T.A. These vitamins were vitamin A, B1, B5, B7, and B9, and the effect of Temperature was significant. Out of all the studied in-situ parameters, dissolved oxygen, available phosphor, total alkalinity, and total dissolved salts strongly negatively impacted Vitamin A, K, B2, B6, and B12. As a result, there was a positive impact on B9, indicating that D.O. and T.H. affected this factor. Specifically, it was revealed that the in-situ parameters exert a statistically insignificant influence on Vitamin C and B3. Finally, I was able to see the impact of Temperature, D.O., T.H., and TDS on water-soluble and fat-soluble vitamins on

4.4.2.2. Effect of Nutrients on Vitamins

Vitamins (Blue Points) position them according to their principal components, PC 1 and PC 2. This positioning represented their similarities or differences concerning the nutrients and chlorophyll. Nutrients and Chlorophyll (Red Arrows) indicated the loadings (correlation coefficients) of the nutrients or chlorophyll parameters with the principal components (Figure 15). The lengths of the arrows showed the degree of influence the respective parameter had on the vitamins, and the orientation of the arrows showed the direction. The variables like NO3, NH4, SRP, TP, and SiO2 in the clusters are µgL-1 and mgL-1; these parameters highly correlate with the vitamins noted above. Some vitamins, such as Vitamin B5 and Vitamin B9, were differentiated to imply that they correlate with different nutrient parameters compared to other vitamins.

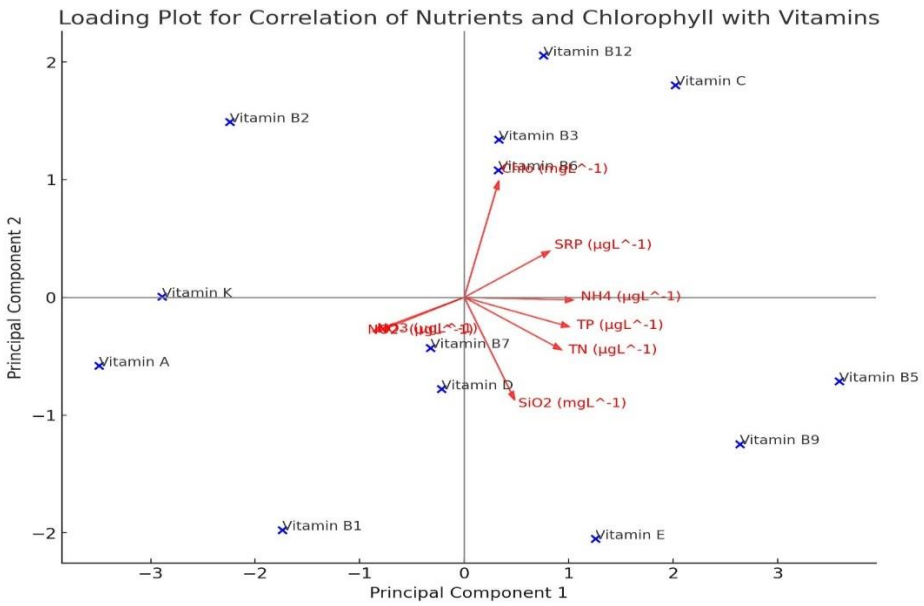

Figure 15. Nutrients and chlorophyll (in red) represented the correlation coefficients of each nutrient or chlorophyll parameter with the principal components.

About the nutrients in the water, it was observed that they affect both the water-soluble as well as the fat-soluble vitamins in both positive and negative ways. For example, all nutrients that hurt Vitamin A were nitrates and nitrites. A similar trend was observed in the TN, SRP, TP, and NH4 effects, which were expected to decrease with an increase in the treatment level. Nonetheless, little impact was observed in vitamin A and Vitamin E, which were influenced only by Silicates, while SRP and NH4 influenced vitamin K.

A Negative effect was observed in water-soluble vitamins, though the change was insignificant. However, deviations were posted in Vitamin C, B1, B3, and B6. Out of all nutrients, silicates had a higher and statistically significant negative correlation with WSVs. TN, TP, SIO_2, and SRP also hurt B2 and B12. However, NO^3 and NO_2 positively affected B5 AND B9. SRP, TP, and NH4 positively correlated with B5, while NO^3, SiO_2, and chlorophyll positively correlated with B12.

4.4.2.3. Effects of Heavy Metals in Sediment on Vitamins

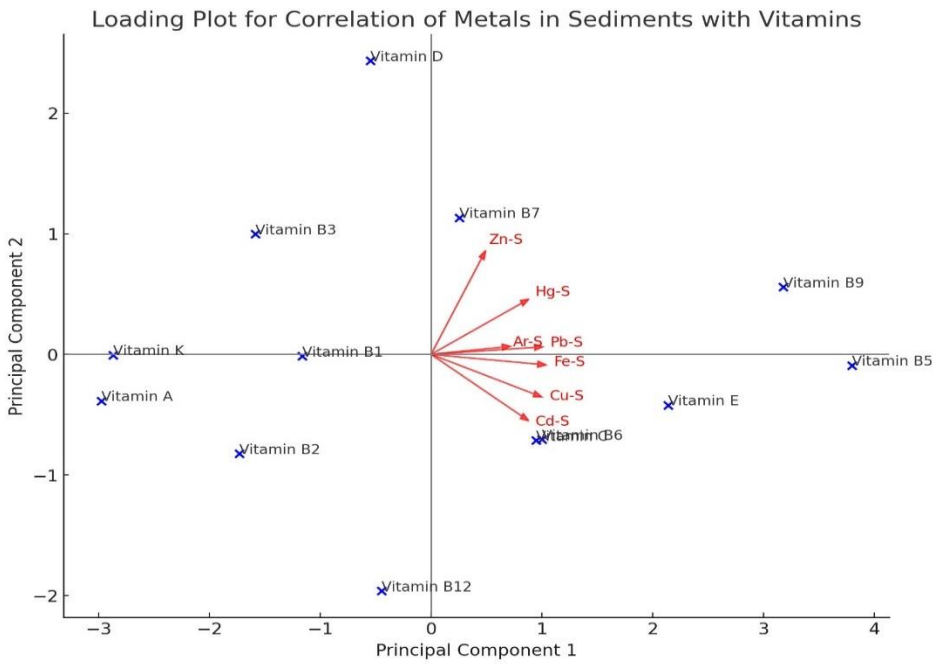

Figure 16. Heavy Metals in Sediments (in red) represented the correlation coefficients of each metal with the principal components.

Vitamins (Blue Points) are shown about the principal components, namely, PC1 and PC2. Their position meant that they were similar or dissimilar concerning the metals in sedimentation. PCA loadings or eigenvalues (correlation coefficients) for Metals in Sediments (Red Arrows) are presented in Figure 16. The longer the arrow and the direction it was drawn, the higher the level and direction of each metal's influence on the vitamins. Ar-S, Hg-S, Pb-S, Fe-S, and Cd-S were clustered together, which implied that the metals had a similar effect or relationship with the vitamins. While positioning other vitamins, Vitamin B9, Vitamin B5, and Vitamin E separately indicated that they may have different associations with these metal parameters than the other vitamins. Concerning the correlation between sediment analysis and Vitamins, they found that all four heavy metals, Ar, Hg, Pb, and Cd, had a negative impact. On the contrary, Ar hurt A, K, B1, B2, and B5, but Ar had a positive effect on vitamins B5, B9, D. Hg reduced vitamins A, D, K, B1, and B2; however, increased vitamins B5, B7, B9, and vitamin D. Contrary to this, Cadmium increased vitamins C, E, B3, B5, B6 and B9 but reduced vitamin A, D, K, B1, B2, B3. The analysis indicated that the relationship between Cd vitamin D and vitamin B had a significant impact.

4.4.2.4. Effects of Heavy Metals in Water on Vitamins

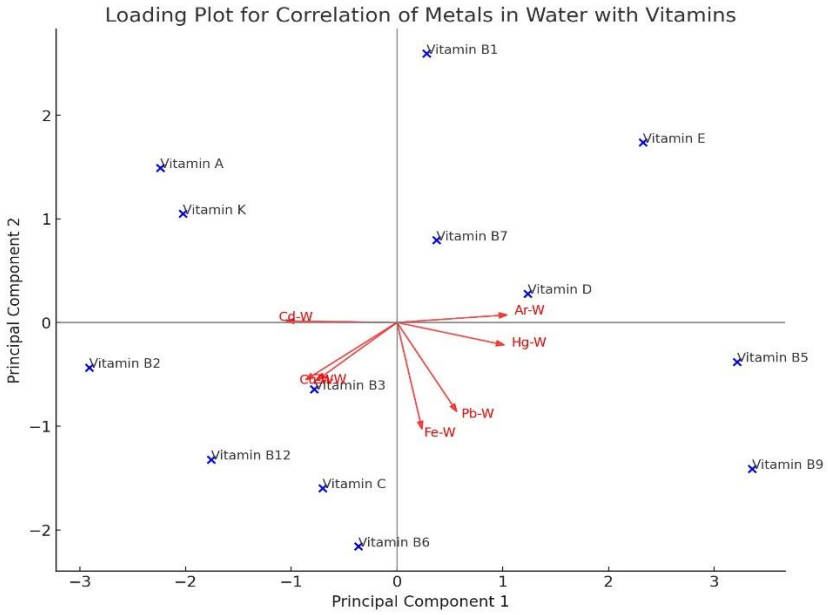

Figure 17. Metals in water (in red) represented the loadings (correlation coefficients) of each metal with the principal components.

Vitamins (Blue Points) are represented by means of their principal component analysis (PC1 and PC2). The position of these points gives a relationship or dissimilarity regarding the metals in water. The loadings (correlation coefficients) of metals in Water (Red Arrows) are shown in Figure 17. The length of an arrow represented the degree of influence each metal had on a given vitamin, while the direction represented the influence. The metal ions such as Ar-W, Hg-W, Pb-W, and Fe-W were clustered closely, implying that they have similar effects or relationships with the vitamins. The vitamins are likely to have been positioned differently, suggesting that Vitamin E, Vitamin B5, and Vitamin B9 had different relations to the metal, as mentioned earlier, parameters than other vitamins.

The heavy metals in the water, therefore, had negligible significant effects on Vitamins in the insect samples, especially Hg and Cd. However, Ar significantly affected vitamins only in a positive way, while Pb influenced vitamins both positively and negatively. Ar impacted parameters such as B2 and B9, while Pb impacted vitamins like A, C, K, B1, B5, and B6.

4.4.3. Effects of Pollution on Fatty Acids

4.4.3.1. a) Effects of in-situ parameters on Saturated Fatty Acids, SFA and Monounsaturated, fatty acids, MUFA

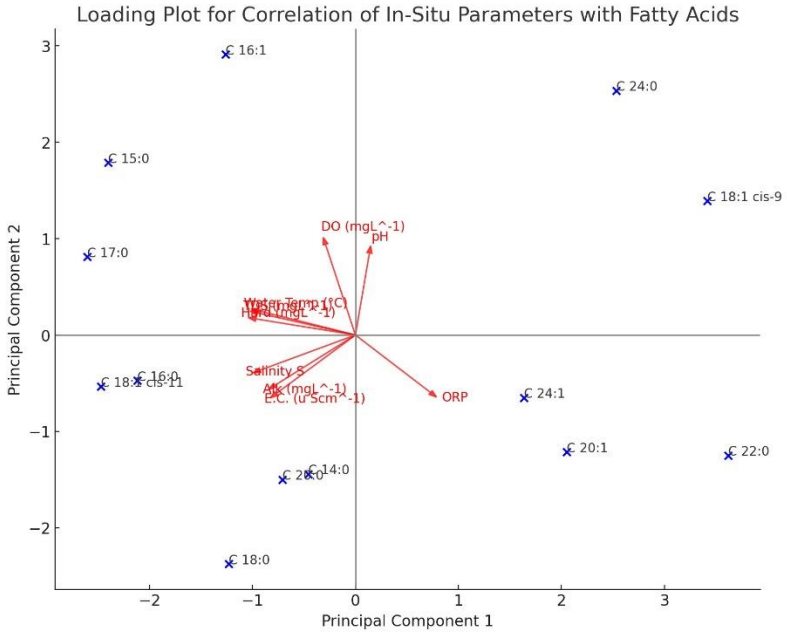

Figure 18. *In-Situ* Parameters (in red) represented the correlation coefficients of each parameter with the principal components

Fatty Acids (Blue Points) are plotted according to their principal components (PC1 and PC2). The positioning of the points indicated their similarities or differences in correlation with the in-situ parameters. In-situ parameters (Red Arrows) represented the loadings (correlation coefficients) of each parameter with the principal components (Figure 18). The length and direction of each arrow indicated the strength and direction of influence each parameter had on the fatty acids. Parameters like Water Temp (°C), pH, DO (mgL-1), and ORP were grouped closely, indicating similar influences or correlation patterns with the fatty acids. Fatty acids such as C 24:0, C 18:1 cis-9, C 16:1, and C 15:0 were positioned differently, suggesting distinct relationships with the in-situ parameters compared to other fatty acids.

In-situ parameters had a significant effect on fatty acids except pH. In particular, in-situ parameters considerably affected SFA (C15:0, C17; C22:0) except for ORP, TH, and water temperature. A correlation was also observed in the influence of TA and TH on C14:0, C18:0, C20:0, C24:0, C15:0, C17:0, and C17:0, respectively. A positive correlation was also

observed in the effect of DO (C15:0), TDS (C15:0, C17:0), EC (C18:0, C20:0), and Salinity(C18:0) on SFA.

In-situ parameters also affected MUFA, except for pH. Temperature had a negative impact except for C24:0, and C18:1, EC affected C18:1 cis 11, C18:1 cis 9, while DO only affected C16:1. Similarly, a positive association was observed in TH (C16:0, C18:1 cis 11), TA (C18:1 cis; C18:1 cis 11), salinity (C18:1 cis 11), and TDS (C16:1, C18:1 cis 11). The most affected MUFA was C18:1 cis 11.

4.4.3.1. b) Effects of in-situ parameters on PUFA Omega -3 and Omega- 6 fatty acids

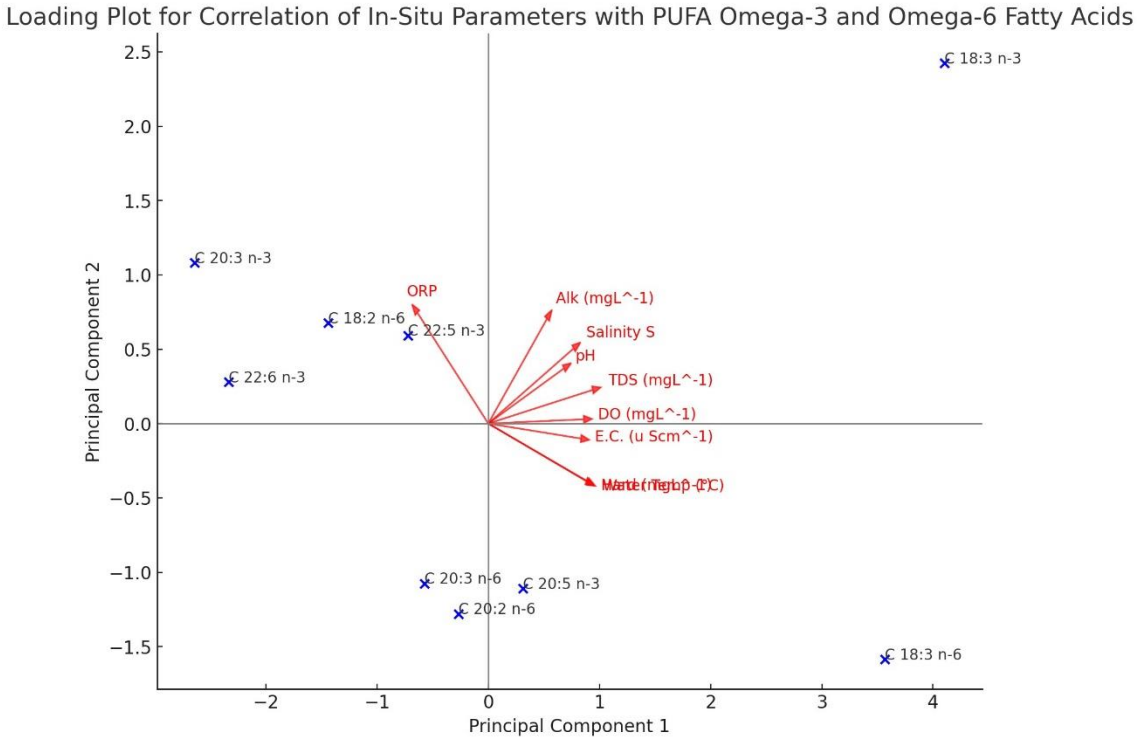

Figure 19. *In-Situ* Parameters (Red Arrows) represented the loadings (correlation coefficients) of each parameter with the principal components (Figure 19).

Fatty Acids (Blue Points) are plotted according to their principal components (PC1 and PC2). The positioning of the points indicated their similarities or differences in correlation with the *in-situ* parameters. *In-situ* parameters (Red Arrows) represented the loadings (correlation coefficients) of each parameter with the principal components (Figure 19). The length and direction of each arrow indicated the strength and direction of influence each parameter had on the fatty acids. Parameters such as ORP, pH, Alk (mgL-1), Salinity S, and TDS (mgL-1) had strong directional influences, suggesting they were significant factors in determining the

distribution of fatty acids along the principal components. Fatty acids like C 20:3 n-3, C 22:6 n-3, and C 18:3 n-6 were positioned differently, indicating distinct relationships with the *in-situ parameters*.

PUFA omega 3 was significantly affected by *in-situ* parameters except for pH, and E.C. D.O. and ORP revealed both negative and positive significant effects on C20:5n-3, respectively. The total hardness had a negative and positive influence on C20:3n-3, C22:5n-3, and C22:6n-3, respectively, while T.A. affected C20:5n-3, C22:5n-3, and salinity C20:5n-3. A strong correlation was observed in the effect of TDS on C22:3n-3 and C22:6n-3.

In-situ parameters had both positive and negative effects on polyunsaturated omega-6 fatty acids. However, the impact of P.H., E.C., D.O., and ORP on *PUFA omega 6* was insignificant. Water temperature, E.C., and T.H. had no significant effect on the fatty acids except for C18:2n-6, C18:2n-6, and C18:2n-6, respectively. Consequently, salinity only impacted C18:2n-60, while TDS affected C20:2n-6 and C20:3n-6. T.H. had the most significant effect on omega-6 fatty acids (C18:3n-6, C18:2n-6, C20:2n-6, and C20:3n-6. In general, the *in-situ* parameters significantly negatively impacted the omega-6 fatty acids.

4.4.3.2.a) Effects of Nutrients and Chlorophyll on Saturated Fatty Acids, SFA and Monounsaturated Fatty acids, MUFAs.

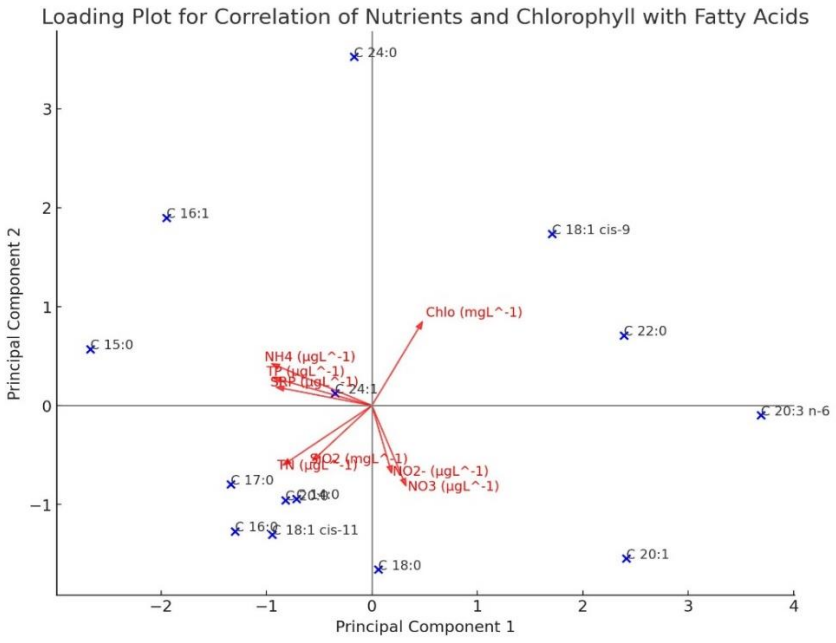

Figure 20. Nutrients and Chlorophyll (in red) represented each nutrient or chlorophyll parameter's correlation coefficients with the principal components.

Fatty Acids (Blue Points) are categorized and shown based on their first two significant axes of variation, PC1 & PC2. The positions were related to the similarities or differences highlighted in their nutritional and chlorophyll values. All nutrient and chlorophyll data (Red Arrows) indicated loadings or correlation coefficients of the individual nutrient or chlorophyll parameters with the principal components (Figure 20). The length of the arrow pointed towards the type and intensity of influence every parameter had on the fatty acids, while the direction explained which way the influence would go. The Chlo (mgL-1) values were significantly related to the first principal component (PC1) and, therefore, exerted a different impact on some of the fatty acids, such as C 20:3 n-6. As factors influencing the distribution of FAA along the principal components, nutrients including NO3 and NO2 and TN and SRP were grouped as having similar properties, such as µgL-1. The ranking of the fatty acids such as C 24:0, C 20:3 n-6, and C 18:1 cis-9 was different and interacted differently with the nutrient factors compared with other fatty acids.

The positive and negative effects of nutrients were established in fatty acids, as outlined in Figure 20. However, some effects were insignificant statistically. For instance, nitrates and nitrites had no significant effect on *SFA*. A positive association was observed in TN (C15:0; C16:0), SRP(C15:0), TP(C15:0), NH4(C15:0), SiO2 and chlorophyll(C14:0). Silicates had a positive impact SFA (C18:0, C20:0 contrary to chlorophyll with negative effects (C18:0, C16:0). Chlorophyll had an effect on C14:0, C16:0, while NH4(C18:0) and SiO2(C22:0. C24:0 positively correlated with the nutrients except for nitrates, nitrites, and total nitrogen. Nutrients affected *MUFA*. However, NO3and TN affected C20:0 and C18: cis 11, respectively, while SRP, TP, TP, and NH4 affected C16:1. Silicates hurt C18:1 cis 9, C20:1, and C24:1. while Chlorophyll affected C18:1 cis 9 and C18:1 cis11(r =-0.51). NO2, SRP, and NH4 affected fatty acids except for C20:1, which NO3 and NO2 negatively influenced.

4.4.3.2.b) Effects of nutrients and chlorophyll on PUFA -Omega-3 and Omega-6 fatty acid

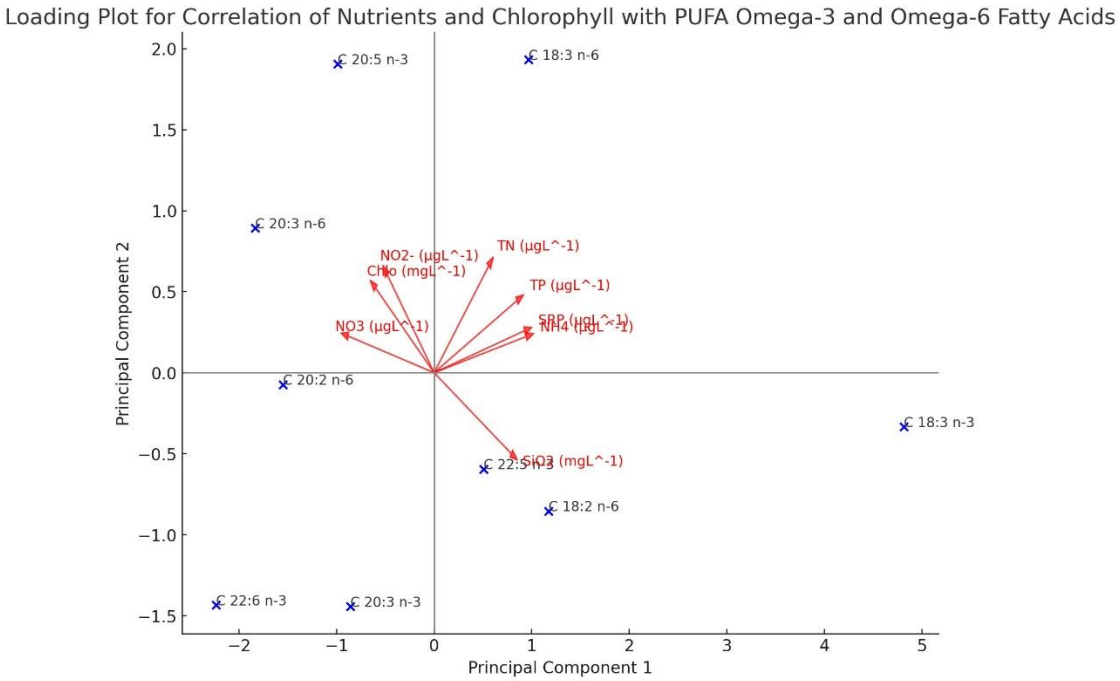

Figure 21. Nutrients and chlorophyll (in red) representing the correlation coefficients of each nutrient or chlorophyll parameter with the principal components

Fatty Acids (Blue Points) refer to a PUFA Omega-3 or Omega-6 fatty acid that is placed based on its principal component 1 and principal component 2. The positioning of the points reflected their similarities or differences to the nutrients and chlorophyll. Principal Component Loadings (Correlation Coefficients) The nutrients and chlorophyll (Red Arrows) in Figure 21 depicted the loadings of each nutrient or chlorophyll parameter with the principal components. The length and direction of each arrow coronates the strength and direction of the influence of each parameter on the fatty acids. Based on these general distances, it is evident that NO3 (µgL-1), NO2- (µgL-1), Chlo (mgL-1), and SRP (µgL-1) were grouped nearer, and these were highly influential for the distribution of fatty acids concerning the two principal components. Concentrations of total dissolved SiO2 (mgL -1) were negatively correlated with the first PFA principal component (RI. PCA1), indicating that it affects fatty acid profile, particularly C 20:3 n- 6 and C 18: 2n- 6, in a unique way. Concentrations of fatty acids, including C 20:5 n-3, C 18:3 n-6, and C 18:3 n-3, were not in the right relationship to the nutrient parameters.

NO2 and chlorophyll had no effect on PUFA omega 3, while NO2, NO3, TP, and TN had no effect on PUFA omega 6. Nutrients did not affect C18:2n-6.

4.4.3.3.a) Effects of Heavy Metals in Sediment on Saturated Fatty Acids, SFA and MUFAs.

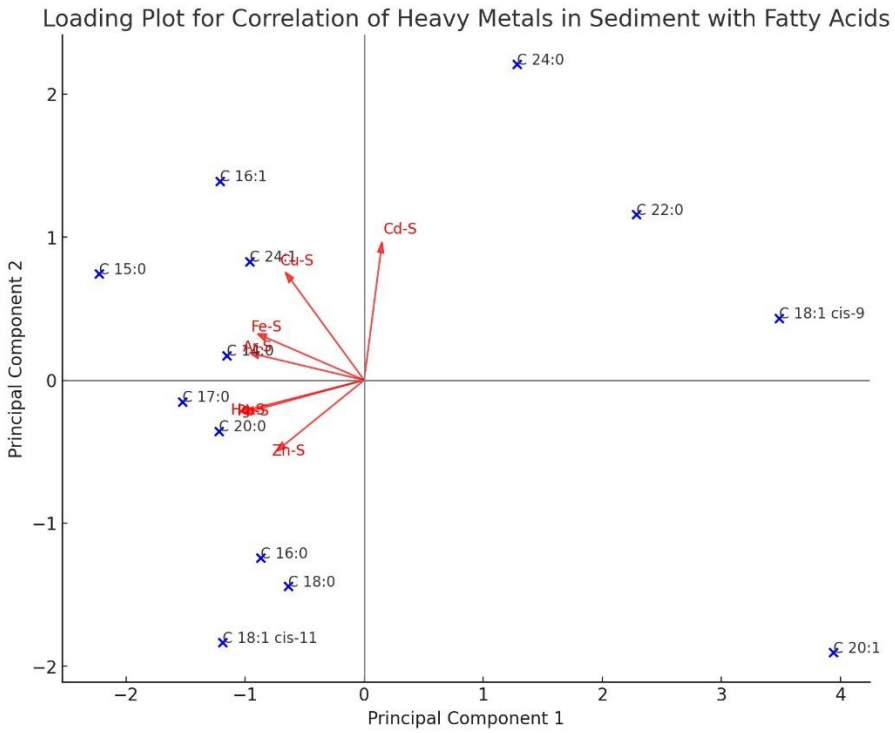

Figure 22. Heavy metals in sediment (in red) represented the correlation coefficients of each metal with the principal components**.**

Fatty Acids (Blue Points) indicated fatty acids, and the positions of these points were determined using the primary components: PC1 and PC2. The positioning of the points referred to their similarities or differences in heavy metals in sediment. Heavy Metals in Sediment (Red Arrows) meant the loadings (correlation coefficients) of each metal on the principal components (Figure 22). The length of every arrow pointed toward the degree of each metal's impact on the fatty acids. In contrast, the direction showed which metal affected them positively or negatively. Cd-S and Cu-S are located closer to the axis of PC1 and PC2, implying that they play a more profound role in the specific FA. Similarly, Ar-S, Fe-S, and Hg-S were situated nearby, meaning that these three factors have a similar impact on the distribution of fatty acids. Other fatty acids, such as C 24:0, C 22:0, and C 18:1 cis-9, were located farther from the middle, indicating that they were related to specific heavy metal parameters differently from the other fatty acids. Heavy metals had an effect on SFA except for C16:0, C22:0, and C24:0-Ar), (C24:0-Hg-s), (C24:0-Pb-s), and Cd-s had insignificant effects. Amongst the MUFA, Ar-s had an except C16:1, C18:1 cis 11, Hg-s had no impact, Pb-s had an effect except C24:1 and Cd-s had no effect except C16:1.

4.4.3.3.b) Effects of Heavy Metals in Sediment on PUFA Omega 3 and Omega Fatty Acids

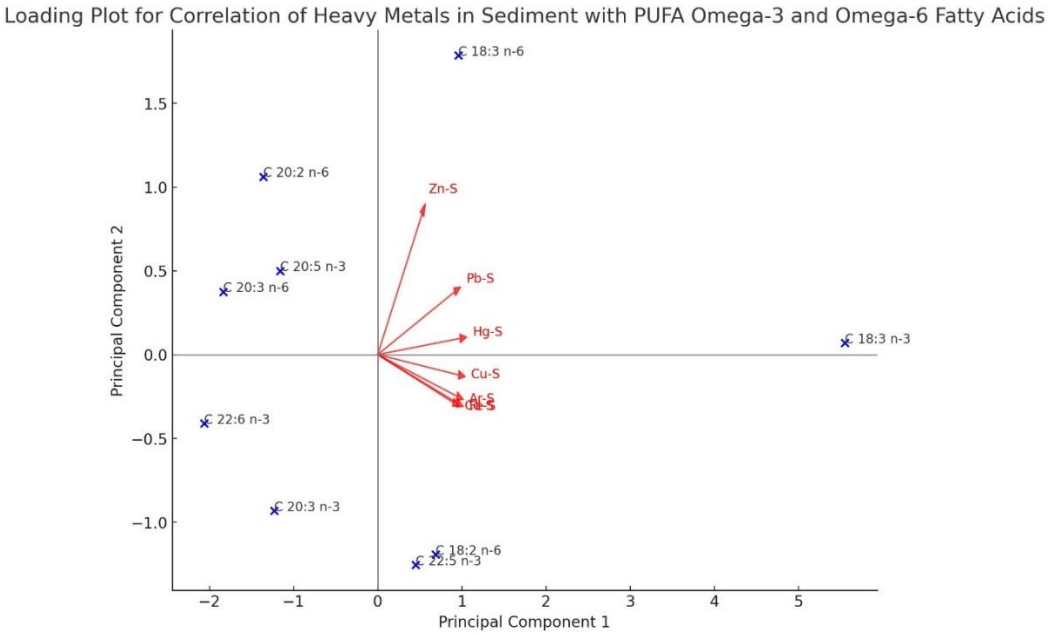

Figure 23. Heavy Metals in Sediment (in red) represented each metal's correlation coefficients with the principal components**.**

Sampled Fatty Acids (Blue Points) quantified PUFA Omega-3 or Omega-6 fatty acid using its principal components (PC1 and PC2). Such positioning of the points demonstrated whether the sites were similar or different regarding the heavy metals in sediment. Loadings (correlation coefficients) of each metal from the principal components were depicted in sediment samples and Heavy Metals in Sediment (Red Arrows) in Figure 23. As found out, the concentration of heavy metals in the sediments was relatively high. The length of each arrow represented the extent of influence a particular metal had on the fatty acids, while the direction pointed to the fatty acids. Pb-S, Hg-S, Zn-S, Cu-S, and Fe-S had different directions, showing that these metals have differential effects on fatty acids. C 18:3 n-3 and C 18:3 n-6 were located at various positions from others, indicating different correlation levelsels of these fatty acids with specific heavy metal parameters with other fatty acids. That heavy metals are grouped in a relatively tight cluster means they had pretty similar patterns of responses to some fatty acids. In contrast, fatty acids are more dispersed, meaning they have different response patterns to the metals.

Ar-s, Hg-s, and Pb-s had a significant effect on Omega 3 and Omega 6 except for C22:5n-3(Ar-s) and C18:2n-6(Ar-S),18:3n-6(Hg-S), C18:3n-6(Pb-S), and C18:3n-3 (Hg-S). Cd-s had the least statistically significant effect on PUFA omega 3 and PUFA omega 6.

4.4.3.4.a) Effects of Heavy Metals in Water on Saturated Fatty Acids, SFA and MUFA

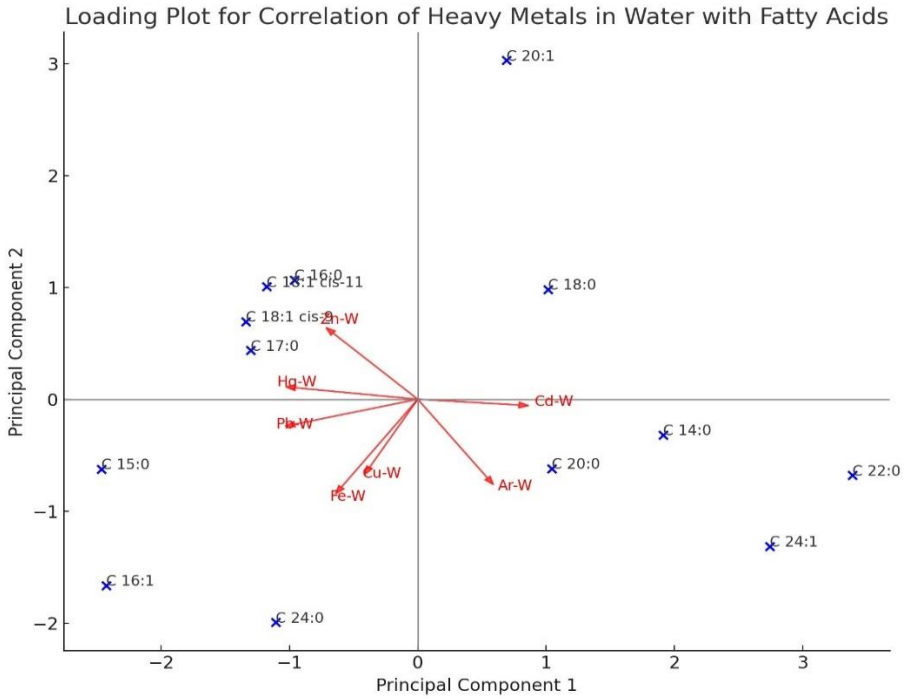

Figure 24. Heavy Metals in water (in red) represented the correlation coefficients of each metal with the principal components.

Fatty Acids (Blue Points) were plotted based on their principal components, Principle Component 1 and Principle Component 2. The position of the points showed their similarity or differences in their relation to the concentration of heavy metals in water. The TP 5 study of Heavy Metals in Water with red arrows indicated the loadings or correlation coefficients of the metals to the principal components of the elements, as shown in Figure 24. The length of each arrow represented the degree of influence the particular metal exercised on the fatty acids. In contrast, the direction of the arrow indicated the direction of that influence the Zn-W, Cd-W, and Cu-W- in all different directions, which signifies that they affect fatty acids differently. Among them, fatty acids like C 22:0, C 24:1, and C 20:1 were far from the center. They indicated different relationships with other fatty acids' various heavy metal parameters. Hg-W, Pb-W, Ar-W, and Fe-W have a similar relationship to the fatty acids, depicted by the

distribution map of the fatty acids showcasing how they differed in moving relation to these metals.

Heavy metals (Ar-w, Hg-w, Pb-w, Cd-w) had a significant effect on SFA (C15:0; C22:0-Hg-W) and(C15:0, C22:0-Cd) and MUFA(C24:1; C20:1; C18:1 cis 9-Ar-w),(C16:1; C24:1-Hg-w), and (C16:1-Pb-w).

4.4.3.4.b) Effects of Heavy Metals in Water on PUFA Omega 3 and Omega 6 Fatty Acids

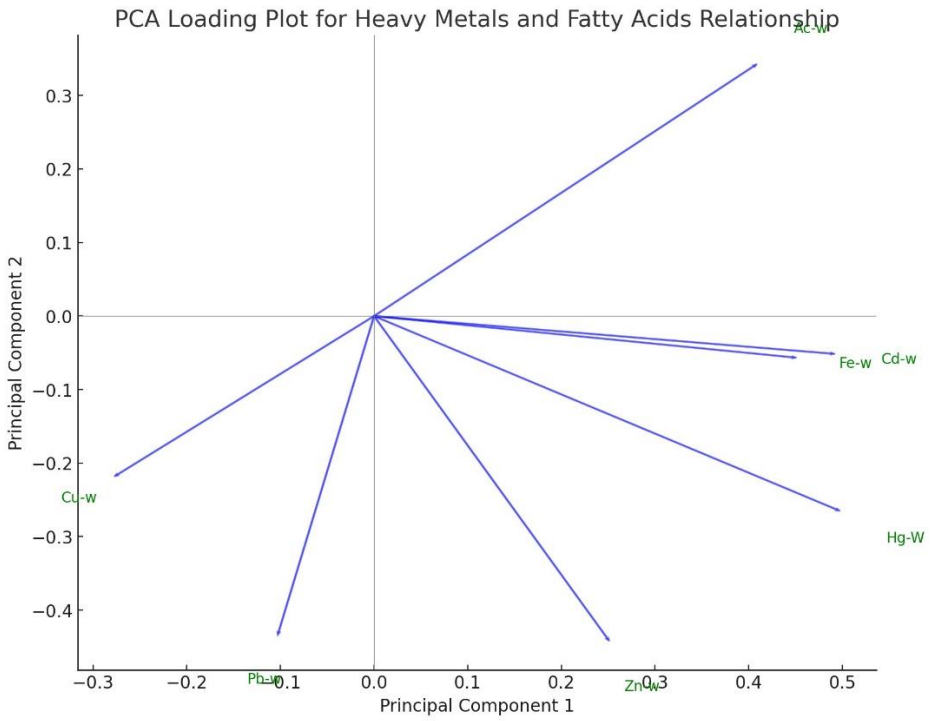

Figure 25. Principal Component 1 (PC1) and Principal Component 2 (PC2) to capture the most variance in the data of heavy metals in water versus PUFA omega 3 and omega 6

Principal Component 1 (PC1) and Principal Component 2 (PC2) capture the most variance in the data (Figure 25). Each arrow represented a heavy metal in water, with the direction and length indicating the contribution to the principal components. Metals like Zn-w and Cd-w had arrows pointing in a similar direction, suggesting that the variables were positively correlated with each other in the context of the data. Metals like Pb-w and Cu-w were positioned in opposite quadrants, indicating a negative correlation. The length represented the strength of the contribution to the principal components. For instance, Zn-w and Hg-W had

longer arrows, suggesting a more substantial contribution to PC1 or PC2.Ar -s, Hg-w, Pb-w, and Cd-w had no statistically significant effect on PUFA omega 3 and PUFA omega 6

4.4.3.5. Effect of Pollution on Micronutrients and macronutrients

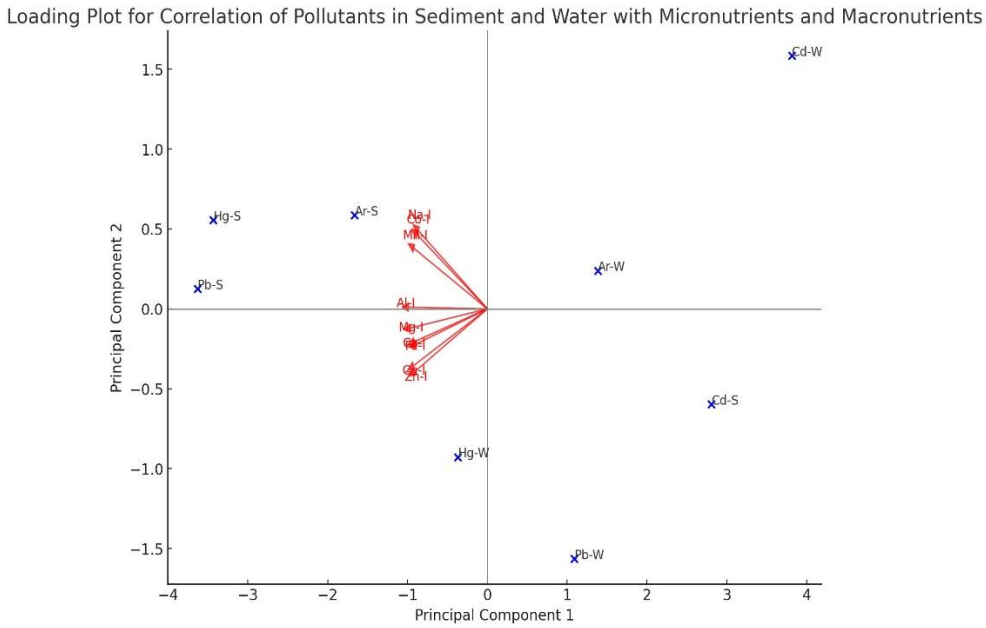

Figure 26. Showing Micronutrients and Macronutrients (in red) represent the correlation coefficients of each nutrient with the principal components

Pollutants in Sediment and Water (Blue Points plotted according to their principal components (PC1 and PC2). The positioning of the points revealed either a similarity or a distinction considering the micronutrients and macronutrients. Micronutrients and Macronutrients (Red Arrows) depicted the loadings (correlation coefficients) with the principal components (Figure 26). As for the size and orientation of each arrow, it pointed out the intensity and nature by which each nutrient affected the pollutants. Dietary factors for Zn-I, Fe-I, Cu-I, and Na-I had different directions, indicating different effects on some contaminants. The combination of some pollutants was similar to micronutrients and macronutrients since others, such as Hg-S and Pb-S, were seen as differently patterned.

4.4.4. Association between pollutants in sediment, in water and in insect samples

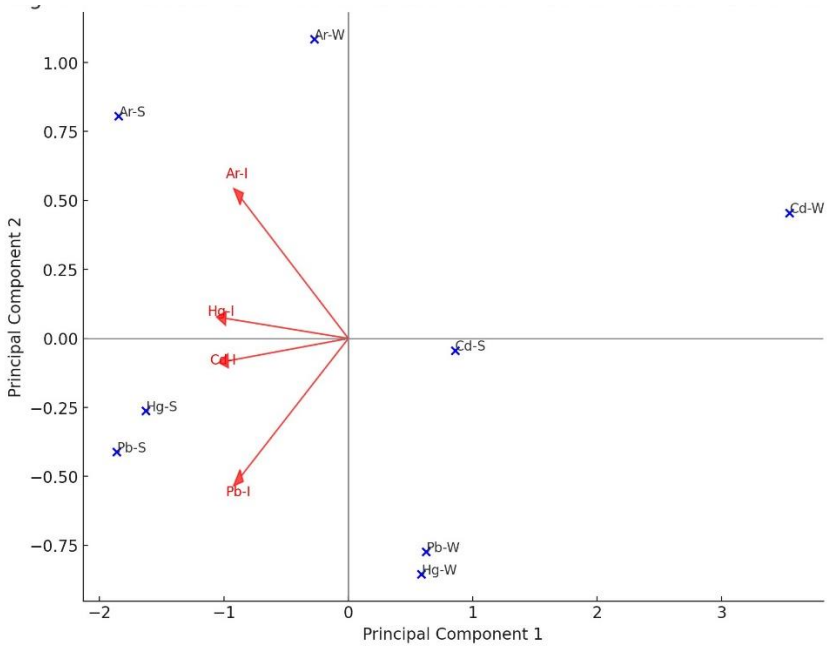

Figure 27. Association between heavy metals in water, sediment and insect samples

The pollutant concentrations in Sediment (Blue Points) are to be related to its principal components (PC1 and PC2). The positioning of these points suggested how similar or different the micronutrients and macronutrients are. Heavy metals in water, sediment, and insect samples (Red Arrows) indicate the principal components' nutrient designs (correlation coefficients). The length and direction of each arrow gave a measure of how much of an influence each nutrient had on the pollutants in sediments. Ar-I and Pb-I were located farther from the origin, which suggests they had a more significant effect applied to the direction of the main components. This indicated that they had different affiliations with the various pollutants. Some pollutants, such as Ar-W and Cd-W, appeared to have similar correlation patterns, while others, like Hg-S and Pb-S, had different patterns.

CHAPTER FIVE
DISCUSSIONS

5.1. Spatial Variations in Water Quality Parameters

The pollution of freshwater ecosystems is a global concern, exacerbated by rapid population growth, urbanization, industrialization, and climate change (U.N. Report, 2019). These factors contribute to the decline in water quality and the disruption of aquatic communities, particularly the insect populations that play critical roles in these ecosystems (Hershey et al., 2010). The increase in anthropogenic activities has contaminated freshwater systems with pollutants, leading to changes in the biological community structures. Tolerant species like chironomids (non-biting midges) often thrive under such conditions due to their adaptability, whereas pollution-sensitive species decline, altering the aquatic food webs and ecosystems. These changes can indirectly impact human food security by affecting the availability of nutritious food resources from marine environments. Therefore, continuous monitoring and evaluation of freshwater ecosystems are crucial for early detection of deterioration and developing strategies to safeguard these ecosystems.

5.1.1. Physical and chemical Characteristics

Water quality parameters from six stations in Winam Gulf, Lake Victoria, showed varied impacts on aquatic life due to natural factors and human activities like industrial effluents and agricultural runoff (Ogola et al., 2003). Temperature ranged from 22.1°C to 27.9°C, affecting metabolic rates and insect distribution. pH levels varied between 6.34 and 8.17, with values outside 6.5-9.0 causing stress. Dissolved oxygen ranged from 5.72 mg/L to 8.62 mg/L, with levels below 5 mg/L risking hypoxia (Bulbul et al., 2022). Electrical conductivity (126.97-201.97 µS/cm) indicated pollution, while total hardness (up to 379.50 mg/L) and alkalinity (up to 167.05 mg/L) affected buffering capacity. Low salinity and variable total dissolved solids (83.92-117.43 mg/L) further influenced aquatic stability and species distribution (Scannell & Jacobs, 2001).

5.1.2. Nutrient Loads and Chlorophyll

Nutrient concentrations in Winam Gulf water samples, including nitrates (NO_3^-), nitrites (NO_2^-), total nitrogen (T.N.), soluble reactive phosphorus (SRP), and ammonium ions (NH_4^+), showed significant spatial variations influenced by proximity to urban centers, agricultural runoff, and sewage effluents. High nutrient levels can lead to eutrophication, resulting in

excessive algae growth, reduced oxygen, and poor water quality (Akinnawo, 2023). For example, Kendu Bay recorded the highest nitrate concentration (32.21 µg/L), while ammonium was highest at Homa Bay (126.23 µg/L). Elevated nutrient levels can cause hypoxia or anoxia, favoring pollution-tolerant species like chironomids. High chlorophyll (CHLO) levels at Homa Bay (4294.05 mg/m³) indicate eutrophication, which can deplete oxygen as algae decompose, creating unsuitable conditions for aquatic insects and altering community dynamics.

5.1.3. Heavy Metals in Water, Sediment, and Insect Samples

Metallic pollution significantly affects water bodies, leading to toxicity and impacting pollution-sensitive aquatic species (Ouma et al., 2022). This study examined toxic metals such as Lead (Pb), Arsenic (As), Cadmium (Cd), Zinc (Zn), and Mercury (Hg) in Winam Gulf, revealing spatial and temporal variations across water, sediment, and insect samples.

5.1.3.1. Heavy Metals in Water Samples

The concentration of heavy metals in water sources fluctuated. Pb and Cd were in high concentrations in urban areas such as Kisumu, Homa Bay, and Kendu Bay and were beyond the recommended WHO standard for water. On the other hand, Mercury (Hg) did not exceed the required limit. Such results depict trends in metallic contamination of water plant habitats that are seasonal and spatial (Akenga et al., 2016).

5.1.3.2. Heavy Metals in Sediment Samples

The concentrations of heavy metals in sediments were relatively higher than in the A. crispate samples for Pb, As, Zn, and Cd, with the exception of Hg. Higher sediment metal concentrations depict industrial effluents and urban water runoff (Jeong et al., 2012; Cui et al., 20`9). Positive relationships between metals in the water and sediment powerfully depict sediment resuspension influencing water quality.

5.1.3.3. Heavy Metals in Insect Samples

Chironomus species had different Patterns of Arsenic (As) and Mercury (Hg) in different sites, while lead (Pb) and Cadmium (Cd) were relatively similar. The positive relationship between the sediment and the metal concentration in the insects also affirmed the fact that these insects are directly affected by the contamination level of sediments since they develop their larvae in the sediment.

5.1.3.4. Ecological and Environmental Implications

The results of the coefficients of the metal concentrations show positive and significant relationships with one another in water, sediment, and insects. Sediment concentrations of toxic metals such as Pb and Cd are high, posing a great danger to aquatic life as well as the food chain, hence the need to control sediment pollution (Timmermans et al., 1998).

5.1.3. 4. Ecological and Environmental Implications

The observed strong positive correlations between metal concentrations in water, sediments, and insects suggest a significant interaction between these aquatic ecosystem components (Chan et al., 2021; Bruno et al.,v2022). The fact that non-biting midge larvae develop in sediments and feed on suspended organic matter from the mud highlights the critical impact of sediment contamination on these insects. Elevated levels of metals like Pb and Cd in water and sediments are particularly concerning as they are categorized as non-essential and toxic (Yilmaz et al., 2018), posing threats to aquatic life and potentially impacting the entire aquatic food web.

5.1.3.5. Effects of Water Quality Parameters on Chironomus Physiology, Biochemistry, and Nutritional Status

Physiological Effects

Chironomus larvae thrive in slightly acidic to neutral pH (6.0 to 7.5); deviations cause stress, affecting growth and osmoregulation (Sela et al.,2021; Fujii et al.,2023). In low pH, larvae may increase mucus production, raising energy expenditure. They tolerate low dissolved oxygen (DO) due to hemoglobin-like pigments, but DO below 2 mg/L causes stress and mortality. Adequate DO (5.72 to 8.62 mg/L) supports growth. High electrical conductivity (EC) and total dissolved solids (TDS) indicate high ion concentrations, affecting osmoregulation and reducing growth. The larvae grow best at 20-30°C; recorded temperatures (22.13°C to 27.94°C) are optimal, but over 30°C increases metabolic stress. Chironomus tolerate varied water hardness, but extreme levels affect osmoregulation and calcium metabolism. Moderate alkalinity maintains ionic balance and physiological health (Senze et al., 2024).

Biochemical Effects

ORP indicates the oxidative or reductive state of the water, which affects the biochemical processes in Chironomus. Higher ORP values suggest a more oxidative environment, which can increase the production of reactive oxygen species (ROS). While Chironomus has mechanisms to detoxify ROS, prolonged exposure to high ORP can lead to oxidative stress, affecting larval survival and biochemical composition (Livingstone, 2001). Parameters like TDS and hardness also indicate the availability of specific ions and minerals. Chironomus larvae require adequate nutrients like calcium, magnesium, and trace metals for normal physiological and biochemical functions. Poor water quality with deficient or excessive nutrients can lead to imbalances in larval biochemistry, affecting enzyme activity, protein synthesis, and lipid metabolism. Exposure to suboptimal water quality (e.g., low DO, high EC, or extreme pH) can induce stress responses in Chironomus larvae, leading to changes in their biochemical composition, such as elevated levels of stress proteins (e.g., heat shock proteins), altered lipid peroxidation, and changes in hemoglobin levels (Jeyachandran et al.,2023; Burt, 1998).

Nutritional Status as Food

Chironomus larvae are rich in protein and hence became the favorite of many aquatic animals in their food list. Different water qualities can alter the protein content; low water pH and high EC inhibit protein formation, influencing the nutritional worth of the proteins in food (Nath et al., 2021). Fats are essential energy sources and integral to fish and other predators' diets. Chironomus larvae in poor water quality conditions probably might have altered lipid fatty acid profile, higher in unsaturated fatty because of stress or lower lipid stores due to higher energy investment in maintaining internal homeostasis (Silva et al.,2022; Strandberg et al.,2020). As in human consumption of mineral content in Chironomus larvae, the nutrients can be directly affected by water hardness and TDS by Muscatello et al., 2022. An optimal range is also beneficial for the growth and accumulation of minerals in the larvae, enabling them to be a healthier source of protein for consumption. The accu- sity of water influences the biochemical content of Chironomus larvae and, therefore, determines the palatability and digestibility of the larvae to the predators (Rodríguez et al.,2024; Sela et al., 2021). For example, larvae that contain higher levels of chemical pollutants or stress proteins are less preferred in terms of palatability or are more accessible to decompose and thus change their position in the food chain.

5.2 Aquatic Insect Community Structure in Winam Gulf

5.2.1 Diversity of Aquatic Insects in Winam Gulf

They remarked that aquatic insects play significant roles in the energy flow and the organizations of the freshwater ecosystems where they are found since they are some of the most important sources of food for the other species in the food chains as proposed by Hershey et al., 2010. They vary and persist based on environmental, biological, and anthropogenic factors that include, water quality, habitat characteristics and pollution. Winam Gulf of Lake Victoria faces pollution and loss of habitat due to urbanization industrialization and agricultural activities. This has altered the structure of the aquatic insects with pollution-tolerant species such as Diptera (Chironomidae) dominating the highly polluted areas such as the Kisumu Bay while pollution-sensitive species like Ephemeroptera are only found in the less polluted areas (Wahizatul et al., 2011). Its stipulation of insect orders such as Hemiptera, Diptera, and Ephemeroptera illustrates their tolerance levels and therefore makes them important biomarkers for water quality and general environmental status.

5.2.1.1. Effect of insitu parameters on Aquatic Insect Community

5.2.1.1.1 Species Richness

Aquatic insects typically thrive within a specific pH range (6.5 to 8.5). Extreme pH levels (either too acidic or too alkaline) can decrease species richness as many insects are sensitive to pH changes. The table shows pH levels ranging from 6.34 to 8.17, which are within a tolerable range, suggesting moderate to high species richness in these sites. High DO levels generally support higher species richness because more oxygen is available for aerobic respiration. The recorded DO levels (5.72 to 8.62 mg/L) suggest that most sites have adequate oxygen, favoring a diverse range of aquatic insects. High EC and TDS can indicate pollution or high mineral content, which might reduce species richness by favoring tolerant species and excluding sensitive ones (Bhattacharya & Das Chatterjee, 2021). The EC and TDS values in the table are moderate, indicating potential for moderate species richness. Very hard or alkaline waters can reduce species richness by limiting species to those that can tolerate such conditions. The values in the table show a range that could allow for moderate species richness.

5.2.1.1.2 Abundance

Air and water temperature influence the metabolism, growth, and reproductive rates of aquatic insects (Bonacina et al.,2022) The temperature ranges (22.13°C to 27.94°C) are generally

conducive to a wide range of aquatic insects, potentially resulting in high abundance.Adequate DO is critical for the survival and abundance of most aquatic insects. Lower levels may limit abundance, particularly of sensitive taxa. The DO levels recorded are mostly above 5 mg/L, which is generally suitable for a healthy insect community (Shafie et al., 2017). These factors affect the availability of certain ions and overall water quality. Elevated levels may reduce abundance by creating stressful conditions. However, the values are within a moderate range, suggesting a potential for moderate to high abundance.

5.2.1.1.3 Diversity

Stability of D. O., pH, and Temperature in these parameters enhance high diversity by providing the organism with different ecological requirements for different species (Zhao et al.,2023). The values in table 1 are relatively constant and thereby support a broad community distribution. It is understood that EC, TDS, and ORP factors play an important role in defining the species diversity. Higher values can lead to reduced diversity because they cause an increase in abundance of species that tolerate pollution. The values of ORP reflect high oxygen concentrations beneficial for diverse species formation (She et al., 2023). The fact that both EC and TDS are moderate means that there is moderate insect species diversity in the stream. Saturated or diluted water has the effect of decreasing the number of organisms that can survive within the area of concentrated salinity. Dissolved level of salinity are very low and ranging between 0. 06mg/L to 0. 08 mg/L which is ideal for supporting high species diversity.

5.2.1.1.4 Composition

Different species are adapted to different pH levels and oxygen concentrations (. Welker et al.,2013; Marium et al.,2023). Slightly acidic to slightly alkaline pH (6.34 to 8.17) and moderately high DO (5.72 to 8.62 mg/L) suggest a community composition with a mix of both sensitive and tolerant species.

Hardness and Alkalinity influence the water's buffering capacity and ionic composition, affecting the types of species present. A range of hardness (126 to 379.5 mg/L) and alkalinity (45.5 to 167.05 mg/L) indicates that the insect community might include species that can tolerate moderately hard and alkaline conditions. Low salinity and moderate TDS suggest that the community composition would include freshwater species adapted to low ionic content. High TDS might shift composition towards more tolerant species (Metzeling, 1993)

5.2.1.2. Effects of Nutrients on Aquatic Insect Communities

Nutrient enrichment (particularly nitrogen and phosphorus) often leads to eutrophication, a process that results in excessive plant and algal growth. This can affect aquatic insect communities in various ways, depending on the level and duration of nutrient input, as well as other environmental factors.

Moderate levels of nutrient enrichment can increase primary productivity (algae and aquatic plants), providing more food resources for herbivorous and detritivorous insects, potentially increasing their abundance and richness (Sterner & Hessen, 1994). Excessive nutrient input can lead to algal blooms, oxygen depletion, and habitat degradation, which can negatively affect aquatic insect diversity, composition, and abundance, particularly sensitive species.

5.2.1.2.1 Richness (Number of Species)

Moderate Nitrates (NO_3^-) and Nitrites (NO_2^-) concentrations can promote diverse plant and algal communities that support a wide range of aquatic insects. However, high levels (as indicated by significant differences in the table) can lead to eutrophication and oxygen depletion, resulting in decreased richness as only tolerant species can survive under such conditions. Ammonium (NH_4^+), available form of nitrogen but can be toxic to many aquatic insects at high concentrations. Elevated NH_4^+ levels can reduce insect species richness by favoring tolerant species (e.g., some Chironomidae) while excluding sensitive taxa. Soluble reactive phosphorus (SRP) and total phosphorus (TP) are key nutrients that drive primary productivity (Weihrauch et al., 2012). High phosphorus levels can cause algal blooms, affecting oxygen levels and reducing insect species richness due to habitat degradation and reduced water quality (Hernández, et al.,2016) .Silica (SiO_2),essential for diatom growth, which forms the base of the food web. Adequate silica levels support diatom productivity, promoting richness in herbivorous and detritivorous insect communities.

5.2.1.2.2 Abundance (Number of Individuals)

Chlorophyll concentrations indicate the level of primary productivity. Moderate levels support increased insect abundance by providing ample food resources (algae and plant detritus). However, very high chlorophyll levels, indicating algal blooms, can result in low oxygen levels, reducing insect abundance. Total Nitrogen (TN) and Total Phosphorus (TP) concentrations reflect the overall nutrient availability in the water. Higher concentrations can lead to an increase in abundance of opportunistic and tolerant species that can exploit abundant

food resources resulting from nutrient enrichment (Tong et al.,2022; Ansari et al., 2011). The distribution of aquatic insects can shift in response to nutrient gradients. For instance, sites with moderate nutrient levels may support a diverse community, while those with high nutrients and poor water quality may be dominated by a few tolerant species.

5.2.1.2.3 Diversity (Variety of Species and Their Evenness)

Nutrient Imbalance impact on diversity (Devlin & Brodie,2023). Nutrient imbalance (e.g., high NO_3^-, NO_2^-, NH_4^+, or SRP) can lead to a decrease in species diversity. Eutrophication often results in a community dominated by a few tolerant taxa (e.g., some Chironomidae, oligochaetes), reducing overall diversity as sensitive species decline. Optimal Nutrient Levels for Diversity. Sites with moderate nutrient levels and good oxygenation can support higher diversity by providing varied niches and stable habitats for a range of species, including both generalists and specialists (Weisser et al., 2017).

5.2.1.2.4 Composition (Types of Species Present)

Community Composition Shifts (Williams-Subiza et al.,2022; Eady et al.,2014). Nutrient enrichment can lead to shifts in community composition. In nutrient-rich environments with low oxygen levels (e.g., from algal blooms and subsequent decomposition), there is often a shift towards communities dominated by pollution-tolerant species such as certain Chironomidae, oligochaetes, and dipterans.

Adequate silica levels support diatom production, which is crucial for insects that feed on diatoms, such as certain mayflies and caddisflies. Low silica levels could alter the composition by favoring other types of primary producers that may not be as beneficial for these insects (Fenoglio et al.,2020).

5.2.1.2.5 Distribution (Spatial and Temporal Spread)

Some of the factors that affect aquatic insect communities include nutrient gradients in the space. The nutrient-rich conditions, such as found in Kisumu and Kendu Bay, allow water quality dismissed by pollution tolerant species, while the moderately nutrient enriched water support a diverse species pool. It also revealed that the influx of nutrients resulting from runoff and rainfall during certain seasons leads to quantitative boost in the insect population but drops as a result of lack of oxygen and habitat pollution. Moderate nutrient enrichment leads to increased primary production and the availability of abundant food sources for insects. However, high nutrients lead to eutrophication that affects species richness, density, and shifts

the community spectrum (Devlin &Brodie,2023; Alexander et al.,2017;Galaviz, 2019). These variations imply spatial changes in insect populations and shift in macronutrient concentrations at some sites, which favor the growth of more eutrophic organisms and tolerant insects. Balancing nutrients is particularly important in countering eutrophication, sustaining insects, and protecting the overall aquatic habitat. Managing nutrients is crucial in maintaining aquatic biological richness and the contribution of aquatic insects.

5.2.1.3. Effects of Heavy Metals on Aquatic Insect Communities

5.2.1.3.1 Heavy Metal Toxicity and Bioaccumulation

Heavy metals are non-biodegradable and can accumulate in aquatic environments, leading to bioaccumulation in marine organisms, including insects (Aziz et al.,2023; Das et al.,2023; Cordeli et al.,2023). Over time, this can result in biomagnification up the food chain (Shah, 2021). The toxicity of heavy metals affects aquatic insects by disrupting physiological processes, leading to mortality, reduced growth, reproductive failure, and behavioral changes. Heavy metals have a Direct Impact on Sensitive Species. Some aquatic insect species are more sensitive to heavy metals than others. Sensitive species may be eliminated from heavily contaminated areas, reducing community richness and diversity.

5.2.1.3.2 Richness (Number of Species)

Elevated concentrations of heavy metals (Arsenic (As), Mercury (Hg), Lead (Pb), and Cadmium (Cd) as indicated in the table for specific sites like Kisumu Bay and Homa Bay) can reduce species richness by causing mortality or inhibiting the reproduction of sensitive species (Irfan et al.,2019; Bănăduc et al.,2024; Bashir et al.,2020; Ngila et al., 2009). For example, mayflies (Ephemeroptera), stoneflies (Plecoptera), and caddisflies (Trichoptera) are often sensitive to heavy metal pollution and may decline or disappear from contaminated sites. It also leads to Threshold Concentrations and Toxicity where when concentrations exceed certain thresholds, as seen in several sites in the table (e.g., Lead in Homa Bay, Arsenic in Kisumu Bay), it can lead to acute toxicity, drastically reducing species richness by favoring only those species that can tolerate high levels of contamination.

5.2.1.3.3 Abundance (Number of Individuals)

Heavy metals such as lead, cadmium, and mercury can affect the population dynamics of aquatic insects by reducing their abundance (Tabassum et al.,2024; Kadim et al.,2022). Sub-lethal concentrations can impair growth, reduce feeding efficiency, and decrease reproductive

success, leading to lower population sizes (Yan et al., 2024). Heavy metals have a Localized Impact on Abundance: Sites with high concentrations of heavy metals (e.g., cadmium in Homa Bay, mercury in Ndere Island) will likely see a reduction in the abundance of sensitive insect taxa, such as certain dipterans (e.g., Chironomidae) and other benthic invertebrates. Conversely, tolerant species, such as some Chironomus species, may increase abundance due to reduced competition and predation.

5.2.1.3.4 Diversity (Variety of Species and Their Evenness)

Heavy metals cause Loss of Sensitive Species. High concentrations of heavy metals can lead to a decline in species diversity. Sensitive species are often the first to decline or disappear, shifting community structure toward a less diverse community dominated by tolerant species (Bååth, 1989). In addition, heavy metals have an Impact on Evenness. Heavy metal contamination can affect species evenness by causing the decline of sensitive species and the proliferation of a few tolerant species. This results in a skewed community structure, where a few taxa dominate the ecosystem. Heavy metals can result in Reduced Alpha and Beta Diversity. High contamination levels reduce alpha diversity (species diversity within a particular area or ecosystem) and beta diversity (species diversity between ecosystems), leading to the homogenization of insect communities across contaminated sites (Gavioli et al.,2022; Menezes et al.,2023).

5.2.1.3.5 Composition (Types of Species Present)

Contamination attributed to heavy metals can lead to Shift in Community Composition (Mitra et al.,2022; Zhang et a,2023; Singh et al.,2022). Sensitive taxa such as mayflies (Ephemeroptera), stoneflies (Plecoptera), and caddisflies (Trichoptera) may be replaced by more tolerant taxa such as certain Chironomidae, oligochaetes, and some dipterans that can withstand or even thrive in contaminated environments (Chang et al., 2014). Dominance of Tolerant Species, at sites with high heavy metal concentrations (e.g., Kisumu Bay, Homa Bay), the insect community may be dominated by tolerant species such as midges (Chironomidae) and worms (Oligochaeta), which can survive in low-oxygen and contaminated environments. This shift reduces the ecological complexity and resilience of the ecosystem.

5.2.1.3.5 Distribution (Spatial and Temporal Spread)

Spatial Variation Due to Heavy Metal Gradients occurs. Aquatic insect communities are likely to vary spatially depending on the concentrations of heavy metals in water, sediments, and the insects themselves. Sites with higher concentrations of heavy metals, as seen in the table (e.g., arsenic in Kisumu Bay, lead in Homa Bay), will likely support communities dominated by tolerant taxa, whereas sites with lower concentrations may have more diverse communities. Temporal Changes also have an effect on distribution of insects. Seasonal changes in water flow, sediment disturbance, and metal inputs can affect the temporal distribution of aquatic insects (Hershey et al., 2010). Heavy metal contamination can result in long-term changes in community structure and function, especially if the metals are persistent and continue to accumulate in the sediments (singh, et al.,2022; Kadim & Risjani,2022; Tchounwou et al.,2012).

5.2.2 Chironomids (Nonbiting Midge Larvae)

The study on aquatic insects also revealed the presence of nonbiting midge larvae, known as chironomids, across all sampling stations in Winam Gulf. Chironomids are known for their high tolerance to polluted environments, such as those with low oxygen levels, nutrient-rich conditions, decomposing organic matter, and winter drawdowns (Podder et al.,2022; Rossaro et al.,2022; Marziali et al.,2024; Misiko et al., 2024). These attributes make chironomids valuable bioindicators for assessing environmental quality and ecosystem integrity. The findings align with previous research, highlighting using chironomids in bioassay assessments of pollution and ecological health.

Chironomids contribute significantly to aquatic food chains and food webs by recycling organic matter, thus supporting various predatory species such as bottom-feeding fish, beetles, dragonflies, birds, and other decomposers (Nicacio & Juen, 2015). This key role in energy flow within aquatic ecosystems has been relatively underappreciated despite chironomids' economic and ecological significance.

5.2.2.1 Phylogenetic Analysis of Chironomids

The present study used phylogenetic analysis and categorized the genus Chironomus into Chironomus transvaalensis, Chironomus pseudothummi, and Chironomus species. As a significant barcode area, this analysis used cytochrome oxidase I (COX1), which was also used in Misiko's study (2024), showing the effectiveness of the COX1 barcode to differentiate

between species with close relationships (Rodrigues et al.,2017; Kher et al., 2011). Consequently, the study shows that the aquatic insects cannot well be identified and classified based on traditional morphological and physiological structures.

5.2.3 Relationships Between Aquatic Insects and Environmental Parameters

Regarding physical factors, it is indexed that aquatic insects' motion and distribution are determined by bodily characteristics and their interaction with chemical and biological factors. Elements like temperature, pH, dissolved oxygen, and heavy metals and pollutants in the water will influence the kind of aquatic insects found in the Winam Gulf and their distribution. Past researches reveal that high concentrations of contaminants such as heavy metals, pesticides, and persistent organic substances upset the ecological balance, leading to the extermination of vulnerable species and lower population multiplication rates (Mitra et al., 2022; Alengebawy et al., 2021; et al.,2023). Still, some species are more able to withstand such extreme conditions and adapt to polluted environments as bio-holographic markers for ecosystems.

5.2.4 Morphological Identification and Phylogenetic Analysis

Morphological identification is still relevant but has disadvantages because many species of aquatic insects are rather diverse and similar in form. Integrating phylogenetic analysis provides a more refined analysis by focusing on the relationship between species and genetic evolution using genetic markers (Brown,2002; Shakya et al.,2020; Zou et al.,2024). This two-tier process not only increases the taxa discrimination but also helps to decode the evolutionary relationships, ecological diversification, and possibly some speciation processes in the aquatic insects. For instance, the comparative study of populations of Chironomus species indicated the role of habitats with high pollution in developmental changes of the species and the forces influencing genetic differentiation (Misiko et al., 2024).

5.2.5 Rate of Substitution and Pairwise Distance in Evolutionary Studies

The rate of substitution and pairwise distance are important figures used in evolutionary studies to explore genetic differentiation and evolution in species (Amanda et al.,2017; Echave et al.,2015). Analyzing these metrics in aquatic insects can provide insights into how evolutionary forces shape life within varying habitats or under stress conditions. For example, a high substitution level in Chironomus species may suggest high adaptative rates to polluted ecosystems. In contrast, pairwise distances enable the comparison of the genetic variability

within and between populations, which is essential for conservation and the sustainable use of freshwater habitats.

Freshwater biomonitoring assessment, the evaluation of species diversification, and the phylogenetic status of aquatic insects with consideration of their habitats give a complete vision of the water ecosystem state (Dijkstra et al.,2014; Weglarz et al.,2021). Aquatic insects like Chironomus species in Winam Gulf of Lake Victoria remain helpful in showing the chances of ecoshift and pollution. Appreciating their diversification patterns, functions, evolution, and environmental interactions is critical for proper management and conservation measures in freshwater ecosystems (Bănăduc et al., 2022; Misiko et al., 2024). The results of this study underscore the significance of integrating conventional populace ecological strategies with newer molecular approaches to strengthen the tracking and conservation of aqua-biotic richness in response to existing environmental constraints.

OBJECTIVE 3: To evaluate the effects of pollution on the nutritional status of selected aquatic edible insects-non-biting midge, and chironomids as food and animal feeds.

5.3. Nutritional Status of Non-Biting Midge

Population growth on the rising trend has been projected to 9.9B by 2050 a fact that is of global concern due to food insecurity. Consequently, the demands for nutritional needs are also on a rising trend, particularly sustainability (Williams et al., 2019). The effects negatively impacting on children under five, adolescents, and maternal populations due to deficiencies. The present study established that insects offer an alternative protein source because of the availability, affordability, environmental friendliness, and nutritive components that are rare in other meat sources. Aquatic insects offer essential nutrients which include: amino acids, essential fatty acids, essential vitamins, and vital macronutrients and micronutrients though their importance is underestimated. Particularly, the non-biting midge -a mosquito-like organism whose larval stage plays a major role in the aquatic food web and food chain. Although, very vital, non-biting midge has received no attention to date despite the completeness of nutritive components.

5.3.1 Amino Acids

Research on non-biting midges (Chironomus species) from various sampling sites in Winam Gulf, Lake Victoria, has revealed significant spatial variations in the concentrations of ten

essential amino acids: arginine, valine, lysine, leucine, methionine, threonine, tryptophan, phenylalanine, isoleucine, and histidine. These amino acids play critical roles in protein synthesis, metabolic pathways, immune function, and stress responses. The analysis, conducted across four sampling sites—Kisumu Bay, Homa Bay, Ndere Island, and Kendu Bay—emphasizes the effects of environmental factors such as pollution, food availability, and habitat conditions on the physiological status of Chironomus species.

5.3.1.1 Ecological and Nutritional Implications

Such spatial changes in concentration of amino acids in Chironomus species are as a result of water quality, pollution, and food conditions. Higher levels of methionine and histidine at some positions connected with HM exposure may be resulted from the oxidative stress; methionine is promoted for detoxification as the precursor of the glutathione and histidine possesses the chelating and antioxidant properties (Jozefczak et al., 2012). Amino acid profiling is useful for evaluating the status of aquatic ecosystems under the conditions of pollution stress (El-SiKaily &Shabaka, 2024; Labine et al.,2023; Zhang et al.,2021; Hook et al.,2014). If the differences of amino acids at the investigated sites are ranked high, then further research of the ecologies and the pollution control measures should be proceeded. These findings are in a similar vein with other studies that argue that amino-acid differences can help in enriching the foods that are strategic nutritional relief for the malnutrition-prone groups in Sub-Saharan Africa (Bell et al.,2024; Olson et al.,2021). Therefore, amino acid profiles are useful bioindicators as a measure of ecosystem health and can inform on how to minimize impacts of pollution on the resident biota of Lake Victoria's Winam Gulf.

5.3.2 Fatty Acids

Meat samples analysed from different sites in Nyanza Gulf and Chironomus species include; 20 fatty acids of which the fatty acids compositional profile consists of eight saturated fatty acids (SFAs), five monounsaturated fatty acids (MUFAs), four polyunsaturated omega-3 fatty acids (PUFAs), and three polyunsaturated omega-6 fatty acids (Strandberg et al., 2020). These fatty acid contents were not constant across four sample stations- Kisumu Bay, Homa Bay, Ndere Island and Kendu Bay hence reflecting the effect of the local environment especially pollution on lipid profiles in aquatic insects.

5.3.2.1 Saturated Fatty Acids (SFAs)

The study revealed eight saturated fatty acids which include C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C22:0 C24:0 at different concentrations in the different sites. Characteristic to saturated fatty acids, C16:0 (Palmitic Acid) was the most prominent in the range from 18.12 µg/ml in Homa Bay to 19.11 µg/ml in Kisumu Bay. The least of the identified SFA was C22:0 (Behenic Acid) that was found in very small amounts and only at Ndere Island and was not discovered at highly polluted sites such as Kisumu and Kendu Bay. Notably, C22:0 and C24:0 were present only in Ndere Island and Homa Bay sites though the latter is a polluted area. Such disparity may point at multiple interactions with its environment, for instance, the lipid metabolism of Chironomus species ((Dvorak, et al., 2023; Kalman et al., 2023; Monteiro et al.,2020; Strandberg et al., 2020).

5.3.2.2 Monounsaturated Fatty Acids (MUFAs)

MUFAs, particularly the C18:1n-9 (Oleic Acid), had comparatively higher levels at the different sites and for which the levels obtained ranged from 12.14 µg/ml at Kisumu Bay and 32.12 µg/ml at Homa Bay. The role of this fatty acid is in cell membrane structure and energy reserve function and increase in concentration may be by adaptation to environmental stress such as pollution (Javdani-Mallak & Iman Salahshoori,2024; Gonçalves et al.,2017). C24:0 was not found in Homa Bay and Kendu Bay and, Kisumu Bay had very low levels of C22:5n-3 and C22:6n-3. The lack of some MUFAs in polluted areas such as Kisumu implies that pollution may greatly affect lipid profile in aquatic insects especially as shown by the shift in MUFA among Chironomus species (Mahboob et al., 2019).

5.3.2.3 Polyunsaturated Fatty Acids (PUFAs)

Concentrations of these fatty acids of Chironomus larvae were also found to vary significantly spatially in the following categories; Monounsaturated fatty acids MUFA: C18:3n-3 (Alpha-Linolenic Acid), PUFA n-3: C20:5n-3 (EPA), C22:5n-3 (DPA), and C22:6n-3 (DHA). For instance, C18:3n-3, was detected in varying proportions: with the lowest concentration of 2.55 µg/ml was recorded in polluted Homa Bay while the highest concentration of 3.55 µg/ml was recorded in cleaner water of Ndere Island. On the other hand, the C22:5n-3 and C22:6n-3 were not detected in highly polluted Kisumu water. This suggests that pollution has a direct effect on omega-3's which are required for cell membranes and the control of inflammation. Omega-

6 fatty acids such as C18:2n-6 were quite stable with the only differences being the lack of C18:3n-6 and C20:2n-6 in fish from Kisumu.

Bioaccumulation of fatty acids and its subgroup, PUFAs, are used as pollution indicators because these molecular structures are vulnerable to oxidation by pollutants such as heavy metals as well as PAHs (Messina et al., 2023). Larger PL-PUFA such as EPA and DHA are decreased suggesting oxidative injury while MUFAs such as oleate are elevated showing a stressed condition of the body (Aldhafiri et al.,2022; Saini et al.,2021). Assessing the fatty acid profiles of Chironomus species at the Nyanza Gulf sites, they have found some differences that result from pollution and give some understanding of the state of the ecosystems and how the stress affects them. Combining fatty acid data with the environment variables will enable the evaluation of the aquatic habitats in Nyanza gulf, Lake Victoria.

5.3.3 Vitamins

Studies on the non-biting midges (Chironomus species) in Nyanza Gulf showed that fat soluble vitamins (A, D, E and K) vary spatially giving the place an altogether unique ecosystem. There were higher concentrations of fat-soluble vitamins as compared to the water-soluble vitamins in the sampled areas such as Kisumu Bay, Homa Bay, Ndere Island and Kendu Bay. Vitamin A obtained the highest value of 0. 34 µg/ml at Kendu Bay while the least 0. 16 µg/ml was at Kisumu Bay probably due to environmental conditions that determined its availability. Mean levels of vitamin D across the islands ranged with Kendu Bay at 20. 90 µg/ml while that of Ndere Island was at 11. 57 µg/ml. Antioxidant vitamins, such as Vitamin E, was the highest at Ndere Island being 31. 40 µg/ml, this might have adapted to the increasing levels of pollution. Another nutrient of clinical importance, vitamin K which is important in coagulation, also showed great variability across the sites. Hypothesising that pollution affects such elements, there was little water-soluble vitamins, when any vitamins and were absent from heavily polluted zones (Shabbir et al., 2022). These results suggest that fat-soluble vitamins may be used as indicators of ecosystem degradation and concentration of pollutants and stressors. Additional research which relates vitamin with other environmental parameters can help in increasing the understanding of ecological processes in Nyanza Gulf.

5.3.4 Micronutrients and Macronutrients in Chironomids-Non-Biting Midge Larvae

Research on Chironomus species (non-biting midges) from Winam Gulf, Lake Victoria, has shown significant spatial variations in micronutrients (Zn, Fe, Cu) and macronutrients (Ca,

Mg, Na, Al, Mn, Co) (Misiko et al., 2024). These differences are influenced by local environmental factors, including pollution from industrial discharges, agricultural runoff, and geochemical processes. High levels of these nutrients in some sites exceed WHO/KEBS water quality standards, posing potential toxicity risks to aquatic life and affecting species diversity by favoring pollution-tolerant species and reducing sensitive ones. For example, high zinc can cause oxidative stress, while elevated iron and copper levels lead to toxicity. Variations in calcium, magnesium, and sodium affect species distribution and biodiversity (GRETEL et al.,2020; Popović, et al.,2022). Non-biting midges, rich in essential nutrients, serve as valuable bioindicators for monitoring freshwater pollution and can also provide sustainable protein and mineral sources to combat malnutrition in regions like Sub-Saharan Africa (Raunio et al., 2011). The study highlights the potential of using these insects to assess ecosystem health and develop management strategies to maintain biodiversity and stability by addressing pollution sources and understanding nutrient variations.

5.4. Effect of Pollution on the Nutritional Status of Non-Biting Midge

The nutritional status of Chironomus species (non-biting midge) is significantly influenced by various environmental factors, including in situ water parameters, nutrient levels, and heavy metal contamination (Gagliardi, 2017). This analysis examines their effects on amino acids, fatty acids, vitamins, and micro- and macronutrients, revealing the complex interactions between environmental conditions and the biochemical composition of these aquatic insects.

5.4.1. Insitu Parameters

5.4.1.1 Effects of in Situ Water Quality Parameters on Amino Acid Composition in Chironomus

Temperature, dissolved oxygen (DO), pH, electrical conductivity (EC), total dissolved solids (TDS), hardness, alkalinity, and oxidation-reduction potential (ORP) significantly influence the metabolic rates, protein synthesis, and amino acid profiles in Chironomus larvae. Optimal temperatures (20-30°C) support efficient protein synthesis and balanced amino acid profiles. DO levels (5.72 to 8.62 mg/L) are sufficient for aerobic metabolism, promoting the maintenance of essential amino acids like lysine and arginine. pH values (6.34 to 8.17) favor protein stability, while moderate EC (126.97 to 201.97 µS/cm) and TDS (83.92 to 117.43 mg/L) levels prevent osmotic stress that could affect amino acid synthesis. Hardness (126 to 379.5 mg/L) and alkalinity (45.5 to 167.05 mg/L) support normal protein metabolism, while ORP (211.43 to 246.35 mV) indicates a suitable oxidative environment for maintaining amino

acid stability. Poor water quality, such as extreme pH or low DO, can lead to stress, altering the nutritional value of Chironomus by affecting amino acid concentrations. Maintaining optimal water quality ensures Chironomus larvae remain a valuable protein source for aquaculture, supporting the growth, immunity, and health of aquatic organisms (Fekadu, 2021).

5.4.1.2. Effects of In Situ Water Quality Parameters on Fatty Acid Composition in Chironomus

Temperature, dissolved oxygen (DO), pH, electrical conductivity (EC), total dissolved solids (TDS), hardness, alkalinity, and oxidation-reduction potential (ORP) significantly influence lipid metabolism and fatty acid composition in Chironomus larvae. Optimal temperatures (20-30°C) support balanced lipid synthesis, while high temperatures may increase saturated fatty acids due to enhanced catabolism. Adequate DO levels (5.72 to 8.62 mg/L) promote aerobic metabolism, preserving unsaturated fatty acids like omega-3 and omega-6 from oxidative damage. A pH range of 6.34 to 8.17 favors the stability of polyunsaturated fatty acids (PUFAs). Moderate EC (126.97 to 201.97 µS/cm) and TDS (83.92 to 117.43 mg/L) prevent osmotic stress that could alter lipid profiles. Hardness (126 to 379.5 mg/L) and alkalinity support enzyme activity essential for lipid metabolism, while moderate ORP (211.43 to 246.35 mV) helps maintain fatty acid stability. Optimal water quality preserves essential fatty acids, such as EPA, DHA, and linoleic acid, in Chironomus, enhancing their value as a nutritious feed source for aquaculture, supporting growth, immunity, and overall health in aquatic organisms (Gladyshev et al., 2009). Poor water quality can degrade these essential fatty acids, reducing their nutritional value.

5.4.1.3. Effects of in Situ Parameters on Vitamin Concentration in Chironomus

Temperature, dissolved oxygen (DO), pH, electrical conductivity (EC), total dissolved solids (TDS), hardness, alkalinity, and oxidation-reduction potential (ORP) significantly influence the synthesis and stability of vitamins in Chironomus larvae (Tabla-Hernandez et al., 2020). Optimal temperatures (20-30°C) promote vitamin synthesis, including B-complex vitamins and Vitamin C, but excessively high temperatures can degrade heat-sensitive vitamins. The recorded water temperature range (23.27°C to 27.94°C) supports efficient vitamin synthesis. Adequate DO levels (5.72 to 8.62 mg/L) are crucial for aerobic metabolism, promoting the retention of antioxidant vitamins like Vitamin C and E, while low DO can lead to oxidative stress and vitamin depletion. The pH range (6.34 to 8.17) generally supports the stability of water-soluble vitamins, such as Vitamin C and B-complex vitamins, which are sensitive to

extreme pH levels. Moderate EC (126.97 to 201.97 µS/cm) and TDS (83.92 to 117.43 mg/L) levels help maintain nutrient uptake efficiency, while high ionic concentrations could impact vitamin absorption. Hardness (126 to 379.5 mg/L) and alkalinity levels influence mineral-vitamin interactions, affecting the bioavailability of fat-soluble and water-soluble vitamins. The ORP range (211.43 to 246.35 mV) indicates a moderately oxidative environment, which generally maintains vitamin stability without excessive degradation. Optimal water quality conditions ensure Chironomus larvae retain a high nutritional value by preserving their vitamin content, making them valuable for aquaculture feed.

5.4.1.4. Effects of in Situ Water Quality Parameters on Micronutrient Composition in Chironomus

Temperature, dissolved oxygen (DO), pH, electrical conductivity (EC), total dissolved solids (TDS), hardness, alkalinity, and oxidation-reduction potential (ORP) significantly impact the uptake, assimilation, and stability of micronutrients in Chironomus larvae. Optimal temperatures (20-30°C) support efficient metabolic processes and enzyme activity, enhancing the absorption of micronutrients like iron, zinc, and copper. The recorded water temperature range (23.27°C to 27.94°C) is optimal for micronutrient metabolism.

Adequate DO levels (5.72 to 8.62 mg/L) are vital for aerobic respiration and maintaining oxidative balance, minimizing oxidative stress-induced degradation of micronutrients such as zinc and copper. The pH range (6.34 to 8.17) supports the solubility and bioavailability of micronutrients and vitamins, as extreme pH can cause precipitation and reduce availability. Moderate EC (126.97 to 201.97 µS/cm) and TDS (83.92 to 117.43 mg/L) levels prevent competitive inhibition among ions, facilitating efficient micronutrient absorption.

Hardness (126 to 379.5 mg/L) and alkalinity (45.5 to 167.05 mg/L) affect the bioavailability of calcium, magnesium, and specific vitamins, supporting normal metabolic processes without causing deficiencies. The ORP range (211.43 to 246.35 mV) suggests a moderately oxidative environment suitable for maintaining micronutrient stability without excessive oxidation.

Chironomus larvae are rich in essential micronutrients like iron, zinc, manganese, copper, calcium, and magnesium, crucial for growth, development, and health in aquaculture species (Schmitt et al., 2019). Optimal water quality conditions enhance the nutritional value of Chironomus larvae by supporting the retention of these essential nutrients, making them a valuable food source in aquaculture. Poor water quality can lead to stress, affecting

micronutrient accumulation and reducing nutritional quality. Maintaining water quality within optimal ranges ensures Chironomus larvae remain a nutritious feed source, promoting the health and growth of aquatic organisms.

5.4.1.5. Effects of in Situ Parameters on Macronutrient Composition in Chironomus

Temperature, dissolved oxygen (DO), pH, electrical conductivity (EC), total dissolved solids (TDS), hardness, alkalinity, and oxidation-reduction potential (ORP) significantly influence the metabolism and composition of proteins, lipids, and carbohydrates in Chironomus larvae. Optimal temperatures (20-30°C) support efficient protein synthesis, lipid metabolism, and carbohydrate storage. The water temperature range (23.27°C to 27.94°C) promotes balanced macronutrient metabolism, enhancing their nutritional quality as aquaculture feed.

Adequate DO levels (5.72 to 8.62 mg/L) are crucial for aerobic respiration, supporting protein synthesis, lipid oxidation, and carbohydrate retention. Low DO can lead to anaerobic metabolism, altering macronutrient composition by reducing carbohydrate content and increasing lipid accumulation. The pH range (6.34 to 8.17) supports enzyme activity, protein stability, and nutrient absorption, preventing nutrient degradation under extreme pH conditions.

Moderate EC (126.97 to 201.97 µS/cm) and TDS (83.92 to 117.43 mg/L) levels prevent osmotic stress, ensuring efficient nutrient uptake. Hardness (126 to 379.5 mg/L) and alkalinity provide a stable protein structure and nutrient absorption environment. ORP values (211.43 to 246.35 mV) suggest a moderately oxidative environment, preventing excessive oxidation of lipids and proteins and preserving their nutritional value.

Chironomus larvae provide high-quality proteins, essential fatty acids, and carbohydrates that support the growth and health of aquatic organisms (Das et al., 2012). Optimal water quality conditions enhance the synthesis and stability of these macronutrients, maintaining their value as feed. Poor water quality (e.g., extreme pH, low DO, high EC, high ORP) can stress larvae, reducing their nutritional quality. Maintaining water quality within optimal ranges ensures Chironomus larvae remain a nutritious and balanced food source in aquaculture.

5.4.2. Nutrients

5.4.2.1. Effects of Nutrients on Amino Acid Concentration in Chironomus as Food and Feed

Among the nitrogen-based nutrients, NO3-, NO2-, NH4+, and total nitrogen (TN) are essential for protein synthesis in Chironomus larvae because nitrogen forms crucial amino acids. Nitrates and nitrites are assimilated into the system, transmuting into ammonium, and the latter is directly utilized in creating amino acids. These moderate amounts of nutrients help form amino acids such as glutamine, but high concentration acts as stress, which changes amino acid formation. Ammonium is well utilized to create strategic amino acids like lysine, but excess leads to toxicity, impacting amino acid composition.

Energy metabolism through ATP of phosphorus-based nutrients, which includes SRP and TP, is essential in synthesizing amino acids. High levels of SRP improve the synthesis of amino acids, but excess phosphorus leads to eutrophication and stress; hence, the metabolism of amino acids tilts towards stress-induced amino acids such as proline. Silica SiO_2 favors diatom growth, a primary food source for Chironomus larvae, leading to a justifiable enhancement of its amino acid content (Dell'Aquila, 2020). Low silica levels limit diatoms' availability, thus impacting the amino acid assemblage.

The CHLO concentration gives information about Chironomus's Chironomus's primary production and food quality. Moderate amounts of chlorophyll increase the biosynthesis of amino acids, but high amounts have been shown to inhibit oxygen availability, hence the amino acid profiles. This also means that oxygen depletion due to high nutrients such as SRP or TP can lead to oxidative deterioration of sensitive amino acids such as cysteine. Protein synthesis requires a favorable ratio of nitrogen, including nitrate (NO_3^-), nitrite (NO_2^-), ammonia (NH_4^+), and total nitrogen (TN) / phosphorus in the forms of soluble reactive phosphorus (SRP) and total phosphorus (TP). When nutrients are in large quantities, eutrophication will occur, leading to changes in amino acid compositions and the biological quality of fish.

5.4.2.2. Effects of Nutrients on Fatty Acid Concentration in Chironomus as Food and Feed

These nitrogenous nutrients (NO_3^-, NO_2^-, NH_4^+, and TN) significantly impact Chironomus larvae's lipid profiles and fatty acid synthesis. Moderate nitrate and nitrite levels favor the

development of phytoplanktons like algae and rich sources of PUFAs like EPA and DHA. This results in the producers being rich in PUFAs, which improves their nutritional quality when ingested by Chironomus larvae (Sokolowski et al., 2013). However, high nitrogen concentrations have adverse effects such as eutrophication, oxygen depletion, saturated fatty acids (SFAs), and low PUFA. Moderate ammonium levels are beneficial for maintaining appropriate synthesis of fatty acids, whereas a high level hits stress and reduces PUFAs while enhancing the level of SFAs.

SRP and TP are phosphorus-based nutrients essential in energy metabolism and lipid synthesis. The appropriate concentration of SRP promotes the synthesis of PUFA. However, toxicity damages the PL in water by stimulating algal blooms and hypoxia, which induce oxidative stress, degrading unsaturated fatty acids. Diatoms contain omega-3, and low silica can alter diets to decrease omega-3-rich diatoms, decreasing PUFA concentrations. CHLO describes the primary production as moderate, which must be involved in increasing fatty acid content, while high levels lead to hypoxic conditions that support SFAs against PUFAs.

Oxygen content affects fatty acid dynamics through its relation to nutrient-induced primary production. Simultaneously, low oxygen from eutrophication can negatively affect omega-3 and omega-6 fatty acid quality. Moderate concentrations of nitrogen, such as NO_3^-, NO_2^-, NH_4^+,

TN and phosphorus, like SRP and TP, are significant nutrients required for good quality fat acids, which is imperative for developing new tissues and the overall health of the latest organisms in the water column. It is important to note that nutrient management benefits the microbial ecosystems and optimizes ((Cottrell, 2020). Thus, the Chironomus larvae's sustainable aquaculture and nutrition value is guaranteed by analyzing their fatty acid composition.

5.4.2.3. Effects of Nutrients on Vitamin Concentration in Chironomus as Food and Feed

The nitrogen-based nutrients (NO_3^-, NO_2^-, NH_4^+, and TN) play a key role in vitamin synthesis and stability for Chironomus larvae. Moderate nitrate and nitrite levels are advantageous for phytoplankton, which produce Vitamin C and B-complex vitamins such as Vitamin B1, B2, B6, B12, and Vitamin E. The Chironomus larvae, which feed on these producers, also contain vitamins. However, if nitrates and nitrites are used in large quantities, they cause eutrophication; water becomes wrong for drinking since it degrades Vitamin C, which is a

water-soluble vitamin; ammonium available in nitrogen stimulates the protein formation and other metabolic activities that encourage the synthesis of B vitamin. Too much ammonium, conversely, has been found to cause toxicity and oxidative stress, which lowers the Vitamin C and E levels (Shilpha et al., 2023).

SRP and TP, derived from phosphorus elements, are significant in energy metabolism and vitamin preservation. Optimal SRP improves the synthesis of several nicotinoprotein-dependent vitamins like vitamin B1 and B6, increasing energy levels; however, high SRP negatively impacts hypoxic stress and vitamin degradation. Moderate TP favors proper vitamin distributions, while high levels can lead to oxidation and decreased vitamin stability. Silica (SiO_2) favors diatoms that contain vitamins such as Vitamin E and A that Chironomus larvae feed on; hence, when they feed on a diet full of diatoms, has rich vitamin content.

Primary productivity can be estimated from the Chlorophyll (CHLO) levels. Moderate chlorophyll helps various foods, which boosts the vitamin component in the diet of Chironomus larvae. High chlorophyll, causing algal blooms and hypoxia, poses the risk of vitamin deterioration due to oxidative stress (Lal & Mogalekar, 2024). Achieving stoichiometric proportions of nitrogen and phosphorus is crucial for synthesizing high-quality vitamins. High levels of nutrients compromise the quality of such vitamins, impairing the value of Chironomus as a feed source. There should be a balance in nutrient levels that facilitates the right concentration of vitamins, which will improve aquaculture's productivity and the aquatic environment's general health.

5.4.2.4. Effects of Nutrients on Micronutrient Concentration in Chironomus as Food and Feed

It has been found that NO_3^-, NO_2^-, NH_4^+, and TN significantly influence the micronutrient uptake and transformation in Chironomus larvae. Moderate concentrations of nitrates and nitrites are conducive for the phytoplankton, which supplies micronutrients such as iron, zinc, copper, and manganese. These micronutrients are accumulated by the Chironomus larvae feeding on these primary producers. However, high quantities of nitrates and nitrites could cause eutrophication and oxygen depletion, thus changing the water quality and the availability of micronutrient iron and manganese (Riaz et al., 2024). Ammonium positively functions in protein synthesis and micronutrient binding and storage due to its optimal level.

Still, high ammonium levels are toxic to the organism and influence micronutrient solubility and availability.

SRP is responsible for rates of primary productivity, while TP influences micronutrient solubility and uptake. Sufficient SRP concentrations promote the uptake of micronutrients by algae and their transfer to Chironomus larvae. However, high SRP triggers eutrophication, resulting in hypoxia, which forms insoluble iron compounds. Silica (SiO_2) helps grow diatoms that provide micronutrients such as zinc and manganese. A high concentration of diatoms in their diet boosts the micronutrient quality of Chironomus larvae for higher-quality feeds.

The amount of chlorophyll (CHLO) represents primary production. Chlorophyll contributed to a moderate level of larval nutrition with minimal effects on the micronutrient concentration of Chironomus larvae. High concentrations can lead to algal blooms and hypoxic conditions, which decreases the solubility and bioavailability of micronutrients (Carvalho et al., 2002). Correct proportions of nitrogen and phosphorus are essential for sustaining the quality of enhanced micronutrients, which are vital for the life of aquatic organisms. There is evidence that high nutrient concentrations can lead to the deterioration of micronutrients, thus lowering Chironomus's nutritional quality as a feed source. Appropriate feeding strategies increase micronutrient content and enhance aquaculture production and the ecosystem's health.

5.4.2.5. Effects of Nutrients on Macronutrient Concentration in Chironomus as Food and Feed

The Nitrogen-basedNitrogen nutrients NO_3^-, NO_2^-, NH_4^+, and TN as the source of Nitrogen are essential for protein synthesis in Chironomus larvae since amino acid and protein formation are essential to their metabolic process. Moderates nitrate and NO2 maintain primary producers that afford Chironomus a protein source for protein synthesis and crucial amino acids (Muangyao, 2020). However, nitrogen levels can result in eutrophication and hypoxia, thus disturbing the stability of proteins and degrading them. *Ammonium* is a nitrogen form that is effectively taken up and directly involved in synthesizing amino acids. However, it is toxic to the plant at a high concentration and interferes with protein synthesis.

SRP and TP are phosphorus-based nutrients that are essential for carrying out lipid metabolism and carbohydrate storage. Optimal SRP concentrations aid in ATP synthesis, transform lipids, and preserve lipids such as glycogen (Cembella et al., 1984). Nevertheless, high SRP can elicit algal blooms and hypoxic conditions, leading to lipids and carbohydrate deterioration. A

moderate TP level benefits macronutrient /water quality stability, while a high TP level is unfavorable for water quality/ macronutrient.

The Apatite: Silica (SiO_2) sustains diatom biomass power-packed with proteins and essential fatty acids. Feeding on diatoms helps increase the protein and lipid composition in the Chironomus larvae, making them more valuable as fishes feed in aquaculture. Chlorophyll (CHLO) levels define the primary productivity of sea and oceans; moderate amounts support a diverse food base, resulting in improved proteins, lipids, and carbohydrates, whereas high amounts result in the depletion of oxygen, leading to the degradation of nutrients (Ebrahimi et al., 2003).

A nitrogen and phosphorus ratio of 1:1 is required for the synthesis and stability of the macronutrient requirements in Chironomus larvae. High nutrient levels can denature proteins and lipids and decrease carbohydrate stocks, making them less nutritious feeds. Husbandry of Chironomus larvae involves nutrition management, which translates to quality production of the fry for aquaculture to enhance the productivity of the ecological system.

5.4.3. Heavy Metals

5.4.3.1. Effects of Heavy Metals on Amino Acid Concentration in Chironomus as Food and Feed

Thus, heavy metals such as lead, cadmium, arsenic, and mercury affect the synthesis of amino acids in chironomus larvae due to inhibiting several enzymes and the onset of oxidative stress (Arambourou et al., 2020). Lead exposure suppresses the activities of enzymes involved in synthesizing sulfur-containing amino acids, such as cysteine and methionine, which decreases the quality of proteins. Cadmium affects the synthesis of stress-responsive amino acids, proline, and inhibits the synthesis of plant growth-promoting amino acids lysine and leucine. They also cause oxidative stress, breaking delicate amino acids such as tryptophan, cysteine, and tyrosine.

Inorganic arsenic and mercury disrupt protein metabolism by chelating on protein and enzyme, thus reducing the efficiency of amino acids. This can reduce levels of essential amino acids such as glutamate and glutamine in the brain. The toxic metals also decrease branched-chain amino acids (BCAAs), vital for muscle protein synthesis and energy metabolism in water-living organisms. Heavy metals such as cadmium and lead have protein denaturing and

cytotoxic properties that break down nitrogen-rich amino acids like arginine and histidine (Bishop, 2020).

Heavy metal stress increases stress-inducible amino acids (proline, alanine) and decreases levels of essential amino acids in Chironomus larvae, reducing the nutritional value of larvae as fish feed. Reducing the threat of heavy metals is vital to maintaining the amino acid components of Chironomus larvae, which will contribute to aquaculture diets and enhance the integrity of the aquatic ecosystems.

5.4.3.2. Effects of Heavy Metals on Fatty Acid Concentration in Chironomus as Food and Feed

Lead, cadmium, arsenic, and mercury, present in the water, interfere with lipid metabolism and fatty acid synthesis first by binding to and inhibiting the enzymes in Chironomus larvae. Lead is a suppressor of enzymes such as desaturases and elongases, which aid in transforming saturated to unsaturated fatty acids, including omega-3 (EPA, DHA) and omega-6 (Arambourou et al., 2020). It inhibits enzyme processes such as acetyl-CoA carboxylase ca, using a decrease in essential fatty acids. Oxidative stress occurs by exposure to heavy metals whereby free radicals are formed to oxidize and break down break down the PUFAs, including omega 3 and omega 6, thus reducing the levels.

Both arsenic and mercury affect lipid accumulation and mobility by disrupting cell and lipoprotein membranes and reducing the availability of essential fatty acids (Genchi et al., 2017). This also alters the lipid profile so that; there arefewer unsaturated fatty acids than stipulated, making Chironomus larvae poorer as a feed source in aquaculture.

Heavy metal contamination of water can affect levels of EFA in Chironomus larvae through synthesis suppression, enhanced degradation, or instability. This may result in fatty acid deficiencies in the organisms that consume them, e.g., fish, for growth, immunity, and general health. The extent of alteration also varies depending on the metal present and its concentration; lead and cadmium preferentially influence PUFA and Hg and alter overall lipid content and membrane stability. Reducing metal accumulation is essential to maintain the nutritional value of Chironomus larvae in aquaculture (Asiminicesei et al., 2024).

5.4.3.3. Effects of Heavy Metals on Vitamin Concentration in Chironomus as Food and Feed

The impact of heavy metal on Chironomus larva and vitamin synthesis Lead, cadmium, arsenic, mercury, and mercury heavy metals affect the biosynthesis of vitamins C and E, inhibiting the enzymes. Lead interferes with synthesizing water-soluble vitamins, including the B-complex vitamins (B1, B6, and B12), through chelating with enzymes, leading to lowered concentrations (Balali-Mood et al., 2021). Cadmium affects Vitamin D in the body and decreases Vitamin C and E synthesis due to oxidation stress. Heavy metals also contribute to oxidative stress, which again results in the breakdown of antioxidants vitamins C and E; for example, mercury and cadmium interfere with Vitamin E as they later fight free radicals, causing lipid peroxidation.

The toxic effect of arsenic and mercury destroys the gut lining, making nutrients such as Vitamin B9 and Vitamin B12 less bioavailable for the body to use for energy and cell division. These metals can also affect lipid metabolism and thus significantly lower the fat-soluble vitamins such as Vitamin A and D, among others. Compelling evidence showed that heavy metals induced oxidative damage to the cell membranes, in which accumulated vitamins interfere with the structure and efficiency of both water- and fat-soluble vitamins.

Contamination by metals: Chironomus larvae contain fewer essential vitamins due to metal contamination, which has minimal nutritional value in feeding. Deficiencies of vitamins that result in a lower population of fish and other aquatic species reduce growth immunities and health (Mohamed, 2023). The toxicity of varied heavy metals depends on their type and vitamin concentration, indicating critical pollution management for the nutrition value of Chironomus larvae in aquaculture and aquatic ecosystems' general health.

5.4.3.4. Effects of Heavy Metals on Micronutrient Concentration in Chironomus as Food and Feed

In Chironomus larvae, metals such as Pb, Cd, As, and Hg interact with and replace essential micrometals for binding with proteins and enzymes, hindering absorption and bioavailability. Lead displaces calcium and Iron needed for physiological activities and hurts the body. Cadmium displaces zinc zinc, which is essential in the functioning and growth of enzymes. Heavy metals also act as oxidants, causing the loss of essential micronutrients such as Iron, copper, manganese, selenium, and others as they scavenge the ROS, thus reducing their level.

Arsenic and mercury both have toxic effects on the mucosa of the stomach and intestine and reduce the absorption of micronutrients such as Iron, Zinc, and manganese. Heavy metals also change the water chemistry, whereby micronutrients such as Iron and manganese become insoluble oxides. These chelate with storage proteins, including ferritin and metallothioneins, thus altering their stability and decreasing stored micronutrient levels.

The increase in heavy metal concentration alters the micronutrient profile to that required to detoxify, decreasing the other micronutrients, resulting in the bio-enthused Chironomus larvae a feed of lower nutritive value (Pietz, 2024). They may lead to malformations in aquatic organisms that feed on them, negatively impacting growth, immune systems, and general health. It also depends on the type and concentration of the heavy metals; hence, pollution management is essential if the nutritional quality of Chironomus larvae is to be preserved to support healthy aquaculture and the overall ecosystems.

5.4.3.5. Effects of Heavy Metals on Macronutrient Concentration in Chironomus as Food and Feed

Heavy metals like lead, cadmium, arsenic, and mercury affect protein synthesis and increase protein degradation in Chironomus larvae by interacting these metals with proteins and enzymes (Arambourou et al., 2020). Lead forms complexes with sulfhydryl groups, inhibiting the ribosomes and protein synthesis, while cadmium distorts proteins through binding to thiol groups and subsequent degradation of proteins. This leads to muscles with low protein content, negatively affecting the larvae's nutritional value for aquaculture feed. Heavy metals also cause oxidative stress, contributing to lipid peroxidation and changing their dietary quality from PUFAs to SFA (Barrera, 2012; Endale et al.,2023).

Heavy metals such as arsenic and mercury affect carbohydrate metabolism by suppressing essential enzymes and depleting glycogen stores (Sabir et al., 2019; Haidar et al.,2023). Heavy metal exposure is toxic to storage organelles, which has implications for the stability and accessibility of macromolecules such as proteins, lipids, and carbohydrates. With the rise in demand for detoxification when the prostate induces heavy metal stress, there is a decrease in nutrient uptake for growth, resulting in an unbalanced macronutrient ratio (Mansoor et al.,2023).

CHAPTER SIX

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

6.1 Summary

6.1.1. Spatial Variations in Water quality parameters

The study revealed that, water quality in Winam Gulf, Lake Victoria affects life forms particularly Chironomus larvae in the region. Six monitoring stations suggest that water quality parameters are different and depend upon natural conditions and man-made changes. Some of the most important physical and chemical variables include temperature, pH, dissolved oxygen, and conductivity in the aquatic environment. Certain locations showed values that were beyond the usual tolerances of biotic organisms in water, which could have had an adverse effect on local species.

This identifies some areas with high nutrient loads leading to cases of eutrophication. It results in low oxygen levels in water and this enable establishment of polluted water organisms such as chironomid larvae. Primarily, analysis of water, sediment and insect samples in this region reveal that heavy metal contamination is present in the gulf. Some of the metals are above the WHO standards especially in the urban areas and therefore can be a threat to the ecosystem. This study found that Chironomus larvae have varying heavy metal concentrations, anomalies closely connected with increased sediment pollution.

It is found out that the physiology, biochemistry, and the nutritional condition of Chironomus larvae are influenced by various water quality indices. They are affected by factors such as pH, dissolved oxygen level and temperature with regard to their growth and stress. Irritation can make changes to the biochemical content of the water affecting enzymes and metabolism in the larvae. However, under suboptimal conditions feeding of Chironomus larvae to other forms could be less nutritious.

The present investigation clearly highlights the interrelationships between water quality, sediment pollution, and benthic animals in Winam Gulf. It raises awareness for integrated management approaches for ecosystems and suggests more studies on the potential impact of pollutants on food chains in water ecosystems. These relationships are essential to know in order to conserve and sustain the health and diversity of this significant freshwater habitat.

6.1.2. Aquatic Insects Community Structure in Winam Gulf

These aquatic insects serve as important bio–metrics of the ecosystem health in the Winam Gulf, Lake Victoria. For instance, quality alterations of water through human intervention including; urbanization and farming exert population pressure on the insects. Sensitive species in the ecosystem are confine to the clean environments while the pollution tolerant species thrive in the polluted environment.

This paper also concludes that environmental parameters are the most important factors likely to influence the distribution, abundance and the species richness of aquatic insects. They include; moderate acidity (pH 6.5-8.5), sufficient dissolved oxygen content (> 5 mg/L) and preferable water temperature (22- 28 °C) for species. On the same account, nutrient increase enhances the insect abundance but excess concentrations lead to eutrophication, which reduces the species diversity and has preference against the vulnerable species.

There is the danger of pollution with heavy metals which can lead to the accumulation of these metals and, therefore, to the reduction of the number of species and their variety. Sensitiveness ratio of Ephemeroptera is comparatively higher than some Chironomidae and thus, it shall be generally replaced by the latter in significantly polluted sites. The difference in heavy metal concentrations with reference to space results in differences of insect diversity in the gulf.

Non biting midge larvae, famously known as chironomids were observed in all the sampling stations hence they are opportune organisms regardless of the prevailing conditions in their habitat. Phylogenetic analysis using COX1 barcoding identified three Chironomus species: The following species of chironomid midges has been identified; C. transvaalensis, C. pseudothummi and Chironomus sp. They outline some of the key problems and methodologies in species recognition with special focus to genetics.

It focuses on the interactions between water beetles and their surroundings enhancing the importance of linking the conventional approach to the assessment of ecosystems with a molecular one. A comprehension of these interactions is beneficial for the conservation and sustainable use of freshwater ecosystems animating the Winam Gulf, in light of the continual changing of the environments.

6.1.3 Nutritional status of non-biting midge

Some midges species, mainly the non-biting midges Chironomus species, found in the Winam Gulf, Lake Victoria, can be potential sources of nutritive foods especially protein foods containing the essential nutrients. In the study it has been found that the nutritional values of spatial differentiation of the samples are greatly influenced by the sampling station.

Comparing the concentration of 10 essential amino acids, the authors came to the conclusion that methionine and histidine may be considered biomarkers of oxidative stress due to pollution. These profiles assist in developing a reliable bioindicator of the health of the ecosystem.

There were 20 different fatty acids identified and their concentrations varied from one site to another. It was found that pollution had a negative impact on ARA: EPA ratios and that monounsaturated fatty acids could be considered as up regulated in relation to the polluted environment.

Fat soluble vitamins A, D, E and K were seen to be present in higher concentration than water soluble vitamins and the prevalence of vitamin deficiency also varied significantly between different sites. They also realized that the levels of vitamin E may be related to adaptive mechanisms of pollution. The results themselves also highlighted that vitamin content may be influenced by environmental factors: areas with a high level of pollution may have lower levels of water-soluble vitamins.

Likewise, the concentrations of the trace metals Micronutrients (Zn, Fe, Cu) and macronutrients (Ca, Mg, Na, Al, Mn, Co) were also determined to be high and spatially variable, in some cases exceeding WHO/KEBS water quality standards. They affected the distribution of different species and the extent of species diversity.

The study is useful in determining the further role of non-biting midges as bio indicators of ecosystem health and as possible source of nutrients. This is because it suggests that future literature should continue studying the relationship between these multiple environmental aspects and nutrient concentration in these organisms for pollution and nutritional management in the region.

6.1.4 Effects of pollution on the nutritional status of the non-biting midge

Summary

The nutritional condition of Chironomus species in Winam Gulf is directly determined by the in-situ water quality parameters, nutrient concentrations and heavy metals. These aspects influenced the proportions of amino acid, fatty, vitamin and micro/macronutrient in the larvae. Environmental factors such as, temperature, dissolved oxygen, pH and conductivity have significant influences in the metabolic activities and nutrients assimilation. Under the right circumstances, nourishment resulted in increased protein production, lipid utilization and, vitamin retention, improving the quality of the larvae. The concentrations of nutrients such as nitrogen and phosphorus compounds influenced primary production and food quantity for Chironomus. Moderate levels ensured that there were balanced nutrient concentrations while high nutrients caused eutrophication hence changing the nutritional value and quality of foods produced. Chironomus larvae are particularly at high risk of receiving heavy metals hence affecting their nutritional quality. Heavy metals such as lead, cadmium, arsenic, and mercury inhibit enzymes, produce free radicals, and affect nutrient transport and utilization. This results to nutrient proportions inequality, lower essential fatty acids and vitamins, shifted protein formation.

The interrelations in these environmental aspects show the need to preserve water quality and address sources of pollution in Winam Gulf. Appropriate environmental conditions maintain Chironomus larvae as a nutritious feed for aquaculture and conserve the ecosystem base for increased productivity. Careful control of these aspects is vital in order to keep Chironomus nutritious for consumers as well as useful for the entire aquatic environment.

6.2 Conclusions and Recommendations

6.2.1. Conclusion and Recommendations 1.

6.2.1.1. Conclusion

The study done in Winam Gulf, Lake Victoria reveals how anthropogenic activities including; urbanization, industrialization, and agricultural pollution affected the freshwater habitat. These activities have caused physical, chemical and nutrient pollution which has changed the water quality parameters and has impacted the aquatic life, especially insects. Differences noted in temperature, pH, dissolved oxygen, electrical conductivity, and nutrient intensity in the sampling stations reflected differences in the environment wich h as significant impact on dispersal and existence of aquatic organisms. Precursors of nutrients such as nitrates and phosphates cause eutrophication, which decreases DO while increasing the number of

pollution-tolerant organisms like chironomids. Moreover, excessive concentration of the lead, arsenic, cadmium, and mercury especially in the sediment and insect samples is a potential threat to the ecology of the environment. The co-relations coefficients of metal concentrations in water, sediments, and insects indicate that the matrices are strongly influenced by a factor that poses considerable risk to the health of aquatic ecosystem. The results highlighted the importance of addressing and implementing measures to mitigate pollution and preserve these lifeline water sources.

6.2.1.2. Recommendations

1. Enhanced Monitoring and Pollution Control: Water quality analysis should be frequently conducted to check changes in the ecosystem because water samples need to be tested for physical, chemical, and nutrients. Improvement of quality requires decreasing the concentration of heavy metals and nutrient loads through managing and controlling industrial wastewater, agricultural leachates, and urban wastewater.

2. Use of Bioindicators for Environmental Assessment: Pollution-tolerant benthic insects such as chironomids and efímícè fauna should be utilised more often in biomonitoring to determine the levels of pollution. They can serve as predictors of changes in the environment and can assist in assessing the effects of pollution on species.

6.2.2. Conclusion and Recommendations 2.

6.2.2.1. Conclusion

Analyzing the abundance of aquatic insects in Winam Gulf shows that it is a diverse community of organisms in a highly pressurized environment due to human interference. The hypothesis of the distribution and abundance of aquatic insects and especially chironomid is important for determining water quality and the state of the ecosystem. The study emphasizes the significance of combining familiar taxonomic identification with novel molecular approaches in offering enriched insights into the species' richness, the evolutionary processes, and the effects of environmental pressures on freshwater organisms. This also implies that pollution-tolerant species dominate the contaminated zones hence the need for both environmental management and conservation measures in Winam Gulf.

6.2.2.2. Recommendations

1. Establish Long-term Biomonitoring Programs: Utilize aquatic insects, especially chironomids, as bioindicators for regular assessment of water quality and ecosystem health.

Implement standardized sampling protocols across multiple sites in Winam Gulf to track changes over time and identify areas of concern.

2. Integrate Genetic Techniques in Biodiversity Assessments: Incorporate DNA barcoding and phylogenetic analysis in routine monitoring to improve species identification accuracy and track genetic diversity changes. This approach will provide deeper insights into species adaptation and evolution in response to environmental stressors.

3. Develop Habitat Restoration Initiatives: Implement projects to restore degraded aquatic habitats, focusing on areas that support pollution-sensitive species. This could include shoreline restoration, creation of buffer zones to reduce runoff, and removal of contaminated sediments where feasible.

By implementing these recommendations, stakeholders can work towards improving the ecological integrity of Winam Gulf, preserving its biodiversity, and ensuring the sustainable management of this vital freshwater ecosystem.

OBJECTIVE 3: To evaluate the effects of pollution on the nutritional status of selected aquatic edible insects-non-biting midge, and chironomids as food and animal feeds.

6.2.3. Conclusion and Recommendations 3.

6.2.3.1. Conclusion

Analyses of non-biting midges (Chironomus species) from Winam Gulf, Lake Victoria show that these organisms have a great usefulness in determining the state of the population and also are an indispensable part of the diet. The differences in amino acids, fatty acids, vitamins, and micro/macronutrients between the different sampling sites can be attributed to the direct relationship between these organisms and the environment with specific focus on effects of pollution and other ecological factors. The nutritional composition of these midges mainly consisting of essential amino acids, omega-3 fatty acids, and vitamins and minerals qualifies this source of protein to help address food security issues. But at the same time, the influence of pollution on the nutritional quality of those organisms is also outlined, thus stressing the relationship between the condition of the environment and the quality of the obtained fish resources.

6.2.3.2. Recommendations:

1. Conduct Human Nutrition Studies: Suggest the use of non-biting midges as a food supplement, or even as a protein source for human consumption which requires further research. This should encompass research on digestibility, availability of nutrients and other related health wise advantages or disadvantages.

2. Integrate Findings into Public Health Policies: Collaborate with the public health agents to utilize the nutritional information acquired from the non-biting midges for the management of malnutrition in the area targeting the essential amino acids and omega 3 fatty acids.

3. Conduct Further Ecotoxicological Research: Examine how environmental pollutants impact nutrient accumulation on non-biting midges and the interactions between certain contaminants and shifts in nutrients.

4. Explore Aquaculture Potential: Consider the possibility to maintain midge larvae in captivity, free from genetic modification, and supply customers with contaminate-free, nourishing food.

6.2.4 Effect of Pollution on the Nutritional Status of Non-Biting Midge

6.2.4.1. Conclusion

The analysis of the influences of the abiotic factors on the nutritional condition of Chironomus species in Winam Gulf presents the intricate connection between the hydrogen andheavy metal concentrations and nutrients and the overall biochemistry of these valuable aquatic invertebrates. Thus, the study signifies the need to adhere to proper environmental conditions in order to enhance the freshness of Chironomus larvae as a food item in the ecosystem and potential feed stuff in aquaculture. It establishes that changes in the water and nutrients concentration as well as heavy metal presence affect the amino acid, fatty acid vitamin, and macro/micronutrient composition, which in turn may greatly affect the health and production of Chironomus and the whole AFP. This highlights the indispensability of holistic ecosystem management strategies that consider the interconnection between system variables and nutritional systems of species such as Chironomus.

6.2.4.2. Recommendations

1. Implement Integrated Water Quality Management: Protect the environment from many stress factors at once by signing, adopting, implementing, and regularly updating WMMP as the ultimate water quality management plan for the Winam Gulf.

2. Establish Biomonitoring Programs Using Chironomus as a Bio-indicator: Suggest the use of Chironomus species as Bio indicators for ecosystem health in Winam Gulf.

3. Carry out Interdisciplinary Investigations on Nutrient Fluxes with Specific Emphasis on how Chironomus interacts with its food chain from the Producers to higher trophic level and other consumers and quantify the processes to generate mathematical models that can be used for ecosystem and aquaculture management.

4. Examine how Chironomus larvae can be utilized in practices of sustainable recirculation aquaculture systems. Find out the best conditions for enhancing the nutritional value of Chironomus through controlling the environmental variables.

6.3. Recommendations and Suggestions for Further Studies

6.3.1. Recommendations and suggestions for further studies 1

1. Long-term Ecological Impact Assessment: Carry out a long-term research in order to assess variations in bottleneck measurements, polluting metals concentrations, and series of aquatic animals or plants. Such a long-term study of the ecosystem would give directions on trends and effects of pollution on the ecosystem in the long run.

2. Bioaccumulation and Biomagnification Analysis: Concentrate more on the heavy metals and other pollutants in the concentrations at different trophic level which include Chironomus larvae and the top consumers. It would also help to explain transfer of cont_EC of contaminants across the food chain.

3. Ecotoxicological Studies: Perform a series of laboratory tests to investigate how particular pollutants affect Chironomus larvae and other of interest, whether singly or in combination, at both the species' early and adult stages.

4. Pollution Source Tracing: To Understanding the sources of many pollutants apply isotope analysis and advanced techniques to identify significant sources of contamination.

The recommendations for further studies would go along way in improving the understanding of the diverse factors that characterise Winam Gulf. These informing data would be useful for defining better and finer tuned conservation and management approaches needed to protect this essential biophysical system.

6.3.2. Recommendations and Suggestions for further studies 2

1. Long-term Ecological Monitoring: Carry out long term research in order to show the changes with time and space of the aquatic insect species.

2. Genetic Adaptation and Speciation: Find out more about the specific genetic factors involved in the tolerance of chironomids and the other tolerant species to the polluted water condition.

3. Ecotoxicological Studies: Conduct controlled experiments to identify the impacts of different pollutants such as heavy metals and nutrients to different aquatic insects.

4. Food Web Dynamics: Investigate the influence of chironomids on the Winam Gulf food web by using stable isotopes to analyse energy flow and the implications of alteration in insect's density to fish stocks.

5. Bioremediation Potential: Investigate the possibility of using some aquatic insects especially chironomids in the process of cleaning up polluted sediments.

6. Molecular Biomarkers: Create and confirm new molecular biomarkers for environmental pollution in aquatic insects and prove its ability to predict environmental stress using gene expression patterns of protein profiles after exposure to several pollutants.

7. Emerging Contaminants: Examine the effects of these new generation pollutants on aquatic insects particularly those that occur in streams.

The suggested studies would greatly advance the knowledge of processes occurring in the Winam Gulf water environment. These would greatly help in coming up with better focused and relevant conservation and management measures that could be appropriate in the protection of this vital freshwater ecosystem and its respective biota.

6.3.3. Recommendations and suggestions for further studies 3

1. Longitudinal Nutritional Profiling: Further research should be carried out to see fluctuations in composition of non-biting midges at various seasons, in a year.
2. Bioaccumulation and Biomagnification Studies: Assess the impact of pollutants in midge nutritional content and food safety as a food source.
3. Genetic and Epigenetic Studies: Find out the role the genes play with relation to the accumulation of nutrients in non-biting midges and how any environmental stressors can trigger epigenetic events that could alter the nutritional value of these midges.
4. Nutrient Transfer in Aquatic Food Webs: Investigate the part played by non-biting midges in transferring nutrients via Winam Gulf' food Chain with special interest to the nutrition values of fish and other organisms.

The recommended studies would go a long way in increasing our knowledge on nutritional ecology of non-biting midges, in Winam gulf region in particular. They would generate useful information for designing and plan more efficient and lasting food sources, as well as designing better management ecosystem practices and gain general knowledge of the synergy between the environmental factors and nutrient cycling in water ecosystems.

6.3.4 Effect of Pollution on the Nutritional Status of Non-Biting Midge

Recommendatio ns and Suggestions for Further Studies:

Long-term Ecological and Nutritional Monitoring: Design a multiple-year analysis program to monitor shifts in Chironomus, diet composition in relation to altered environmental values.

Molecular and Physiological Adaptation Mechanisms: Research the basis of genetic and physiological flexibility that Chironomus species use to adjust their: unsuitable physiological reactions, Chironomus populations

Nutrient Transfer and Bioaccumulation Studies: Identification of the possible advantages/ disadvantages and implications of employing Chironomus as food for fish in aquaculture sectors emphasizing the ability of these insects to mediate nutrient/ foreign substance cycling.

1. Ecotoxicological Studies on Emerging Contaminants: Investigate the impacts of emerging contaminants (e.g., microplastics, pharmaceuticals, personal care products) on the

nutritional status of Chironomus and develop biomarkers for early detection of emerging contaminant impacts on aquatic ecosystem

- Nutritional Enhancement and Sustainable Aquaculture Applications: Explore methods to enhance the nutritional value of Chironomus for sustainable aquaculture applications (optimized cultivation conditions, use of probiotics or specialized feeds, economic feasibility and environmental sustainability).

The suggested studies would help uplift the knowledge of the multifaceted relationships between the physical environment conditions and the diets of Chironomus species in Winam Gulf. They would generate useful information for enhancing better and specific conservation measures, enhancing the ways of managing ecosystems, and for assessing the possibilities of rational using of aquatic stocks. The prospective interdisciplinarity of such studies would imply the cooperation of ecologists, nutritionists, geneticists, and specialists in aquaculture that would naturally contribute towards the more efficient utilizing and managing of the Winam Gulf resources.

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` **APPENDICES**

APPENDIX I PUBLICATIONS

1. Monicah Florence Misiko[1, 2], Taurai Bere[3], Darius O. Andika[1,2], Patrick Okoth[5], Benson Onyango[4] (2023).**Spatial Variations In Aquatic Insect Community Structure In Winam Gulf, Lake Victoria, Kenya.**:International Journal Of Ecology.
2. **Misiko, M. F.,** Andika, D., Angienda, P. O., & Onyango, B. (2024). A Microcosm Analysis of Species-Specific Responses of Chironomidae on Heavy Metal Pollution in The Nyanza Gulf of Lake Victoria. East African Journal of Environment and Natural Resources, 7(1), 401-422. https://doi.org/10.37284/eajenr.7.1.2179.
3. Monicah Florence Misiko[1, 2], Taurai Bere[3], Darius O. Andika[1,2], Patrick Okoth[5], Benson Onyango[4] (2023). **Chironomid Larvae as Food in the Lake Victoria Basin, Kenya.**: International Journal Of Agricultura Scientia.(under review)
4. Monicah Florence Misiko[1,2], Taurai Bere[4], Darius Andika[1,2], Patrick Okoth,[5] Paul Oyieng Angienda[6] And Benson Onyango[3](2022).**Sequences Banked in NCBI Data Base,Assigned Accession No.ON455096-ON455103:Cytochrome c oxidaseI(CO1) gene,partial cds,mitochondrial.**

APPENDIX II FASTA SEQUENCES

FASTA SEQUENCES FROM CHIRONMOMUS ISOLATE FROM THE SAMPLING STATIONS CH1 TO CH8, COX 1

Chironomus transvaalensis isolate CH1_kisumu cytochrome c oxidase subunit I (COX1) gene, partial cds, mitochondrial

Genbank: ON455096.1

Genbank Graphics

>ON455096.1 Chironomus transvaalensis isolate CH1_kisumu cytochrome c oxidase subunit I (COX1) gene, partial cds, mitochondrial

TCTTTAAGTATGCTTATTCGAGCAGAATTAGGACGACCTGGAACATTTATTGGTG
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CTAATTGGTGGATT
TGGTAATTGATTAGTTCCTCTTATATTAGGAGCCCCTGATATGGCGTTTCCTCGA
ATAAATAATATAAGT
TTTTGACTTCTCCCTCCTTCATTAACTCTTTTACTTTCTAGTTCTTTTGTAGAAAA
TGGAGCAGGAACCG
GTTGAACTGTTTATCCCCCCCTTTCTGCAGCAATTGCTCACAGTGGGGCTTCTGT
TGATTTAGCAATTTT
TTCTCTACATTTAGCAGGTGTTTCATCAATTTTAGGATCAGTAAATTTTATTACT
ACAGTTATTAATATA
CGAGCTAATGGAATTACTCTTGATCGAATACCTTTATTTGTTTGATCAGTTGTAA
TTACAACTGTTCTTT
TATTACTATCTCTTCCTGTATTAGCAGGGGCTATTACTATACTTCTTACTGATCG
AAATTTAAATACAT

Chironomus pseudothummi isolate CH2_kendubay cytochrome c oxidase subunit I (COX1) gene, partial cds, mitochondrial

Genbank: ON455097.1

Genbank Graphics

>ON455097.1 Chironomus pseudothummi isolate CH2_kendubay cytochrome c oxidase subunit I (COX1) gene, partial cds, mitochondrial

ATCTTTAAGAATGCTTATTCGAGCAGAATTAGGACGACCTGGAACATTTATTGG
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TTCTAATTGGTGGTT
TTGGTAATTGACTAATTCCCCTAATACTAGGAGCCCCAGATATGGCCTTCCCACG
AATAAATAATATAAG
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ATTACTACTGTATTA
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GAAATTTAAATACAT
C

Chironomus sp. isolate CH3_kendubay cytochrome c oxidase subunit I (COX1) gene, partial cds, mitochondrial

Genbank: ON455098.1

Genbank Graphics

>ON455098.1 Chironomus sp. isolate CH3_kendubay cytochrome c oxidase subunit I (COX1) gene, partial cds, mitochondrial

TCTTTAAGAATGCTTATTCGAGCAGAATTAGGACGACCTGGAACATTTATTGGT
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TATTACTTTCTCTCCCAGTATTAGCTGGA
//

Chironomus pseudothummi isolate CH4_kendubay *cytochrome c oxidase subunit* I (COX1) gene, partial cds, *mitochondrial*

Genbank: ON455099.1

Genbank Graphics

>ON455099.1 Chironomus pseudothummi isolate CH4_kendubay cytochrome c oxidase subunit I (COX1) gene, partial cds, mitochondrial

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Chironomus pseudothummi isolate CH5_kendubay cytochrome c oxidase subunit I (COX1) gene, partial cds, mitochondrial

Genbank: ON455100.1

Genbank Graphics

>ON455100.1 *Chironomus pseudothummi* isolate CH5_kendubay cytochrome c *oxidase subunit* I (COX1) gene, partial cds, *mitochondrial*

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GGAATTACTTTAGACCGAATACCTTTATTTGTTTGATCAGTTGTTATTACTACTG
TATTACTATTACTTT
CTCTCCCAGTATTAGCTGGAGCTATTACAATACTTCTTACAGATCGAAATTTAAA
TACAT

Chironomus pseudothummi isolate CH6_kendubay *cytochrome c oxidase* subunit I (COX1) gene, partial cds, mitochondrial

Genbank: ON455101.1

Genbank Graphics

>ON455101.1 *Chironomus pseudothummi* isolate CH6_kendubay *cytochrome* c *oxidase* subunit I (COX1) gene, partial cds, mitochondrial

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ATTTATAATGTAGTAG
TAACCGCACATGCATTTATTATAATTTTCTTCATAGTTATACCAATTCTAATTGG
TGGTTTTGGTAATTG
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CTCTCCCAGTATTAGCTGGAGCTATTACAATACTTCTTACAGATCGAAATTTAAA
TAC

Chironomus sp. isolate CH7_NdereIsland cytochrome c oxidase subunit I (COX1) gene, partial cds, mitochondrial

Genbank: ON455102.1

Genbank Graphics

>ON455102.1 Chironomus sp. isolate CH7_NdereIsland cytochrome c oxidase subunit I (COX1) gene, partial cds, mitochondrial

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TGCTTCAGTTGATTTAGCTATTTTTTCCCTCCATTTAGCTGGTATTTCTTCTATTTT
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CTATTGTAATTACAACAGTATTATTACTACTTTCACTCCCAGTTTTAGCTGGAGC
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//

S

Chironomus pseudothummi isolate CH8_HomaBay cytochrome c oxidase subunit I (COX1) gene, partial cds, mitochondrial

Genbank: ON455103.1

Genbank Graphics

>ON455103.1 *Chironomus pseudothummi* isolate CH8_HomaBay *cytochrome* c oxidase subunit I (COX1) gene, partial cds, mitochondrial

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AC

APPENDIX III GEN BANK SEQUENCES

GENBANK OF THE CHIRONOMUS ISOLATE FROM THE SAMPLING STATION CH1 TO CH8, COX 1

Chironomus transvaalensis isolate CH1_kisumu *cytochrome c oxidase* subunit I (COX1) gene, partial cds, mitochondrial

Genbank: ON455096.1

FASTA Graphics

Go to:

LOCUS ON455096 559 Pb DNA linear INV 15-MAY-2022

DEFINITION *Chironomus* transvaalensis isolate CH1_kisumu *cytochrome* c oxidase subunit I (COX1) gene, partial cds, mitochondrial.

ACCESSION ON455096

VERSION ON455096.1

KEYWORDS.

SOURCE mitochondrion Chironomus transvaalensis

ORGANISM *Chironomus transvaalensis*

Eukaryote, Metazoan, Ecdysozoa, Arthropoda, Hexapoda, Insecta, Pterygota, Neoptera, Endopterygota, Diptera, Nematocera, Chironomoidea, Chironomidae, Chironominae, Chironomus.

REFERENCE 1 (bases 1 to 559)

AUTHORS Misiko, F.M., Angienda, P.O., Onyango, B. and Bere, T.

TITLE Direct Submission

JOURNAL Submitted (07-MAY-2022) Food Security and Sustainable Agriculture, Jaramogi Oginga Odinga University of Science and Technology, Bondo, Bondo 40601, Kenya

COMMENT ##Assembly-Data-START##

Sequencing Technology: Sanger dideoxy sequencing

##Assembly-Data-END##

FEATURES Location/Qualifiers

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CDS <1..>559
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/product="cytochrome c oxidase subunit I"
/protein_id="UQE68956.1"

/translation="SLSMLIRAELGRPGTFIGDDQIYNVVVTAHAFIMIFFMVMPILI

GGFGNWLVPLMLGAPDMAFPRMNNMSFWLLPPSLTLLLSSSFVENGAGTGWTVY
PPLS

AAIAHSGASVDLAIFSLHLAGVERSESSILGSVNFITTVINMRANGITLDRMPLFVWS
VVIT
TVLLLLSLPVLAGAITMLLTDRNLNT"
ORIGIN
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61 caaatttata atgttgttgt tacagctcac gcttttatta taatttttt catagttata
121 cctattctaa ttggtggatt tggtaattga ttagttcctc ttatattagg agcccctgat
181 atggcgtttc ctcgaataaa taatataagt ttttgacttc tccctccttc attaactctt
241 ttactttcta gttcttttgt agaaaatgga gcaggaaccg gttgaactgt ttatcccccc
301 ctttctgcag caattgctca cagtggggct tctgttgatt tagcaatttt ttctctacat

361 ttagcaggtg tttcatcaat tttaggatca gtaaatttta ttactacagt tattaatata

421 cgagctaatg gaattactct tgatcgaata cctttatttg tttgatcagt tgtaattaca

481 actgttcttt tattactatc tcttcctgta ttagcagggg ctattactat acttcttact

541 gatcgaaatt taaatacat

//

Chironomus pseudothummi isolate CH2_kendubay cytochrome c oxidase subunit I (COX1) gene, partial cds, mitochondrial

GenBank: ON455097.1

FASTA Graphics

Go to:

LOCUS ON455097 561 bp DNA linear INV 15-MAY-2022

DEFINITION *Chironomus pseudothummi* isolate CH2_kendubay cytochrome c oxidase subunit I (COX1) gene, partial cds, mitochondrial.

ACCESSION ON455097

VERSION ON455097.1

KEYWORDS.

SOURCE mitochondrion *Chironomus pseudothummi*

ORGANISM *Chironomus pseudothummi*

Eukaryote, Metazoan, Ecdysozoa, Arthropoda, Hexapoda, Insecta, Pterygota, Neoptera, Endopterygota, Diptera, Nematocera, Chironomoidea, Chironomidae, Chironominae, Chironomus.

REFERENCE 1 (bases 1 to 561)

AUTHORS Misiko, F.M., Angienda, P.O., Onyango, B. and Bere, T.

TITLE Direct Submission

JOURNAL Submitted (07-MAY-2022) Food Security and Sustainable Agriculture, Jaramogi Oginga Odinga University of Science and Technology, Bondo, Bondo 40601, Kenya

COMMENT ##Assembly-Data-START##

Sequencing Technology: Sanger dideoxy sequencing

##Assembly-Data-END##

FEATURES Location/Qualifiers

source 1.561

/organism="Chironomus pseudothummi"

/organelle="mitochondrion"

/holotype="genomic DNA"

/isolate="CH2_kendubay"

/db_xref="taxon:72528"

/country="Kenya"

gene <1..>561

/gene="COX1"

CDS <1..>561

/gene="COX1"

/codon start=2

/translatable=5

/product="*cytochrome c oxidase* subunit I"

/protein_id="UQE68957.1"

/translation="SLSMLIRAELGRPGTFIGDDQIYNVVVTAHAFIMIFFMVMPILI

GGFGNWLIPLMLGAPDMAFPRMNNMSFWLLPPSLTLLLSSSFVQNGAGTGWTVYP
PLS

AAIAHSGASVDLAIFSLHLAGVERSESSILGXVNFITTVINMRANGITLDRMPLFVW
SVVIT

TVLLLLSLPVLAGAITMLLTDRNLNTS"

ORIGIN

1 atctttaaga atgcttattc gagcagaatt aggacgacct ggaacattta ttggtgatga

61 tcaaatttat aatgtagtag taaccgcaca tgcatttatt ataattttct tcatagttat

121 accaattcta attggtggtt ttggtaattg actaattccc ctaatactag gagccccaga

181 tatggccttc ccacgaataa ataatataag tttttgactt cttcccccat ctcttacact

241 tttactttca agttcattcg tacaaaatgg agcaggaaca ggatgaacag tttatccccc

301 tctttcagcc gcaattgctc atagaggagc ctctgtagat ttagcaattt tttctcttca

361 tctagccgga gtttcatcta ttttaggtts tgtaaatttt attaccacag ttattaatat

421 acgggcaaac ggaattactt tagaccgaat acctttattt gtttgatcag ttgttattac

481 tactgtatta ctattacttt ctctcccagt attagctgga gctattacaa tacttcttac

541 agatcgaaat ttaaatacat c

//

Chironomus sp. isolate CH3_kendubay *cytochrome c oxidase* subunit I (COX1) gene, partial cds, mitochondrial

Genbank: ON455098.1

FASTA Graphics

Go to:

LOCUS ON455098 519 bp DNA linear INV 15-MAY-2022

DEFINITION *Chironomus sp.* isolate CH3_kendubay *cytochrome* c oxidase subunit I (COX1) gene, partial cds, mitochondrial.

ACCESSION ON455098

VERSION ON455098.1

KEYWORDS.

SOURCE mitochondrion Chironomus sp.

ORGANISM *Chironomus sp.*

Eukaryota, Metazoa, Ecdysozoa, Arthropoda, Hexapoda, Insecta, Pterygota, Neoptera, Endopterygota, Diptera, Nematocera, Chironomoidea, Chironomidae, Chironominae, Chironomus.

REFERENCE 1 (bases 1 to 519)

AUTHORS Misiko, F.M., Angienda, P.O., Onyango, B. and Bere, T.

TITLE Direct Submission

JOURNAL Submitted (07-MAY-2022) Food Security and Sustainable Agriculture, Jaramogi Oginga Odinga University of Science and Technology, Bondo, Bondo 40601, Kenya

COMMENT ##Assembly-Data-START##

Sequencing Technology: Sanger dideoxy sequencing

##Assembly-Data-END##

FEATURES Location/Qualifiers

source 1.519

/organism="*Chironomus sp."*

/organelle="mitochondrion"

/mol_type="genomic DNA"

/isolate="CH3_kendubay"

/db_xref="taxon:7152"

/country="Kenya"

gene <1..>519

/gene="COX1"

CDS <1..>519

/gene="COX1"

/codon_start=1

/transl_table=5

/product="cytochrome c oxidase subunit I"

/protein_id="UQE68958.1"

/translation="SLSMLIRAELGRPGTFIGDDQIYNVVVTAHAFIMIFFMVMPILI

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PLS

AAIAHSGASVDLAIFSLHLAGVERSESSILGSVNFITTVINMRAKGITLDRMPLFVWS
VVIT

TVLLLLSLPVLAG"

ORIGIN

1 tctttaagaa tgcttattcg agcagaatta ggacgacctg gaacatttat tggtgatgat

61 caaatttata atgtggtagt aaccgcacat gcatttatta taattttctt catagttata

121 ccaattctaa ttggtggttt tggtaattga ctaattcccc taatactagg agccccagat

181 atggccttcc cacgaataaa taatataagt ttttgacttc ttccccccatc tcttacactt

241 ttactttcaa gttcattcgt agaaaatggg gcaggaacag gatgaacagt ttatcccccct

301 ctttcagccg caattgctca tagaggagcc tctgtagatt tagcaatttt ttctcttcat

361 ctagccggag tttcatctat tttaggttct gtaaatttta ttaccacagt tattaatata

421 cgggcaaaag gaattacttt agaccgaata cctttatttg tttgatcagt tgttattact

481 actgtattac tattactttc tctcccagta ttagctgga

//

Chironomus pseudothummi isolate CH4_kendubay cytochrome c oxidase subunit I (COX1) gene, partial cds, mitochondrial

Genbank: ON455099.1

FASTA Graphics

Go to:

LOCUS ON455099 559 bp DNA linear INV 15-MAY-2022

DEFINITION Chironomus pseudothummi isolate CH4_kendubay cytochrome c oxidase subunit I (COX1) gene, partial cds, mitochondrial.

ACCESSION ON455099

VERSION ON455099.1

KEYWORDS.

SOURCE *mitochondrion Chironomus pseudothummi*

ORGANISM Chironomus pseudothummi

Eukaryota, Metazoa, Ecdysozoa, Arthropoda, Hexapoda, Insecta, Pterygota, Neoptera, Endopterygota, Diptera, Nematocera, Chironomoidea, Chironomidae, Chironominae, Chironomus.

REFERENCE 1 (bases 1 to 559)

AUTHORS Misiko, F.M., Angienda, P.O., Onyango, B. and Bere, T.

TITLE Direct Submission

JOURNAL Submitted (07-MAY-2022) Food Security and Sustainable Agriculture, Jaramogi Oginga Odinga University of Science and Technology, Bondo, Bondo 40601, Kenya

COMMENT ##Assembly-Data-START##

Sequencing Technology: Sanger dideoxy sequencing

##Assembly-Data-END##

FEATURES Location/Qualifiers

source 1.559

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/organelle="mitochondrion"
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/db_xref="taxon:72528"
/country="Kenya"
gene <1..>559
/gene="COX1"
CDS <1..>559
/gene="COX1"
/codon_start=2
/transl_table=5
/product="cytochrome c oxidase subunit I"
/protein_id="UQE68959.1"

/translation="SLSMLIRAELGRPGTFIGDDQIYNVVVTAHAFIMIFFMVMPILI

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PLS

AAIAHSGASVDLAIFSLHLAGVERSESSILGSVNFITTVINMRANGITLDRMPLFVWS
VVIT
TVLLLLSLPVLAGAITMLLTDRNLNT"
ORIGIN
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61 tcaaatttat aatgtggtag taaccgcaca tgcatttatt ataattttct tcatagttat
121 accaattcta attggtggtt ttggtaattg actaattccc ctaatactag gagccccaga
181 tatggccttc ccacgaataa ataatataag tttttgactt cttcccccat ctcttacact
241 tttactttca agttcattcg tagaaaatgg ggcaggaaca ggatgaacag tttatccccc
301 tctttcagcc gcaattgctc atagaggagc ctctgtagat ttagcaattt tttctcttca
361 tctagccgga gtttcatcta ttttaggttc tgtaaatttt attaccacag ttattaatat
421 acgggcaaat ggaattactt tagaccgaat acctttattt gtttgatcag ttgttattac

481 tactgtatta ctattacttt ctctcccagt attagctgga gctattacaa tacttcttac

541 agatcgaaat ttaaataca

//

Chironomus pseudothummi isolate CH5_kendubay cytochrome c oxidase subunit I (COX1) gene, partial cds, mitochondrial

Genbank: ON455100.1

FASTA Graphics

LOCUS ON455100 550 bp DNA linear INV 15-MAY-2022

DEFINITION *Chironomus pseudothummi* isolate CH5_kendubay cytochrome c oxidase subunit I (COX1) gene, partial cds, mitochondrial.

ACCESSION ON455100

VERSION ON455100.1

KEYWORDS.

SOURCE *mitochondrion Chironomus pseudothummi*

ORGANISM Chironomus pseudothummi

Eukaryote, Metazoan, Ecdysozoa, Arthropoda, Hexapoda, Insecta, Pterygota, Neoptera, Endopterygota, Diptera, Nematocera, Chironomoidea, Chironomidae, Chironominae, Chironomus.

REFERENCE 1 (bases 1 to 550)

AUTHORS Misiko, F.M., Angienda, P.O., Onyango, B. and Bere, T.

TITLE Direct Submission

JOURNAL Submitted (07-MAY-2022) Food Security and Sustainable Agriculture, Jaramogi Oginga Odinga University of Science and Technology, Bondo, Bondo 40601, Kenya

COMMENT ##Assembly-Data-START##

Sequencing Technology: Sanger dideoxy sequencing

##Assembly-Data-END##

FEATURES Location/Qualifiers

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/organelle="mitochondrion"

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/db_xref="taxon:72528"
/country="Kenya"
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/gene="COX1"
CDS <1..>550
/gene="COX1"
/codon_start=1
/transl_table=5
/product="cytochrome c oxidase subunit I"
/protein_id="UQE68960.1"

/translation="MLIRAELGRPGTFIGDDQIYNVVVTAHAFIMIFFMVMPILIGGF

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AAI

AHSGASVDLAIFSLHLAGVERSESSILGSVNFITTVINMRANGITLDRMPLFVWSVVI
TTVL
LLLSLPVLAGAITMLLTDRNLNT"
ORIGIN
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61 aatgtggtag taaccgcaca tgcatttatt ataatttttct tcatagttat accaattcta
121 attggtggtt ttggtaattg actaattccc ctaatactag gagccccaga tatggccttc
181 ccacgaataa ataatataag tttttgactt cttcccccat ctcttacact tttactttca
241 agttcattcg tagaaaatgg ggcaggaaca ggatgaacag tttatccccc tctttcagcc
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361 gtttcatcta ttttaggttc tgtaaatttt attaccacag ttattaatat acgggcaaac
421 ggaattactt tagaccgaat acctttattt gtttgatcag ttgttattac tactgtatta
481 ctattacttt ctctcccagt attagctgga gctattacaa tacttcttac agatcgaaat
541 ttaaatacat
//

Chironomus pseudothummi isolate CH6_kendubay cytochrome c oxidase subunit I (COX1) gene, partial cds, mitochondrial

Genbank: ON455101.1

FASTA Graphics

Go to:

LOCUS ON455101 548 bp DNA linear INV 15-MAY-2022
DEFINITION *Chironomus pseudothummi* isolate CH6_kendubay *cytochrome* c oxidase subunit I (COX1) gene, partial cds, mitochondrial.
ACCESSION ON455101
VERSION ON455101.1
KEYWORDS.
SOURCE mitochondrion Chironomus pseudothummi
ORGANISM *Chironomus pseudothummi*
Eukaryota, Metazoa, Ecdysozoa, Arthropoda, Hexapoda, Insecta, Pterygota, Neoptera, Endopterygota, Diptera, Nematocera, Chironomoidea, Chironomidae, Chironominae, Chironomus.
REFERENCE 1 (bases 1 to 548)
AUTHORS Misiko, F.M., Angienda, P.O., Onyango, B. and Bere, T.
TITLE Direct Submission
JOURNAL Submitted (07-MAY-2022) Food Security and Sustainable Agriculture, Jaramogi Oginga Odinga University of Science and Technology, Bondo, Bondo 40601, Kenya
COMMENT ##Assembly-Data-START##
Sequencing Technology: Sanger dideoxy sequencing
##Assembly-Data-END##
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/organelle="mitochondrion"
/mol_type="genomic DNA"
/isolate="CH6_kendubay"

/db_xref="taxon:72528"
/country="Kenya"
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/gene="COX1"
CDS <1..>548
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/codon_start=1
/transl_table=5
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/protein_id="UQE68961.1"

/translation="MLIRAELGRPGTFIGDDQIYNVVVTAHAFIMIFFMVMPILIGGF

GNWLIPLMLGAPDMAFPRMNNMSFWLLPPSLTLLLSSSFVENGAGTGWTVYPPLS
AAI

AHSGASVDLAIFSLHLAGVERSESSILGSVNFITTVINMRANGITLDRMPLFVWSVVI
TTVL
LLLSLPVLAGAITMLLTDRNLNT"
ORIGIN
1 atgcttattc gagcagaatt aggacgacct ggaacattta ttggtgatga tcaaatttat
61 aatgtagtag taaccgcaca tgcatttatt ataatttttct tcatagttat accaattcta
121 attggtggtt ttggtaattg actaattccc ctaatactag gagccccaga tatggccttc
181 ccacgaataa ataatataag tttttgactt cttcccccat ctcttacact tttactttca
241 agttcattcg tagaaaatgg agcaggaaca ggatgaacag tttatccccc tctttcagcc
301 gcaattgctc atagaggagc ctctgtagat ttagcaattt tttctcttca tctagccgga
361 gtttcatcta ttttaggttc tgtaaatttt attaccacag ttattaatat acgggcaaac
421 ggaattactt tagaccgaat acctttattt gtttgatcag ttgttattac tactgtatta
481 ctattacttt ctctcccagt attagctgga gctattacaa tacttcttac agatcgaaat
541 ttaaatac
//

Chironomus sp. isolate CH7_NdereIsland cytochrome c oxidase subunit I (COX1) gene, partial cds, mitochondrial

Genbank: ON455102.1

FASTA Graphics

Go to:

LOCUS ON455102 514 bp DNA linear INV 15-MAY-2022
DEFINITION Chironomus sp. isolate CH7_NdereIsland cytochrome c oxidase subunit I (COX1) gene, partial cds, mitochondrial.
ACCESSION ON455102
VERSION ON455102.1
KEYWORDS.
SOURCE mitochondrion Chironomus sp.
ORGANISM Chironomus sp.
Eukaryota, Metazoa, Ecdysozoa, Arthropoda, Hexapoda, Insecta, Pterygota, Neoptera, Endopterygota, Diptera, Nematocera, Chironomoidea, Chironomidae, Chironominae, Chironomus.
REFERENCE 1 (bases 1 to 514)
AUTHORS Misiko, F.M., Angienda, P.O., Onyango, B. and Bere, T.
TITLE Direct Submission
JOURNAL Submitted (07-MAY-2022) Food Security and Sustainable Agriculture, Jaramogi Oginga Odinga University of Science and Technology, Bondo, Bondo 40601, Kenya
COMMENT ##Assembly-Data-START##
Sequencing Technology: Sanger dideoxy sequencing
##Assembly-Data-END##
FEATURES Location/Qualifiers
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/organism="Chironomus sp."
/organelle="mitochondrion"
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/isolate="CH7_NdereIsland"

/db_xref="taxon:7152"
/country="Kenya"
gene <1..>514
/gene="COX1"
CDS <1..>514
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/transl_table=5
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/protein_id="UQE68962.1"

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MLLTDRNLNTS"

ORIGIN

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      181 cttctcttac tttacttctt tcaagtagta ttgtagaaaa cggagcagga actggttgaa
      241 cagtttatcc tccattgtct tctagaattg ctcatagagg tgcttcagtt gatttagcta
      301 ttttttccct ccatttagct ggtatttctt ctattttagg ttctgtaaat tttattacaa
      361 ctgttattaa tatacgatca agaggaatta cattagatcg aatacctta tttgtttggt
      421 ctattgtaat tacaacagta ttattactac tttcactccc agttttagct ggagcaatta
      481 caatattatt aactgatcga aatttaaata catc
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//

Chironomus pseudothummi isolate CH8_HomaBay cytochrome c oxidase subunit I (COX1) gene, partial cds, mitochondrial

Genbank: ON455103.1

FASTA Graphics

Go to:

LOCUS ON455103 547 bp DNA linear INV 15-MAY-2022

DEFINITION Chironomus pseudothummi isolate CH8_HomaBay cytochrome c oxidase subunit I (COX1) gene, partial cds, mitochondrial.

ACCESSION ON455103

VERSION ON455103.1

KEYWORDS.

SOURCE *mitochondrion Chironomus pseudothummi*

ORGANISM Chironomus pseudothummi

Eukaryota, Metazoa, Ecdysozoa, Arthropoda, Hexapoda, Insecta, Pterygota, Neoptera, Endopterygota, Diptera, Nematocera, Chironomoidea, Chironomidae, Chironominae, Chironomus.

REFERENCE 1 (bases 1 to 547)

AUTHORS Misiko, F.M., Angienda, P.O., Onyango, B. and Bere, T.

TITLE Direct Submission

JOURNAL Submitted (07-MAY-2022) Food Security and Sustainable Agriculture, Jaramogi Oginga Odinga University of Science and Technology, Bondo, Bondo 40601, Kenya

COMMENT ##Assembly-Data-START##

Sequencing Technology: Sanger dideoxy sequencing

##Assembly-Data-END##

FEATURES Location/Qualifiers

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/organism="Chironomus pseudothummi"

/organelle="mitochondrion"

/mol_type="genomic DNA"

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/db_xref="taxon:72528"

/country="Kenya"

gene <1..>547

/gene="COX1"

CDS <1..>547

/gene="COX1"

/codon_start=3

/transl_table=5

/product="cytochrome c oxidase subunit I"

/protein_id="UQE68963.1"

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TVLL

LLSLPVLAGAITMLLTDRNLNT"

ORIGIN

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    121 ttggtggttt tggtaattga ctaattcccc taatactagg agccccagat atggcctttc
    181 cacgaataaa taatataagt ttttgacttc ttccccccatc tcttacactt ttactttcaa
    241 gttcattcgt agaaaatggg gcaggaacag gatgaacagt ttatcccccct ctttcagccg
    301 caattgctca tagaggagcc tctgtagatt tagcaatttt ttctcttcat ctagccggag
    361 tttcatctat tttaggttct gtaaatttta ttaccacagt tattaatata cgcacaaacg
    421 gaattacttt agaccgaata cctttatttg tttgatcagt tgttattact actgtattac
    481 tattactttc tctcccagta ttagctggag ctattacaat acttcttaca gatcgaaatt
    541 taaatac
```

//

S

APPENDIX IV ACCESSION NUMBERS

ACCESSION NUMBERS

TABLE…. BELOW ARE ACCESSION NUMBERS FROM NCBI

SAMPLE STATION	ACCESSION NUMBERS	SEQUENCE IDENTIFY
CH 1	JQ025715.1	Chironomus transvaalensis isolate CH1_kisumu cytochrome c oxidase subunit I (COX1) gene, partial cds, mitochondrial Genbank: ON455096.1 Chironomus transvaalensis isolate EM5 cytochrome oxidase subunit I gene, partial cds, mitochondrial Sequence ID: JQ025715.1Length: 631
CH2	MZ657916.1	Chironomus nr. pseudothummi ZMUO 024913 voucher ZMUO.024913 cytochrome oxidase subunit 1 (COI) gene, partial cds, mitochondrial Sequence ID: MZ657916.1Length: 658
CH3		Chironomus sp. isolate CH3_kendubay cytochrome c oxidase subunit I (COX1) gene, partial cds, mitochondrial

		Genbank: ON455098.1
CH4	MZ657916.1	Chironomus nr. pseudothummi ZMUO 024913 voucher ZMUO.024913 cytochrome oxidase subunit 1 (COI) gene, partial cds, mitochondrial Sequence ID: MZ657916.1Length: 658
CH5	MZ657916.1	Chironomus nr. pseudothummi ZMUO 024913 voucher ZMUO.024913 cytochrome oxidase subunit 1 (COI) gene, partial cds, mitochondrial Sequence ID: MZ657916.1Length: 658
CH6	MZ657916.1	Chironomus nr. pseudothummi ZMUO 024913 voucher ZMUO.024913 cytochrome oxidase subunit 1 (COI) gene, partial cds, mitochondrial Sequence ID: MZ657916.1Length: 658
CH7	KX051969.1	Chironomidae sp. sc_06001 cytochrome oxidase subunit 1 (COI) gene, partial cds, mitochondrial Sequence ID: KX051969.1Length: 658
CH8	MZ657916.1	Chironomus nr. pseudothummi ZMUO 024913 voucher ZMUO.024913 cytochrome oxidase

		subunit 1 (COI) gene, partial cds, mitochondrial Sequence ID: MZ657916.1Length: 658

APPENDIX V SUMMARY OF ANOVA TABLES ON NUTRITION

```
> summary(anova.aminoacid)
             Df Sum Sq Mean Sq F value Pr(>F)
Stations      3  0.037  0.0122   0.252  0.859
aminoacid     9 20.901  2.3224  48.029 <2e-16 ***
Residuals   107  5.174  0.0484
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Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
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```
> summary(anova.fatiacids)
             Df Sum Sq Mean Sq F value Pr(>F)
Stations      3     19     6.5   1.794  0.149
fatiacids    21  12378   589.4 163.295 <2e-16 ***
Residuals   238    859     3.6
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Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

```
> summary(anova.vitamins)
             Df Sum Sq Mean Sq F value Pr(>F)
Stations      3      9     3.0   1.487  0.221
vitamins     12  10155   846.3 413.772 <2e-16 ***
Residuals   138    282     2.0
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

APPENDICES VI AMINO ACIDS ANALYSIS-PEAKS

KEY: Sample 1-Kisumu Bay*Sample 2-Homabay*Sample 3-Ndere Island*Sample 4-Kendubay

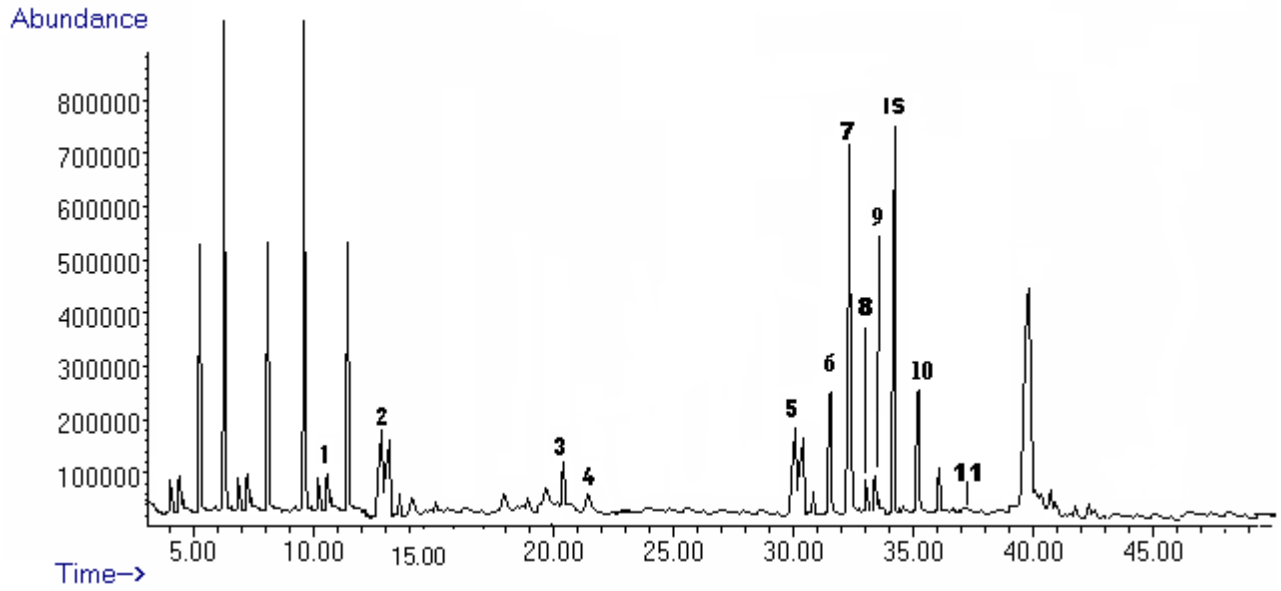

Figure: Sample 1-Kisumubay. Acids Analysis in Nonbiting Midge from Kisumu Bay.

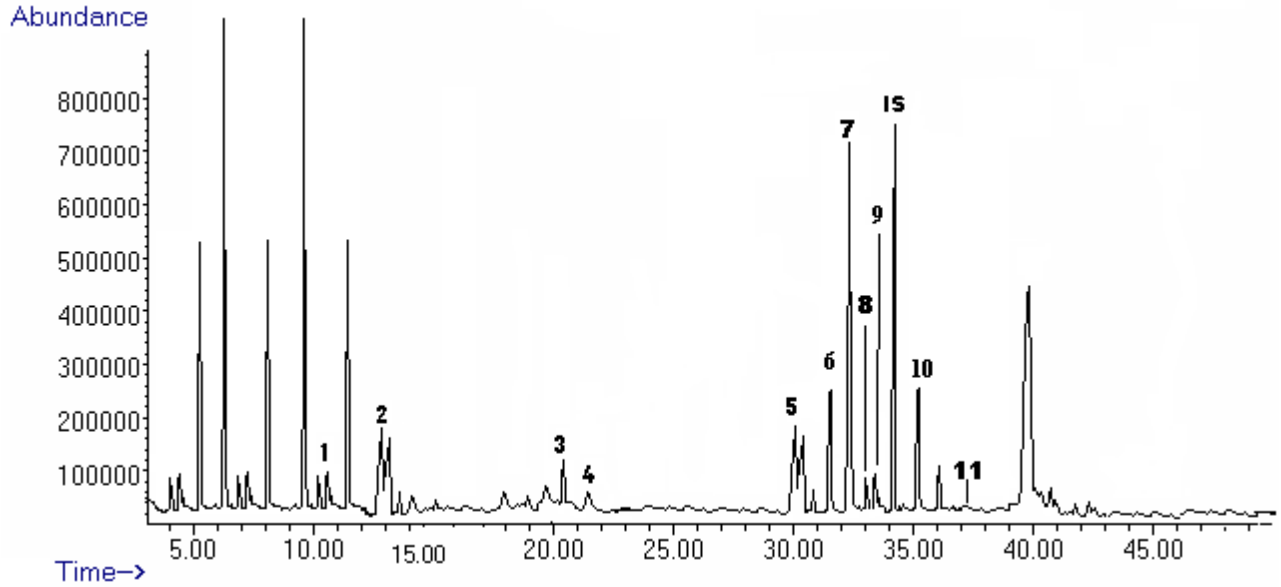

Figure –Sample 2. Amino Acids Analysis in Nonbiting Midge from Homabay.

:

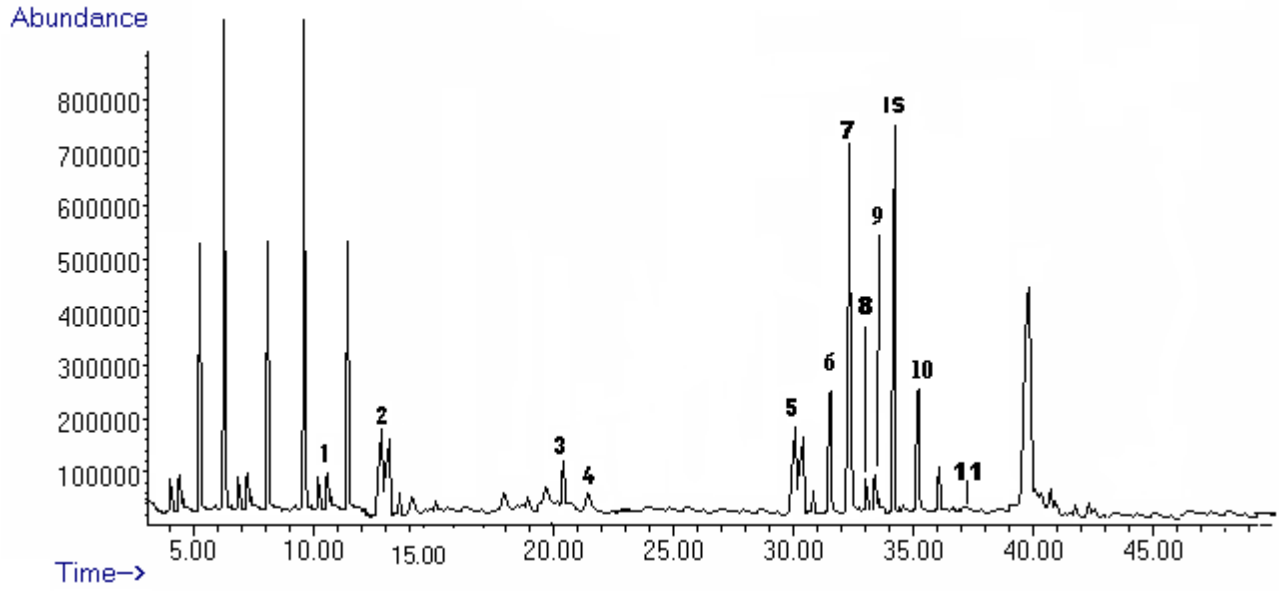

Figure: Sample 3-Ndere Island. Amino Acids Analysis in Nonbiting Midge from ND ere Island

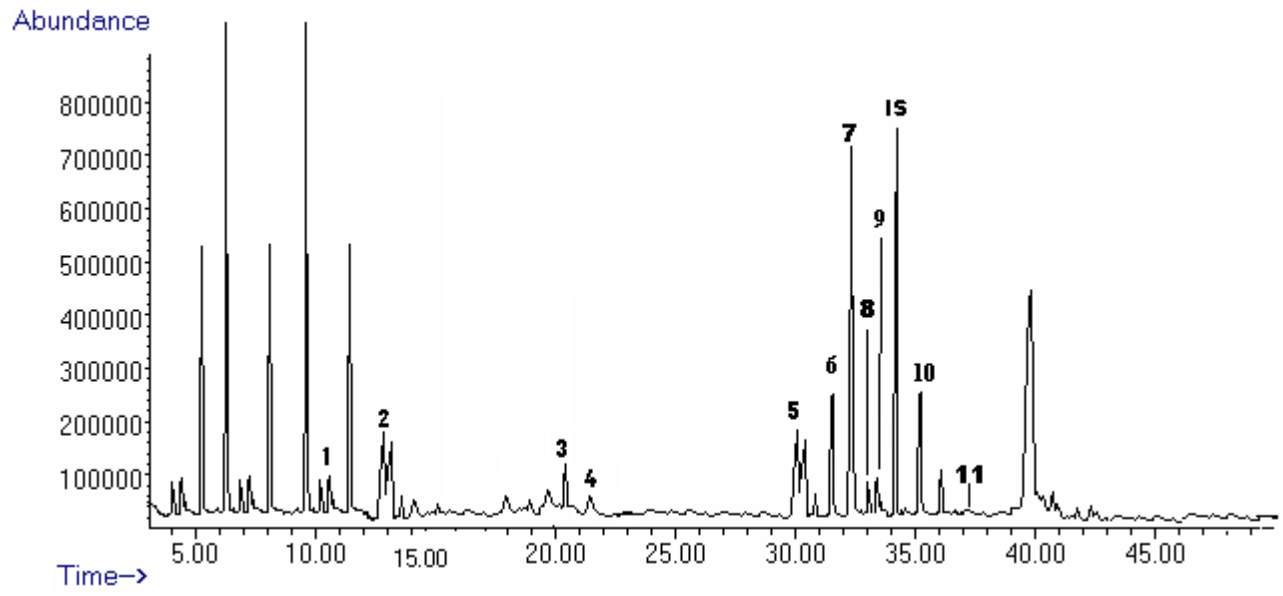

Figure: Sample 4-Kendubay.Amino Acid Analysis in Winam gulf