uPREVALENCE OF *LISTERIA* AND *VIBRIO* SPECIES AND POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) IN BIVALVES (*Ergeria radiata*) FROM BAYELSA STATE, NIGERIA

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A THESIS WRITTEN IN THE DEPARTMENT OF MICROBIOLOGY AND SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHYLOSPHY (Ph.D) OF THE UNIVERSITY OF BENIN, BENIN CITY.

JANUARY 2025

CERTIFICATION

Wet certify that this work was carried out by Vivian Osewonyenmwen OGBEBOR (Mrs.) of sthe Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City.

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CERTIFICATION OF THESIS

We, the undersigned, attest and declare that the thesis of Vivian Osewonyenmwen OGBEBOR (Mrs) titled "Prevalence of *Listeria* and *Vibrio* species and Polycyclic Aromatic Hyhrocarbons (PAHs) in bivalves (*Ergeria radiata*) from Bayelsa state, Nigeria" has successfully passed the anti-plagiarism test and does not violate any copyright regulations.

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DEDICATION

I dedicate this reseach to God Almighthy for his love, infinite mercy and grace upon my life and for making this research work a success. I also dedicate this work to my dearly beloved late father, whose love, guidance, support and encouragement continue to inspire me, his legacy lives on through my work.

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ABSTRACT

The consumption of bivalves, such as mussels, clams, and oysters, is widespread across the globe, particularly in Coastal regions. Due to the natural habitat of bivalves and feeding habitat, there is a high possibility of the meat being contaminated with microorganisms and toxic elements. Hence, this research investigated the prevalence of *Listeria* and *Vibrio* species, and polycyclic aromatic hydrocarbons (PAHs) in bivalves (*Ergeria radiata*).

A total of three hundred (300) edible bivalves (*Ergeria radiata*) were obtained during March 2023 to February, 2024 from Ekowe community Coastal waters and from wet markets (Swali and Opolo) using sterile containers. Bacterial were enumerated using standard microbiological procedures. Isolates of *Vibrio* and *Listeria* were identified by polymerase chain reaction (PCR) technique. Antibiotic susceptibility of the isolates was determined using Kirby-Bauer disc diffusion method. Plasmid profiling and curing of *Vibrio* and *Listeria* isolates were determined by standard methods. Proximate analysis of *Ergeria radiata* was determined using standard analytical chemical methods. Mineral and heavy metal compositions were determined by Atomic Absorption Spectroscopy (AAS), while Na and K were analyzed by flame photometry. Polycyclic aromatic hydrocarbons were analyzed by Gas Chromatography-Mass Spectrometry (GC/MS). Standard methods were used to determine the physicochemical properties of the Coastal water samples. The data obtained were statistically analyzed using one way analysis of variance (ANOVA) using SPSS version 20.0. Duncan multiple range test was used to separate the means at $p<0.05$ significant differences.

The results of this study revealed that higher bacterial counts were observed in the wet season compared to the dry season. The total heterotrophic bacterial counts for the wet season ranged from $20.48 \pm 1.62 \times 10^5$ cfu/g - $30.06 \pm 1.63 \times 10^5$ cfu/g while for dry season it ranged from $10.88 \pm 1.09 \times 10^5$ cfu/g – $18.18 \pm 1.10 \times 10^5$ cfu/g. The *Vibrio* counts for wet season ranged from $16.82 \pm 1.17 \times 10^5$ cfu/g - $19.90 \pm 1.27 \times 10^5$ cfu/, while for dry season it ranged from $9.96 \pm 0.95 \times 10^5$ cfu/g – $13.58 \pm 1.12 \times 10^5$ cfu/g. *Listeria* counts for wet season ranged from $8.08 \pm 0.50 \times 10^5$ cfu/g – $11.52 \pm 0.76 \times 10^5$ cfu/g, while for dry season it ranged from $6.12 \pm 0.67 \times 10^5$ cfu/g – $9.72 \pm 0.8 \times 10^5$ cfu/g - $9.72 \pm 0.81 \times 10^5$ cfu/g.The *Vibrio* species isolated were *Vibrio parahaemolyticus*, *Vibrio cholerae*, *Vibrio vulnificus*, and *Vibrio fluvialis* with the accession numbers PP832852, PP382853, PP832854 and PP832855, respectively. *Listeria* species isolated were *Listeria innocua* PP832856, *Listeria monocytogenes* PP832857, and *Listeria ivanovii* PP328560. The *hlyA* virulence gene was

detected in all the *Listeria* species for both wet and dry season. The virulence gene *ctxA* was not detected in *Vibrio parahaemolyticus* for wet season, but was detected in dry season. The penicillin binding protein (*pbp2b*) resistant gene was detected in all *Listeria species*. The *aph(2")-If* antimicrobial resistant gene was detected in *Vibrio parahaemolyticus* and *Vibro fluvialis* for wet season, while in dry season, it was detected in all the *Vibrio* species. There was no seasonal variation in the values obtained for proximate composition, minerals. and heavy metal content. Protein ranged from 7.42 ± 0.72 % - 7.59 ± 0.6 %, fat (0.74 ± 0.05% - 0.77± 0.9%), K (510 ± 0.52 mg/kg - 520 ± 0.13 mg/kg), and Pb (0.03 ± 0.01 mg/kg – 0.047 ± 0.16 mg/kg). Polycyclic aromatic hydrocarbons (PAHs) concentration of *Ergeria sradiata* was low for both wet and dry season, and was below the permissible limit of 200 ppm set by World Health Organization (WHO). The physicochemical properties of the Coastal waters for wet season exceeded the permissible limit set by WHO. Bivalve (*Ergeria radiata)*, although nutritionally rich, harbors potentially pathogenic bacteria of public health concern, particularly when they are consumed raw or not properly cooked.

CHAPTER ONE

INTRODUCTION

1.0 Background of study

Sea food product especially bivalves mollusc constitute a great diet in Niger Delta (Moruf *et al.,* 2021). Bivalves are soft bodied animal protected by shell. Bivalvia have two hinge shells called valves. Bivalves belong to the phylum Mollusca and class Bivalvia and the most important species of bivalvia are the clams, mussels, oysters, clockles and scallops. Bivalves are found in both fresh water and marine environment (Can *et al*., 2015).

Bivalve molluscs are especially prone to act as transmitters of disease-causing enteric and diarrheagenic bacteria, because the waters bivalve inhabit are often exposed to contamination by fecal matter from sewer drains or from infected individuals. It has been widely shown that the consumption of contaminated seafood can cause diseases in humans. Mollusc can accumulate microorganisms including pathogens from water environment, as filter feeding organisms and can concentrate bacteria in high number, and the number of microorganisms present in water depend on anthropogenic factors (Peter, 2022).

In marine environment, bivalve mollusc constitute habitats for bacteria of the Vibrionaceae family *Vibrio* spp are responsible for bacillary necrosis in a wide range of species of bivalve larvae. *Vibrio* spp produce exotoxins (ciliostatic factors and hemolysins), which cause deciliation, loss of velar epithelia, abnormal swimming behavior, thus are associate with the main diseases affecting worldwide bivalves production (Zhang *et al*., 2022).

Listeria monocytogenes is a bacterial pathogen responsible for the life-threatening disease listeriosis. The most common cause of listeriosis is considered to be ingestion of food

contanminated by *L. monocytogenes* from unclean food production equipment (Ferreira *et al*., 2014). *Listeria monocytogenes* is a ubiquitous environmental bacterium that has been associated with a wide variety of environments, such as rivers, soil, vegetation, food processing environments and urban areas (Vivant *et al*., 2013). It is also among the deadliest foodborne pathogens and knowledge about its presence and diversity in potential sources is crucial to effectively track and control it in the food chain (Liao *et al*., 2021).

Over the years, water pollution has become one of the major environmental challenges facing most of the states in Southern Nigeria, particularly the Niger Delta region. Niger Delta is one of the most prominent regions in Nigeria with vast aquatic ecosystems greatly blessed with variety of fish species both Fin and Shell fishes (Koh *et al*., 2 019). Amongst other states in the region, Bayelsa State Coastal waters are faced with massive environmental pollutions due to increased Coastal population, rapid urbanization, oil and gas explorations, heavy rainfall throughout the year and other array of anthropogenic activities which are capable of deteriorating the aquatic ecosystem (Akagbue *et al.*, 2021). Most of these activities generate heavy metals that cause environmental degradation, often times, these heavy metals which are non-biodegradable, accumulate in organs and tissues of aquatic organisms like bivalves through their diets and respiration, thereby posing a serious health risk to human and other animals when consumed (Kinuthia *et al*., 2020).

Large increases in population and industrial activities have compromised sanitary conditions in Coastal areas. This situation has led to considerable changes in the physicochemical and biological characteristics of marine ecosystems, thus creates a potential public health risk from the consumption of marine organisms. Bivalve molluscs growing in Coastal waters can become contaminated because the organizations feed by filtering microscopic material from the water around them (Udoh *et al*., 2017).

If seafood products from contaminated sites are consumed raw or only slightly cooked, or are stored or handled inappropriately. They can cause outbreaks of diseases that can lead to public health challenges (García and Sánchez, 1998). Outbreaks of infections associated with bivalve molluscs have been notified in only 12 countries in Europe, Asia, North America and Australia. Most reports have come from the U.S.A. Although the largest outbreak to date occurred in Shanghai in 1988, where 290,000 persons contracted hepatitis A after eating clams (Potasman *et al.*, 2002). In contrast with other bivalve-associated infections, this epidemic stood out because it caused 47 deaths. Other large outbreaks occurred in Australia, where 1,800 persons became ill in 1979, in the U.S.A. in 1986 and in Japan in 1991 (Potasman *et al.*, 2002).

Bivalve mollusc are currently one of the cheapest sources of animal protein consumed by the average Nigerian and it accounts for about 50% of total intake (Amosu *et al*., 2017). Most species of bivalve molluscs consumed in Nigeria are harvested from the brackish water that is exposed to varying amounts of chemical and environmental contaminants such as industrial chemicals, toxic residues from various anthropogenic activities (Nwaichi and Ntorgbo, 2016). Pollution of the Coastal waters in the Niger Delta has continued to attract great attention. This is due to the high level of environmental degradation posed by petroleum production and exploitation along the coastline (Zabbey and Babatunde, 2015; Wala *et al*., 2016). Petroleum hydrocarbon from human-mediated activities are usually incorporated into sediments where it can persist for years gradually releasing toxic substances into the immediate and remote environments (Zabbey and Babatunde, 2015). Some of the deleterious effects caused by the dietary intake of these contaminants include diarrhea and gastrointestinal disorders, immune suppression, neurological disorder, reproductive impairment, developmental retardation, cardiovascular disorder, liver disease, infertility and miscarriage (Mochungong and Zhu, 2015). The groups most vulnerable to dietary exposure of the contaminants are child-bearing

women, children below twelve years and subsistence fish farmers. In order to understand and characterize the risks presented by chemical toxins in the environment to human and ecological receptors, most researchers use benthic organisms such as bivalves as biomonitors of the levels and long-term influences of chemical toxins within the ecosystem (Sarkar *et al*., 2008). According to Conte *et al*. (2014) sea food generally is perceived as healthy food and as an alternative source of proteins.

Niger Delta is most known for oil and gas production in Africa and it is recognized as the most polluted environment for shellfish harvesting (Potasman *et al.*, 2002). The ability of bivalve to bioaccumulate and bioconcentrate contaminants leaves those at the highest trophic level at the greatest concentration and risk (Figueira *et al*., 2011). Fishery products are considered the major sources of human contact to pollutants such as polychlorinated biphenyls, dioxins, organochlorines polycyclic aromatic hydrocarbons, some heavy metals and other environmental toxic substances. The prevailing widespread of contanminants pollution has severely impacted negatively on the food product especially seafood obtained from the Coastal waters of this region (Conte *et al*., 2014). Research has determined that there is bioaccumulation of benzo(a)pyrene (BaP), other hydrocarbons and heavy metals in toxic levels in major high protein seafood such as periwinkle (*Tympanotonus fuscatus*), mudskipper and other seafood (Ordinioha and Brisibe, 2013). Yakubu, (2017) reported a benzene concentration of 0.155-48.2 μg m^{-3} in this area and this concentration represents 1:10,000 cancer risk, as benzene and other polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) are known carcinogens.

Persistent organic pollutants (POPs) are organic compounds that subsist in the environment, bio-accumulate in the food chain, with the possibility of causing harmful effects to the health of living organisms and the environment. This group of priority pollutants consists of polycyclic aromatic hydrocarbons (PAHs). Therefore, bivalve shellfish harvested from these

locations can induce potential deleterious health effects to the consumers (Zabbey and Babatunde, 2015). polycyclic aromatic hydrocarbons (PAHs) are stable organic chemicals that persist in the environment and bioaccumulate through the food chain (Ukwo *et al*., 2022). Industrial contaminants in marine ecosystems and biota include different group of compounds that have entered the sea through natural and anthropogenic activities (Ryder *et al.,* 2014). Some of these contaminants are also formed during volcanic activities, bush burning, incomplete combustion of petroleum and related compounds as well as during waste incineration (Ukwo *et al*., 2022).

Bacteria such as *Listeria* and *Vibrio* species, have shown remarkable abilities emerging new pathogenic strains. Studying the prevalence and identifying these bacteria with molecular techniques, provides valuable insights into their potential virulence, resistant genes and mechanism of antibiotics resistance. Such information is crucial for designing targeted control measures, developing efficient diagnostic tools and formulating precise treatment protocols to combat infections caused by these organisms.

Bayelsa state, Nigeria is known for its rich biodiversity and abundant marine resources, making it a prime location for studying the impact of environmental contaminants on aquatic organisms. However, limited research has been conducted on the prevalence of *Listeria* and *Vibrio* species as well as polycyclic aromatic hydrocarbons (PAHs) in bivalves (*Egeria radiata*) from this region. Therefore, this research aimed to contribute significantly to the scientific knowledge base in Nigeria and provide essential data for informed decision-making by local authorities and policymakers.

The consumption of bivalves, such as mussels, clams, and oysters, is widespread across the globe, particularly in Coastal regions. These bivalves have a high filtration capacity, which makes the organism susceptible to accumulating microorganisms and environmental

contaminants, including pathogenic bacteria and polycyclic aromatic hydrocarbons (PAHs.) The presence of these bacteria, *Listeria* and *Vibrio* species, in bivalves can pose serious health risks to humans who consume them raw or undercooked. Therefore, investigating the prevalence of these bacteria is essential for ensuring food safety and preventing potential outbreaks of foodborne diseases. Nigeria Coastal areas, including Bayelsa state have a significant dependence on bivalve as a major source of protein and livelihood, making it essential to access the potential risks posed by these bacteria and Polycyclic aromatic hydrocarbons (PAHs) in these organisms.

1.2 Aim and Objectives

The aim of this study was to investigate the prevalence of *Listeria* and *Vibrio* species and polycyclic aromatic hydrocarbons (PAH) in bivalves (*Ergeria radiata*) from Bayelsa State.

The specific objectives were to;

1. enumerate, and identify *Listeria* and *Vibrio* species in bivalves (*Ergeria radiata*) collected from Baylsa Coastal communities.
2. investigate antibiotic susceptibility and plasmid profile of the isolates.
3. screen for antimicrobial resistant and detect virulence genes.
4. determine the proximate, mineral composition and heavy metals concentration in the bivalves (*Ergeria radiata*)
5. assess for polycyclic aromatic hydrocarbons (PAHs) in the bivalves (*Ergeria radiata*)
6. determine the physiochemical properties of the Coastal water.

CHAPTER TWO

LITERATURE REVIEW

2.0 Description of a Bivalve

A bivalve is an animal belonging to the class Bivalvia. The word 'bivalve' comes from the Latin *bis* ('two') and *valvae* ('leaves of a door'). That is because all bivalve animals have two shells that can open and close like door (Potasman *et al*., 2002). Bivalves are aquatic animals, and different species of bivalves can be found in freshwater, saltwater, brackish water and a slightly salty, water. They typically live on the seafloor, on seabeds or attach themselves to the sides of hard surfaces, like rocks or manmade pipes. These animals are abundant throughout the world, and because of their wide availability, they are very commonly consumed form of seafood (Potasman *et al*., 2002).

2.1 Size range and diversity of Bivalves

Bivalves range in size from about one millimetre (0.04 inches) in length to the giant clam of South Pacific coral reefs, *Tridacna gigas*, which may be more than 137 centimetres (54 inches) in length and weigh 264 kilograms (582 pound). They may have a life span of about 40 years (Ponder and Lindberg, 2008). The shell morphology and hinge structure are used in its classification. In most surface-burrowing species (the hypothetical ancestral habit) the shells are small, spherical or oval, with equal left and right valves. In deeper-burrowing species the shells are laterally compressed, permitting more rapid movement through the sediments (Desdouits *et al.,* 2023; Johnson *et al*., 2020). The shells of the most efficient burrowers, the razor clams *Ensis* and *Solen*, are laterally compressed, smooth, and elongated. Surface-burrowing species may have an external shell sculpture of radial ribs and concentric

lines, with projections that strengthen the shell against predators and damage (Ponder and Lindberg, 2008).

A triangular form, ventral flattening, and secure attachment to firm substrates by byssal threads (byssus; proteinaceous threads secreted by a gland on the foot) have allowed certain bivalves to colonize hard surfaces on wave-swept shores. The byssus is a larval feature that is retained by adults of some bivalve groups, such as the true mussels (family Mytilidae) of marine and estuarine shores and the family Dreissenidae of fresh and estuarine waters (Vaughn, 2012). Such a shell form and habit evolved first within sediments (endobyssate), where the byssus serves for anchorage and protection when formed into an enclosing nest. Other bivalves have used the byssus to attach securely within crevices and thus to assume a laterally flattened, circular shape. The best example of this is the windowpane shell *Placuna*. (Vaughn, 2012). This form has allowed the close attachment of one valve to a hard surface, and although some groups still retain byssal attachment (family Anomiidae), others have forsaken this for cementation, as in the true oysters (family Ostreidae), where the left valve is cemented to estuarine hard surfaces. Some scallops (family Pectinidae) are also cemented, but others lie on soft sediments in Coastal waters and at abyssal depths (Strayer *et al*., 1999). By limiting shell thickness (which reduces weight), smoothing the shell contours (which reduces drag), and assuming an aerofoil-like leading edge, such scallops can awkwardly swim several metres at a time (Ponder and Liberg, 2008).

In other species, such as the clams, the foot has become modified for rapid and effective digging, and the folds of the mantle tissue have developed into long siphons. Both these features allow the animals to burrow deeply within sand, mud, and other substrates (even into wood and rock). They are protected from predators within such substrata but are still able to feed and breathe using their long siphons (Ponder and Lindberg, 2008). Bivalve shell and

body form is thus intimately related to habitat and the relative degree of exposure to predation. From the simple burrowing, equivalent ancestor, the various bivalve groups have repeatedly evolved an elongated, triangular or circular shell; thus, similar body adaptations have been responses to similar modes of life (Ponder and Lindberg, 2008; Stewart *et al.,* 1998).

2.2 Bivalves distribution and abundance

Most bivalves are marine and occur at all depths in or upon virtually all substrates. In shallow seas, bivalves are often dominant on rocky and sandy coasts and are also important in offshore sediments. They occur at abyssal and hadal depths, either burrowing or surface-dwelling, and are important elements of the midoceanic rift fauna (Strayer, *et al*., 1999). In addition, bivalves bore into soft shales and compacted muds but may be important also in the bioerosion of corals.They thus, occur at all latitudes and depths, although none are planktonic. There are also estuarine bivalves, and two important families, the Unionidae and Corbiculidae, are predominantly freshwater with complicated reproductive cycles. There are no terrestrial bivalves, although some high-intertidal and freshwater species can withstand drought conditions (Strayer *et al.,* 1999). Bivalves abundance varies considerably since they comprise more than 15,000 living species (Potasman *et al.,* 2002). Commensal and parasitic species are small, often highly host-specific, and comprise some of the rarest animals. Others, such as cockles and clams on soft shores and mussels and oysters on rocky coasts, can occur in densities high enough that they dominate entire habitats and assume important roles in nutrient cycle (Strayer, 2014).

2.3 Bivalve reproduction and life cycles

Although most bivalve species are gonochoristic (that is, they are separated into either male or female members) and some species are hermaphroditic (they produce both sperm and eggs), sexual dimorphism is rare. In gonochoristic species there is usually an equal division of the sexes (Breton *et al*., 2011). Simultaneous hermaphroditism occurs when sperm-producing tubules and egg-producing follicles intermingle in the gonads (as in the family Tridacnidae), or the gonads may be developed into a separate ovary and testis, as in all representatives of the subclass Anomalodesmata (Breton *et al*., 2011). In consecutive hermaphroditism, one sex develops first. Typically, this is the male phase (protandry), but in a few cases it is the female (protogyny). This is most clearly seen in the wood-boring family Teredinidae, where young males become females as they age. Rhythmical consecutive hermaphroditism is best known in the European oyster, *Ostrea edulis*, in which each individual undergoes periodic changes of sex. Alternative hermaphroditism is characteristic of oysters of the genus *Crassostrea*, in which most young individuals are male. Later the sex ratio becomes about equal, and finally most older individuals become female (Potasman *et al*., 2002; Breton *et al*., 2011).

Bivalve sperm have two flagellae. Most eggs are small, and synchronized spawning results in the discharge of both types of gametes into the sea for external fertilization. Hermaphrodites usually bring in sperm from another individual through the incurrent siphon. The embryos are then brooded, and brooding typically occurs within the ctenidia. There the fertilized eggs, well endowed with yolk, develop directly (without a larval stage), and the young are released as miniature adults. Although ctenidial incubation is most common, there are other patterns: egg capsules are produced by *Turtonia minuta*; a brood chamber is plastered to the shell of

the palaeotaxodont *Nucula delphinodonta*; and in members of the Carditidae the female shellis modified into a brood pouch (Stewart *et al*., 1996; Ponder and Lindberg, 2008).

For most marine species, however, the fertilized egg undergoes indirect development first into a swimming trochophore larva and then into a shelled veliger larva. The veliger has a ciliated velum for swimming and also for trapping minute particles of food. Following a period in the plankton, which varies from hours in some species to months in others, the veliger descends to the seafloor, where it metamorphoses into the adult form: the velum is lost, the foot develops and usually secretes one or two byssal threads for secure attachment, and the ctenidia develop (Hatha *et al.,* 2005).

In the freshwater Unionidae the released larva, called a glochidium, often has sharp spines projecting inward from each valve. The larva attaches to either the gills or fins of passing fish and becomes a temporary parasite. Eventually, it leaves the fish, falls to the lake floor, and metamorphoses into an adult (Dame, 2011) In saltwater bivalves, fertilization and reproduction usually occur in the water outside of the animal. Sperm and eggs are released into the water, where the sperm can then fertilize the egg. Many bivalves release hundreds of sperm and eggs into the water in mass spawning events. Others (Ponder and Linderberg, 2008).

2.4 Ecology and habitats

Most bivalves are found in Coastal seas, but their diversity is greatest on continental landmasses, where large rivers create suitable deltaic habitats and the continental shelf is broad (Johnson *et al*., 2020). The division and lateral compression of the shell into two valves is clearly related to the adoption of a burrowing mode of life, which is achieved by a muscular foot. Primitive forms were detritivorous, whereas modern bivalves are suspension

feeders that collect food particles from seawater using ciliated ctenidia (modified gills). The burrowing, filter-feeding mode of life restricts bivalves to aquatic environments (Yeager *et al*., 1994; Dame, 2011).Retention of the larval anchoring byssus into adult life has freed many bivalves from soft substrates, allowing them to colonize hard surfaces. This has also been achieved by cementation, as, for example, in oysters.There are no pelagic bivalves, except for *Planktomya hensoni*, which is still benthic as an adult but has an unusually long planktonic larval stage. Some bivalves can swim, albeit weakly, when removed from the sediment, as some file shells. True swimming is, however, seen only in the family Pectinidae (scallops) but is used mostly as an escapereaction (Dame, 2011; Ansell and Hawkins, 2021).

Many representatives of the superfamily Galeommatoidea are commensal, a few are parasitic, and both have thus become miniaturized. Most bivalves are found in Coastal seas, but their diversity is greatest on continental landmasses, few are found in the various subclasses and two are most important ecologically: the Heterodonta are modern burrowers that include cockles, clams, shipworms, and giant clams and feed primarily on suspended material (Cranford *et al.,* 2011). In contrast, the Pteriomorphia, an older group that is epibyssate (that is, anchored to rocks) dominates hard substrates. The subclass is made up of oysters, mussels, jingle shells, and others. Some of their older representatives are endobyssate (that is, anchored to material within a burrow or dugout), exposing their evolutionary history (Ponder and Lindberg, 2008). Most of these two classes occupy a wide diversity of subhabitats, with simple reproductive strategies, external fertilization, and planktonic larvae to effect wide dispersion. The Palaeoheterodonta (a group that includes the unionids) are exclusively freshwater species, but all have significantly more complicated life cycles (Dame, 2011).The Palaeotaxodonta (or Protobranchia) are Coastal and deepwater detrivores, always infaunal. They share this diversity of habitat with the Anomalodesmata, which have radiated along two lines: shallow-water species that are highly specialized, are hermaphroditic, occupy narrow

niches, and have a short planktonic stage and deep-sea species that are more specialized, most being predators (Stewart, 1998). Most bivalves are primary consumers, typically exploiting organic material. The two dominant bivalve subclasses are high in the diet of many predators. Some 60 million years ago great adaptive radiation, notably in the Bivalvia, took place with a similar radiation in predatory crustaceans, starfishes, and snail (Stewart, 1998). It is thought that such predation pressure effectively drove the Bivalvia underground with the resultant evolution of many antipredation devices on the shell spines, ridges, and teeth or of the habit of burrowing to great depths. A similar pressure on coral reefs a led to deep boring into the fabric of the coral and the evolution of a host–borer intimacy on islands (Dame, 2011).

2.5 Locomotion

Unlike in other molluscan groups, locomotion in bivalves is used only when dislodgement occurs or as a means to escape predation. The bivalve foot, unlike that of gastropods, does not have a flat creeping sole but is bladelike (laterally compressed) and pointed for digging (Yeager *et al*., 1994). The muscles mainly responsible for movement of the foot are the anterior and posterior pedal retractors. They retract the foot and effect back-and-forth movements. The foot is extended as blood is pumped into it, and it is prevented from overinflating by concentric rings of circular, oblique, and longitudinal muscle fibres, which also help to direct pedal extension and permit fine mobility (Gosling, 2004). During burrowing, the foot is greatly extended anteriorly from between parted shell valves. Taking a grip on the substratum, typically by dilation of the tip, the pedal retractors pull the shell downward. This is accompanied by sharp closure of the shell valves, forcing water out of the mantle cavity into the burrow, helping to fluidize the sediment, and making movement

through it more efficient. So effective is this mechanism that fast burrowers, when removed from the sediment, can swim short distances (Gosling, 2004).

2.6 Importance of Bivalves

The total marine catch of molluscs is twice that of crustaceans, and the great majority of this is bivalve. Some three million metric tons (6,615,000,000 pounds) of bivalves are harvested throughout the world each year. Virtually all bivalves, with the possible exception of the thorny oyster *Spondylus*, are edible and fall into the main categories of oysters, mussels, scallops, and clams. A number of species are raised commercially (Potasman *et al*., 2002).The most important edible oysters are representatives of the genus *Crassostrea*, notably *C. gigas* in the western Pacific, *C. virginica* in North America, and *C. angulata* in Portugal. Most mussels are cultivated on ropes suspended from floats. The European mussel *Mytilus edulis* has been introduced into the northern Pacific, and the practice now flourishes widely in Japan and China (Haag and Williams, 2014). Most scallops, *Pecten*, *Placopecten*, and *Amusium*, are caught by offshore trawlers, although cultivation is being attempted. A wide variety of clams are cultivated such as, *Mya arenaria* and *Mercenaria mercenaria* in the North Atlantic and *Venerupis japonica* and *Tapes philippinarum* in the Pacific (Potassman *et al,* 2002). In some parts of the world, red tides, caused by large numbers of toxic protozoan dinoflagellates, are lethal to fish and certain invertebrates. Bivalves, by virtue of their filter-feeding apparatus, concentrate toxins and, if eaten by humans, can cause paralysis or death (Potasman *et al*., 2002).

Bivalves of the genera *Pinctada* and *Pteria* have been collected in many tropical seas for the natural pearls they may contain, although in many countries, most notably Japan, pearl oyster fisheries have been developed (Potassman *et al,* 2002). The outer shell of the windowpane

oyster, *Placuna placenta*, is called the capiz shell. It is used, primarily in the Philippines, in the manufacture of lampshades, trays, mats, and bowls. In developing countries, many kinds of bivalve shells are used in the manufacture of jewelry and ornaments. The shells of bivalves are used in craftwork, and the manufacture of jewellery and buttons. Bivalves have also been used in the biocontrol of pollution (Kellog *et al*., 2012). Bivalves appear in the fossil record first in the early Cambrian more than 500 million years ago and the total number of known living species is about 9,200 (Potasman *et al*., 2002).

2.7 Characteristics of Bivalves

The most important feature of a bivalve is its two shells, called valves. These shells act like a protective armor, guarding the bivalve's soft inner body parts. They can open their shells to move and feed, but at the first hint of a threat, they will rapidly close their shells (Cranford *et al*., 2011). The muscle that helps them to close their valves is called the foot. This foot is not like a human foot. Instead, it is a large muscle that joins the two valves together and helps to open and close them. In that respect, the foot moves much more like one of your hinged joints, such as your knee or elbow (Gonsling, 2004).

Unlike many animals, bivalves do not have mouths and teeth with which to eat. Instead, they have a tube-like body part called a siphon that takes in microscopic particles of food dissolved in the water. The food goes then into the stomach for processing, and any oxygen in the water is passed along to their gills, which function similar to fish gills, for respiration.Any waste products and gases, like carbon dioxide, are also passed out of the body using a different siphon. The siphon that takes in nutrients is often called the in-current siphon, whereas the wastes exit through the out-current siphon. Notably, bivalves do not have eyes. Though some have afew primitive eyes, but most do not. Considering that they often live in dirty, cloudy water, which has more particles for them to feed on, they probably would not be able to see most of the time anyway. There are many species of bivalves, both currently

living and fossilized. Oysters and scallops are examples of bivalves, but so are clams and mussels and these are the most common bivalves living today (Potasma *et al.,* 2002; Ponder and lindberg, 2008).

2.8 Feeding in bivalves

Most bivalves are filter feeders, using their gills to capture particulate food such as phytoplankton from the water. The protobranchs feed in a different way, scraping detritus from the seabed, and this may be the original mode of feeding used by all bivalves before the gills became adapted for filter feeding (Cranford *et al*., 2011). Feeding in bivalves involves pumping water through a set of ctenidia, removal of particles from suspension, and trans- port of collected material to the mouth. The digestive tract of typical bivalves consists of an oesophagus, stomach, and intestine. A number of digestive glands open into the stomach, often via a pair of diverticula; these secrete enzymes to digest food in the stomach, but also include cells that phagocytose food particles, and digest them intracellularly (Evan and Shumway, 2004).

2.9 *Ergeria radiata* (water snail)

Scientific Classification of *Ergeria radiata*

Clam species belong to the kingdom , Animalia, ; Phylum, Mollusca; Class, Bivalvia; Order, veneroida; Family,Donoacidae; Genus,Ergeria; specie, *Ergeria radiata*..

2.9.1 Description of a clam (*Ergeria radiata*)

Clams are marine molluscs with two valves or shells. Like all molluscs a clam has a mantle which surrounds its soft body. It also has a muscular foot which enables the clam to burrow itself in mud or sand. The soft tissue above the foot is called the visceral mass and contains the clam's body organs. Giant clams have four or five large, inward, vertical folds in their

thick, heavy shell. The shell does not have scutes and consists of two valves. Once they are fully grown they cannot close their shell completely (Ponder and Lindberg, 2008).

2.9.2 Benefit of clam (*Ergeria radiata*)

They are an excellent source of lean protein, providing the body with the building blocks it needs for muscle growth and repair. Clams (*Ergeria radiata*) are also loaded with vitamins such as B12, essential for nerve function, and vitamin C, which boosts the immune system and promotes skin health (Udoh *et al*., 2017).

2.9.3 Nutritional importance of clam (*Ergeria radiata*)

Clam (*Ergeria radiata*) meat which is also a type of snail meat tastes good and is good for the body. It serves as a special delicacy in the diet (Akinnusi, 1996). Studies conducted on clam (*Ergeria radiata*) indicated that its meat is particularly rich in protein, iron, calcium, and phosphorous (Wright *et al.,* 2018). The fresh flesh of the clam contains at least 70 % water while its dry mass consists of high quality protein. These compare well with values obtained from conventional animal proteins sources such as beef, broiler meat, goat meat, mutton, pork and fish. Clam (*Ergeria radiata*) meat is low in sodium, fat and cholesterol (Wright *et al*., 2018).

For thousands of years, clam meat, a type of snail meat has been used for human consumption and its shell for jewelry (Haag and Williams, 2014). Nitrogenous substances are among the most indispensable constituents in the diet of man, so also is iron and calcium elements. These are found in abundance in clam meat. The methionine content of clami is low but their arginine and lysine contents are very high even higher than 6.5 % and 7.5 %, respectively as compared to whole egg (Wright *et al*., 2018). Due to the high content of iron, it is often recommended for use in the treatment of iron deficiency anaemia which is very common in the tropics either due to nutritional deficiency or disease such as hookworm

disease or pregnancy. In evaluating the nutritive value of clam meat, it was found that the chemical composition varies with species and locality of its habitat which translates to differences in the mineral composition of the clam (Hatha *et al.,* 2005).

2.9.4 Common bivalves

Commonly consumed molluscan bivalves include clams, mussels, oysters, and scallops. These commercially important species had a dockside landing value in 2015 of approximately $870 million (Potassman *et al*., 2002). Although they comprise about 15% of the total seafood value in the United States, consumer preference for crustacean shellfish (shrimp comprise nearly 25% of all seafood consumed in the United States) suggests that bivalve molluscan shellfish are an underappreciated seafood commodity in the United States (Lund, 2013). In fact, seafood is the least consumed protein food overall on a weekly per capita basis in the United States (Ansai *et al.,* 2025).

2.9.5 Macronutrients composition of Bivalves

Essential nutrients are those nutrients that are not produced by the human body in adequate amounts to sustain life and include macronutrients, such as carbohydrates, fats (fatty acids and cholesterol), proteins (amino acids), fiber, and water. Other essential nutrients include vitamins and minerals. Nonessential nutrients are metabolized in the body (Townsend and Subramanian, 2023).

2.9.6 Water

Water is an essential nutrient in any living system. On average, an adult human body contains about 60% water. Dehydration by as little as 2% of the body weight will result in impaired physiological response (Borghi *et al*., 1996). Water is often involved in biochemical mechanisms and is needed to maintain body temperature. Water fills the spaces between cells, lubricates and cushions joints, and carries waste through urination, perspiration, and bowel movements. Molluscan shellfish consist of approximately 75% water (Borghi *et al.*, 1996).

2.9.7 Protein

In general, molluscan shellfish are considered to be abundant in protein and comparable with land-based protein sources (Wright *et al*., 2018). For example, clams, mussels, oysters, and scallops contain 9.0%–13.0%, 12.6%–13.0%, 8.9%–14.3%, and 14.8%–17.7% protein in their raw meat respectively (Venugopal and Gopakumar, 2017). Molluscan shellfish protein is considered high-quality protein because of its essential amino acid profile and classification as a highly digestible protein source (Miletic *et al.*, 1991).

2.9.8 Carbohydrates

Molluscan shellfish contain moderate levels of carbohydrates in their tissue as they may be consumed live or shortly after death. Although molluscan shellfish are generally considered low in carbohydrate content, raw scallops, oysters, and mussels have been reported to contain between 3% and 5% carbohydrate. By contrast, carbohydrate levels in crustacean shellfish are nearly 0% in raw crab, shrimp, and lobster (Wylie-Rosett *et al.*, 2004).

2.10 Molluscan Shellfish (Bivalve) and Seafood Safety

Although nutritional benefits related to consumption of molluscan shellfish are clearly evident, there are food safety considerations related to these seafood products, particularly when consumed raw. Filter-feeding molluscs can concentrate pathogens and toxins that are detrimental to human health. Seafood is responsible overall for less than 7% of illnesses or deaths from foodborne outbreaks in the United States (Painter *et al*., 2013). These data excluded *Vibrio vulnificus,* which is the leading cause of death from infectious disease related to seafood consumption; however, the incidence of disease is rare (124 cases in 2014) relative to *Salmonella* (>million cases/year) (Onifade *et al*., 2011).

Seafood safety concerns are largely related to risks associated with the consumption of raw shellfish. Many of these risks could be avoided simply by cooking the product; however, a

culture of consuming oysters raw dates back to early human populations, as evidenced by the discovery of massive shell middens that are an archaeological testament to raw oyster consumption. These mounds of mollusc shells are generally located either nearshore or underwater and are thought to be directly associated with molluscan shellfish consumption in villages along Coastal areas. Molluscan shellfish were both a food source and a useful tool, as shell by-product was used for cutting and digging applications (Chung *et al.*, 2024).

2.11 Shellfish Safety Monitoring

In general, safety of shellfish in the United States is monitored at harvest and is based on water quality standards determined by the FDA and the Interstate Shellfish Sanitation Conference and described in the National Shellfish Safety Program Guide (Ansai *et al*., 2025). Coastal states in the United States track faecal indicator bacterial (faecal coliforms or *Escherichia coli*) to determine open and closing of harvest areas. Heavy rainfall can cause nonpoint source pollution and may instigate closures. California now monitors *Vibrio parahaemolyticus* levels that indicate increased risk and precipitate cessation of shellfish harvesting (Baker-Austin *et al*., 2024). Some states also monitor directly for toxic algal species. For example, Florida Department of Environmental Protection does periodic sampling for algal species and maintains a hot line for reporting blooms. Health considerations for *Vibrio vulnificus* are not addressed by monitoring, as the organism is found naturally in Coastal waters and is not a result of faecal contamination. The presence of *V. vulnificus* (or other pathogens) does not change the sensory attributes of molluscan shellfish, as these are not spoilage organisms and would be essentially impossible to detect even in an oyster with high levels of *Vibrio* spp. by visual inspection, taste, or smell (Mouzin *et al.,* 1997). Bacterial and viral contamination of both raw and cooked seafood during postharvest processing (PHP) or storage have been reported and also present risks that are not prevented by the current system of monitoring (Baker, 2016). It appears that most of the general public

understands that there is a certain level of risk when raw oysters are consumed, but a survey conducted in 2002, noted that there were several health risks that were not well known to raw oyster consumers with certain underlying medical conditions, such as diabetes. In addition, some raw oyster consumers have the general knowledge that oysters may collect and concentrate bacteria and virus particles naturally because of the way they feed (Uchida *et al*., 2017). Warnings on menus serving raw fish and shellfish draw attention to these risks and have been validated by successful lawsuits against seafood restaurants that ensure labels are informative and visible. However, it should be noted that unlike other food industry, shellfish suppliers and retailers have long maintained a tracking system that tags the location and harvest date for all oysters to facilitate trace-back investigations and prevent large outbreaks (Wright *et al*., 2018).

2.12 Post-Harvest Processing (PHP)

To address problems associated with the consumption of raw molluscan shellfish, the National Shellfish Sanitation Program promoted and, in some cases, mandated harvest time–temperature controls, specific storage parameters, and Postharvest Processing methods to reduce the food safety risk (Baker, 2016). One method commonly used in Europe is depuration or wet storage using sanitized seawater to ‘‘flush’’ out filter-feeding shellfish (Fang *et al*., 2015). Although this method is effective in removing faecal associated bacteria, *Vibrio* spp. are not eliminated. PHP methods now in practice in the United States include thermal processing, freezing, irradiation, and high hydrostatic pressure (Wright and Schneider, 2010). These methods are generally effective and will reduce levels of *Vibrio* spp. to the required non-detectable level; however, they all have the disadvantage of killing the mollusc, and thus require additional storage and packaging protocols for the product to be served as a ‘‘on the half shell.’’ Alternatively, shell stock can be transferred from estuaries with relatively low salinity (1–10 ppt) and high *Vibrio* levels to more off-shore sites with

higher salinity and lower *Vibrio* levels. The process is called relaying and has been shown to be effective in some cases but may not be suitable for all *Vibrio* species, as some (*Vibrio parahaemolyticus* in particular) has higher salinity tolerance. If PHP is used to reduce human pathogens in molluscan shellfish, the dealer must use the process under a seafood hazard analysis critical control point plan and validate that it achieves a minimum 3.52 log reduction of viable bacteria and reduces the level of *Vibrio vulnificus* and *V. parahaemolyticus* to nondetectable levels (Baker, 2016). A novel method using a derivative of chitin in the form of chitosan microparticles was recently described (Fang *et al*., 2015) and was effective against all the three major pathogenic *Vibrio* species to achieve the required reductions while leaving the oyster viable; however, efficacy of chitosan for *Salmonella* was inhibited in the presence of sea salt (Fan *et al.,* 2017).

2.13 Cooking of bivalve

Fried and steamed oysters are commonly consumed in the United States, but raw remains the number one method of consumption (Anacleto *et al*., 2014). In other countries and cultures, clams, mussels, and scallop meats are also frequently consumed raw. In the United States, clams, mussels, and scallops are mostly prepared by sautceing, frying, broiling, and incorporated in a soup or chowder (Anacleto *et al*., 2014).

The nutritional content for cooked food appears to increase after cooking because of water loss. Cooked seafood will lose some level of moisture (even if steamed or boiled), resulting in nutrient concentration effects unless thermal degradation or loss from edible tissue. Some B vitamins are particularly heat labile (Nettleton and Exler, 1992). Common cooking procedures and their effect on levels of environmental contaminants was studied, results suggest that common cooking methods (boiling, frying, grilling, and roasting) may reduce the level of organic contaminants in the cooked product because these residues are present in fatty tissues that melt during cooking and thus are not consumed (Ukwo *et al*., 2020).

Guidelines for cooking molluscan shellfish to ensure food safety was described by FDA, (2007). Unless previously processed, shellfish are sold live and should be examined before cooking for open shells that do not close, as product with open shells are likely dead. Dead shellfish decompose rapidly, increasing the likelihood of contamination with pathogenic bacteria (Ukwo *et al*., 2020). Some health-related considerations from consuming fried molluscs include increasing the fat concentration from oil uptake in the muscle tissue. Also, most fried foods are breaded which increases in carbohydrates, calories, and additional frying oil uptake. Furthermore, frying oil can become oxidized after sufficient use, and oxidized fat consumption is also associated with negative health implications due to formation of toxic aldehydes (Viau *et al*., 2016). Soybean oil, canola oil, or frying oil blends, commonly used for frying seafood, are also subject to auto-oxidation after if improperly stored. Volatile off-flavors are formed because of accelerated oxidative rancidity during repeated frying that forms aldehydes, ketones, alcohols, carboxylic acids, and other organic compounds which are associated with fishy, painty, cardboardy, green, grassy and many other off-flavors (Domingo, 2016). There are several positive aspects to frying foods, as it introduces additional flavors, reduces moisture, changes in texture, and eliminates pathogenic bacteria. A restaurant or food processor commonly change the oil enough to minimize any off-flavors that come from the frying process. It is also unlikely that toxic levels of aldehydes would not be Pconsumed at high enough concentrations to have health implications because the consumer would likely not eat it because of the rancid flavor of the fried seafood (Domingo, 2016).

2.14 Persistent Organic Pollutant (POP)

Persistent Organic Pollutant (POPs) are lipophilic chemicals that can pass through biological phospholipid membranes and bio-accumulate in fatty rich tissues of humans (Ukwo *et al*., 2022). Initially described for their deleterious effects on reproductive function and

carcinogenicity, there is now growing body of evidence showing that exposure to POPs leads to metabolic diseases. First, many epidemiological studies performed in the US, Europe and Asia have led to the common finding that there is an increased body burden of POPs in people with diabetes (Wang *et al*., 2008). For instance, diabetes has been associated with elevated serum levels of 2,3,7,8-tetrachlorodibenzodioxin (TCDD), 2,2',4,4',5,5'-hexachlorobiphenyl (CB153), coplanar polychlorinated biphenyls (PCBs; CB77, CB81, CB126, CB169), p,p'-diphenyldichloroethene (DDE), oxychlordane and *trans*-nonachlor (Goncharov *et al*., 2011).. In addition, bio-accumulation of PCBs has been linked to non-alcoholic fatty liver disease (NAFLD) and elevated blood pressure (Goncharov *et al*., 2011). Recently, prospective studies have furthermore shown an increased risk of diabetes in persons exposed to POPs, especially organochlorine pesticides (Lee *et al*., 2008). Second, there is evidence for a causal relationship between POP exposure and metabolic disorders linked to insulin resistance. Animals exposed to environmental levels of POP mixtures through the intake of non-decontaminated fish oil (obtained from farmed Atlantic salmon) exhibited insulin resistance, glucose intolerance, abdominal obesity and NAFLD (Ruzzin *et al.,* 2010).

Persistent organic pollutants (POPs), including dioxins, furans, polychlorinated biphenyls (PCBs), and organochlorine pesticides, are chemicals mainly created by industrial activities, either intentionally or as by-products (Fisher *et al*., 1999). Because of their ability to resist environmental degradation, these substances are omnipresent in food products, and found all around the world, even in areas where they have never been used like Antarctica (Fisher *et al*., 1999). Thus, virtually all humans are daily exposed to POPs. In the general population, exposure to POPs comes primarily from the consumption of animal fat like fatty fish, meat and milk products; the highest POP concentrations being commonly found in fatty fish (Fisher *et al.,* 1999). During the last half of the 20th century, the global environment has

become contaminated with a number of persistent, fat-soluble chemical contaminants, commonly referred to as the Persistent Organic Pollutants (POPs) (Fisher, 1999). Contamination of the global environment with a complex mixture of POPs resulted from deliberate discharges, applications and inadvertent formation of by-products of incomplete combustion or industrial processes (Ukwo *et al.,* 2022)..

According to the World Health Organization, about 24% million of annual deaths are attributable to preventable environmental causes, accounting for 22% of the global burden of disease (life years lost) and 23% of total premature deaths (WHO, 2016) . Persistent Organic Pollutants (POPs) represent a relevant health concern (Ukwo *et al*., 2022). POPs are characterized by a great resistance to chemical degradation, lipophilicity and, thus, a high biomagnification and bioaccumulation potential in living organisms (Fisher, 1999). In addition, POPs are semi-volatile so that they can be transported over long distances in successive cycles of deposit and reemission, consequently, can be found in regions far from their sources (Potasman *et al*., 2002). POPs include polychlorinated biphenyls (PCBs), as well used in industrial processes as refrigerants, lubricants, hydraulic fluids, among other commercial applications and organochlorine pesticides (OCPs) as well as polycyclic aronmatic hydrocarbon (PAH) used in agricultural activities as insecticides and as vector control (Fisher, 1999). The production of PCBs and OCPs begun in the 1920s, and were extensively used worldwide from 1940s until 1970s–1980s, when their use and production were severely restricted on the basis of their persistence and suspected health effects. However, they are still present in old equipment and/or used for public health campaigns, as well as in virtually all the ecosystems as a consequence of their high persistence (Fisher, 1999).

Consequently, the general population is exposed to relatively low but frequent doses of OCPs and PCBs, with diet being considered the main source of exposure (Fischer *et al*., 1999).

Despite the impossibility to completely avoid exposure, certain dietary strategies have shown to decrease exposure levels (Patel *et al*., 2009). Therefore, chronic exposure to OCPs and PCBs could be considered, to some extent, as potentially modifiable. Low-dose OCP and PCB exposure is suspected to cause endocrine/ metabolic disruption and, has been linked to a number of highly prevalent chronic conditions related to the Metabolic Syndrome (Grun *et al*., 2009). Although with not entirely congruent results, a number of epidemiological studies have evidenced associations of OCP/PCB exposure with several cardiovascular risk factors/conditions, including hypertensive disorders (Grun *et al*., 2009) stroke risk (altered serum lipids and obesity/adiposity (Muller *et al*., 2008) ventricular mass (carotid intima-media thickness (cIMT) (Richardson *et al*., 2009) homocysteine levels (Grun *et al*., 2009) or myocardial infarction incidence among others. The abovementioned conditions are closely inter-related, which hamper the establishment of causal relationships between OCP and PCB exposure risk.

2.14.1 Global issue of persistent organic pollutant

Persistent organic pollutants (POPs) are toxic chemicals that adversely affect human health and the environment around the world. Because they can be transported by wind and water, most POPs generated in one country can and do affect people and wildlife far from where they were used and released. They persist for long periods of time in the environment and can accumulate and pass from one species to the next through the food chain (Hong *et al*., 2006).

To address this global concern, the United States joined forces with 90 other countries and the European Community to sign a groundbreaking United Nations treaty in Stockholm, Sweden, in May 2001. Under the treaty, known as the Stockholm Convention, countries agreed to reduce or eliminate the production, use, and/or release of 12 key POPs, and it was specified under the Convention a scientific review process that has led to the addition of other

POPs chemicals of global concern (Crinnion, 2012). Many of the POPs included in the Stockholm Convention are no longer produced in the countries under the stockholm convention. However, U.S. citizens and habitats can still be at risk from POPs that have persisted in the environment from unintentionally produced POPs that are released in the United States, from POPs that are released elsewhere and then transported here (by wind or water, for example), or from both. Although most developed nations have taken strong action to control POPs, a great number of developing nations have only fairly recently begun to restrict their production, use, and release (Crinnion, 2012).

The Stockholm Convention adds an important global dimension to our national and regional efforts to control POPs. Though the United States is not yet a Party to the Stockholm Convention, the Convention has played a prominent role in the control of harmful chemicals on both a national and global level (Fisher *et al*., 1999). For example, Environmental protection agency (EPA) regulation with states have significantly reduced the release of dioxins and furans to land, air, and water.. In addition to assessing dioxins, EPA has also been working diligently on the reduction of DDT from global sources. The United States and Canada signed an agreement for the Virtual Elimination of Persistent toxic substances in the Great Lakes to reduce emissions from toxic substances (Hong *et al*., 2006). The United States has also signed the regional protocol of the United Nations Economic Commission for Europe on POPs under the Convention on Long-Range Transboundary Air Pollution which addresses the Stockholm Convention on POPs and other chemicals (Crinnion, 2012).

In addition to signing the POPs-related agreements the United States has taken part, the Unite States has also provided ample financial and technical support to countries across the globe supporting POPs reduction. A few of these initiatives include dioxin and furan release inventories in Asia and Russia, and the reduction of PCB sources in Russia (Crinnion, 2012).

Many POPs were widely used during the boom in industrial production after World War II, when thousands of synthetic chemicals were introduced into commercial use. Many of these chemicals proved beneficial in pest and disease control, crop production, and industry. These same chemicals, however, have had unforeseen effects on human health and the environment. Many people are familiar with some of the most well-known POPs, such as PCBs, DDT, and dioxins. POPs include a range of substances that include: intentionally produced chemicals, currently or once used in agriculture, disease control, manufacturing, or industrial processes. Examples include PCBs, which have been useful in a variety of industrial applications (e.g., in electrical transformers and large capacitors, as hydraulic and heat exchange fluids, and as additives to paints and lubricants), and DDT, which is still used to control mosquitoes that carry malaria in some parts of the world. Unintentionally produced chemicals, such as dioxins, result from some industrial processes and from combustion (for example, municipal and medical waste incineration and backyard burning of trash) (Kim *et al*., 2023; Li *et al*., 2022; UNEP, 2024).

DDT is likely one of the most famous and controversial pesticides ever made. An estimated 4 billion pounds of this inexpensive and historically effective chemical have been produced and applied worldwide since 1940. In the United States, DDT was used extensively on agricultural crops (Fischer *et al*., 1999), particularly cotton, from 1945 to 1972. DDT was also used to protect soldiers from insect-borne diseases such as malaria and typhus during World War II, and it remains a valuable public health tool in parts of the tropics. The heavy use of this highly persistent chemical, however, led to widespread environmental contamination and the accumulation of DDT in humans and wildlife - a phenomenon brought to public attention by Rachel Carson in her 1962 book, Silent Spring. A wealth of scientific laboratory and field data have now confirmed research from the 1960s that suggested, among other effects, that high levels of DDE (a metabolite of DDT) in certain birds of prey caused

their eggshells to thin so dramatically they could not produce live offspring One bird species especially sensitive to DDE was the bald eagle. Public concern about the eagles' decline and the possibility of other long-term harmful effects of DDT exposure to both humans and wildlife prompted the Environmental Protection Agency (EPA) to cancel the registration of DDT in 1972. The bald eagle has since experienced one of the most dramatic species recoveries in our history (Crinnion, 2012).

The United States has taken strong domestic action to reduce emissions of POPs. For example, none of the original POPs pesticides listed in the Stockholm Convention is registered for sale and distribution in the United States today and in 1978, Congress prohibited the manufacture of PCBs and severely restricted the use of remaining PCB stocks. In addition, since 1987, EPA and the states have effectively reduced environmental releases of dioxins and furans to land, air, and water from U.S. sources. These regulatory actions, along with voluntary efforts by U.S. industry, resulted in a greater than 85 percent decline in total dioxin and furan releases after 1987 from known industrial sources (EPA 2001). To better understand the risks associated with dioxin releases, EPA has been conducting a comprehensive reassessment of dioxin science and will be evaluating additional actions that might further protect human health and the environment (EPA, 2001).

2.14.2 Polycyclic aromatic hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants generated primarily during the incomplete combustion of organic materials (e.g. coal, oil, petrol, and wood (Ma *et al*., 2024). Emissions from anthropogenic activities predominate; nevertheless, some PAHs in the environment originate from natural sources such as open burning, natural losses or seepage of petroleum or coal deposits, and volcanic activities (Berríos-Rolón *et al.,* 2025). Major anthropogenic sources of PAHs include residential heating, coal gasification

and liquefying plants, carbon black, coal-tar pitch and asphalt production, coke and aluminum production, catalytic cracking towers and related activities in petroleum refineries as well as motor vehicle exhaust (Ma *et al*., 2024).

Polycyclic Aromatic Hydrocarbon (PAH) are stable organic chemicals that persist in the environment and bioaccumulate through the food chain (Ukwo *et al*., 2022). PAHs are found in the ambient air in gas-phase and as sorbet to aerosols. Atmospheric partitioning of PAH compounds between the particulate and the gaseous phases strongly influences their fate and transport in the atmosphere and the way they enter into the human body (Ma *et al*., 2024). The removal of PAHs from the atmosphere by dry and wet deposition processes are strongly influenced by their gas/particle partitioning. Atmospheric deposition is a major source for PAHs in soil (Ma *et al*., 2024). Several remediation technologies have been tested in efforts to remove these environmental contaminants (Ma *et al*., 2024). Among them, bioremediation is showing particular promise as a safe and cost-effective option. In spite of their xenobiotic properties, a variety of genera of gram-positive and gram-negative bacteria, fungi and algae have been isolated and characterized for their ability to utilize PAHs (Yunker- Marke *et al*., 2015). The sources of PAHs can either be petrogenic i.e., released from petroleum products or pyrogenic due to the combustion of biomass (Yunker- Marke *et al*., 2002).

The Niger Delta region of Nigeria is impacted due to contamination traceable to oil and gas activities. Polycyclic Aromatic Hydrocarbon (PAH) is a component (0.2–7%) of hydrocarbon (Ukwo *et al*., 2022) and is a major group of chemicals of concern in the environment due to its carcinogenic and mutagenic potencies. Researchers have reported the presence of PAH in the region (Li *et al*., 2025). PAHs sources can be petrogenic emanating from petroleum-related activities or pyrogenic (pyrolytic), from the incomplete combustion of diesel fuel and engine oil, wood, coal, biomass of forest, grass fires, waste incinerators, and fossil fuels that are used in industrial operations and power plants (Ashraf *et al*., 2013). Due to their low

vapour pressure and solubility in sea water PAH attach to suspended organic matter and eventually sink to the bottom of sediments and remain persistent for years (Ukwo *et al.,* 2022). The sediment is home for most bivalves such as *Arca senilis*, a filter feeding mollusc of the order Arcoida in the family Arcidae. *Arca senilis* satisfies basic biomonitoring conditions due to wide distribution along Coastal areas, sessile lifestyle, easy to handle it is also a filter feeder with the ability to accumulate heavy metals and contaminants without appreciable metabolism (Ashraf *et al*., 2013). In view of the above, the organism gives a time-integrated indication of environmental contamination because it accumulates considerable level of contaminants during indiscriminate filter feeding. It is a major sea food (rich in protein and vitamins) in the south coast of Nigeria and consumed in a variety of delicacies. Average consumption of seafood in some Coastal communities of the Niger Delta region of Nigeria is about 370 g per week in terms of dry weight of sea food per meal consumed (Berríos-Rolón *et al.,* 2025). Bioaccumulation and toxic effects associated with such marine organisms and onward transmission to humans via the food chain is a major concern due to negative effects. The alternation in gametogenesis, gender determination, and growth (Udom *et al.,* 2025) and increased risk of cancer and mutation are major adverse effects on living organisms (Manoli *et al.,* 2000). The carcinogenic PAHs include Benzo [a] anthracene(BaA) , Chrysene (Chr) , Benzo [k] fluoranthene(BkFL), Benzo [a] pyrene (BaP), Benzo [b] fluoranthene (BbF)L, Indeno [1, 2, 3-cd] pyrene (Ind), Dibenzo [a, h] anthracene (DBA) and Benzo [g, h, i] perylene(BP)while the non-carcinogenic PAHs include Naphthalene (Nap), Acenaphthylene (AcPY), Acenaphthene (AcP), Fluorine (Flu), Phenanthrene (Phe), Anthracene (Ant), Fluoranthene (FL), Pyrene (Pyr) (Ghosal *et al*, 2016).

2.15 Physiochemical properties of Coastal water

The Coastal ecosystems harbour a rich diversity of marine flora and fauna because of their higher productivity (Saravanan *et al*., 2013). Rapid urbanization and industrial growth has

shown a significant impact on Coastal ecosystems, such as estuaries and the surrounding Coastal areas. The presence of a dense human population in their watersheds contaminates the environment (Jha *et al*., 2015). Coastal environment reference characteristics are necessary to provide a better management solution for the Coastal ecosystem (Barbier *et al*., 2011). Coastal ecosystems have been seriously impacted by land- and coast-based pollution, including wastewater, runoff effluents, runoff from agricultural waste and petroleum product as well as anthropogenetic activities around the evironment. Conversely, the marine environment, as a complex system is mainly influenced by various physical, chemical and biological processes (Okoro and Akande, 2025). As it is more stable compared to the coastalwaters, where the interaction with the terrestrial zone is more effective in bringing about variations in different physico-chemical parameters influenced by anthropogenic activities surrounding it. Hence a thorough knowledge of hydrography is indispensable to estimate the quality of the environment and its influence on biological fertility. Physical parameters play an important role in the biochemistry of the water body. Subtle changes in physical conditions can have profound effects on the watquality, which may in turn affect the spatial and temporal distribution of nutrients (Jha *et al*., 2015).

Additionally, changes in physical Coastal water process can affect weather patterns and climatic variability. Important physical and chemical parameters influencing the aquatic environment are temperature, rainfall, pH, salinity, dissolved oxygen and carbon dioxide. Others are total suspended and dissolved solids, total alkalinity and acidity and heavy metal contaminants. These parameters are the limiting factors for the survival of aquatic organisms (flora and fauna). The physical and chemical properties of water immensely influence the uses of a water body for the distribution and richness of biota and also influence both vertical and horizontal migration of aquatic organisms. And also affects their distribution, diversity and feeding (Okoro and Akande, 2025). The quality of water in any ecosystem provides

significant information about the available resources for supporting life in that ecosystem. Good quality water resources depend on a large number of physico-chemical parameters and biological characteristics. So, monitoring of these parameters is essential to identifying the magnitude and source of any pollution load. Physical Properties of Coastal Water (Leizuou *et al*., 2017). Some of the physicochemical properties include; pH , water temperature, turbidity, total dissolve solid (TDS) biological oxyen demand (BOD), dissole oygen (DO), electrical conductivity (EC), alkalinity, hardness,, chloride phosphate(PO_4), sulphite (SO_4), nitrite (NO_3).

Colour: The colour of Coastal water indicates the amount of organic and inorganic matter, sediments in the Coastal water and also due to microbial diversity and water could result, cloudy in colour or in appearance (Okoro and AKande, 2025).

Odour and temperature: coastalwater by itself does not have any smell but the things that live in it certainly do. The odour of coastalwater is due to variety of substances in the Coastal water, including dissolved gases, organic matter, inorganic compounds, microorganisms and their activities also the human activities surrounding it.The temperature of coastalwater can vary depending on a number of factors, including the depth, the latitude, and the time of year (Okoro and AKande, 2025).

Turbidity: Turbidity is a measure of the clarity of water, and it is determined by the amount of suspended particles in the water. Suspended particles can include sediment, algae, bacteria, and other matter.Turbidity is an important water quality parameter that can be used to assess the health of the marine ecosystem and to monitor water quality. The turbidity generally measured in NTU (Nephelometric Turbidity Units) (Jha *et al*., 2015).

Electrical conductivity: Electrical conductivity is a measure of the ability of water to pass or conduct an electrical current. The electrical conductivity of coastalwater is determined by the

amount of dissolved ions in the water. The higher the concentration of dissolved ions, the higher the electrical conductivity. Salinity is the amount of salt dissolved in the water. The higher the salinity, the higher the electrical conductivity. Temperature also affects the electrical conductivity of sea water. The higher the temperature, the lower the electrical conductivity.The electrical conductivity generally determined by conductivity meter and unit is µ S/m (Barbier *et al*., 2011).

Total dissolved solids (TDS): Total dissolved solids (TDS) is a measure of the total concentration of dissolved inorganic and organic substances in a liquid. TDS is expressed in milligrams per liter (mg/L) or parts per million (ppm). The TDS of a water source can vary depending on a number of factors, including the source of the water, and the surrounding environment. The TDS in coastalwater is composed of a variety of minerals, including sodium, chloride, magnesium, calcium, sulfate, and bicarbonate. The exact composition of the TDS can vary depending on the location of the Coastal water.The numerous kinds of pollutants,oil spills,salts are responsible for increase in the level of dissolved solids in the water (Saravanan *et al*., 2013). High TDS can signal poor water quality that is unsuitable for sustaining healthy aquatic ecosystems and the bivalves within them. Stress from high TDS can lead to reduced growth rates, impaired reproduction, and increased susceptibility to diseases and other stressors in bivalves (Saravanan *et al*., 2013).

pH: The pH of Coastal water is typically around 8.1. This makes Coastal water slightly alkaline. Dissolved oxygen: coastalwater contains dissolved oxygen, which is essential for marine life. The amount of dissolved oxygen in Coastal water can vary depending on a number of factors, such as temperature, salinity, and currents (Zhou *et al*., 2007).

Nutrients: Coastal water contains a number of nutrients, such as nitrogen, phosphorus, and potassium. These nutrients are essential for the growth of marine plants and algae High levels of phosphates, nitrites, and sulphites in Coastal waters can negatively affect bivalves by

altering water quality, leading to eutrophication, agal bloom and reduced dissolved oxygen, which impairs bivalve health and survival Phosphate and nitrite are key nujtrients linked to pollution from runoff, while excessive sulphates can also harm aquatic life. These pollutants can cause stress, reduce biodiversity, and compromise the ecological functions of bivalves as filter feeders and habitat modifiers in polluted Coastal ecosystems. Phosphates and nitrites act as nutrients, and their accumulation in Coastal waters can lead to eeutrophication. This process results in excessive growth of algae and phytoplankton, which consume large amounts of dissolved oxygen when they decompose, creating hypoxic conditions. Low dissolved oxygen is harmful to bivalves, which are sensitive to oxygen depletion (Jha *et al*., 2015).

Alkalinity: Alkalinity is a measure of the ability of water to neutralize acids. It is a measure of the concentration of alkaline substances in water, such as bicarbonate, carbonate, and hydroxide ionsAlkalinity is crucial for bivalves in Coastal waters because it affects their ability to form shells, as they rely on calcium carbonate for growth (Okoro and Akande, 2025).

2.16 Enteric illness associated with consumption of raw bivalve (clam and oyster)

According to Potassman *et al.* (2002) the New York State Health Department has received reports of at least 14 separate outbreaks of gastroenteritis associated with consumption of raw clams in the year 1998. Approximately 150 persons have been affected. Typical symptoms of diarrhea and abdominal cramps beginning 12- 72 hours after eating clams, with nausea, vomiting, and fever occurring less often. In three of these outbreaks, seven individuals subsequently developed hepatitis A 21- 37 days after eating clams (Potasman *et al*., 2002). Three other persons developed hepatitis A without initial gastrointestinal symptoms. Eight of 10 cases was verified by the presence of IgM antibody (Potasman *et al*., 2002).

2.17 *Vibrio*

Vibrio spp. are ubiquitous in freshwater, estuarine, and saltwater aquatic milieus (Osunla and Okoh, 2017). They are curved Gram negative bacilli, oxidase and catalase positive, and are generally sensitive to vibrio static agents. Their motility is conferred by polar flagella (Deen *et al*., 2019). *Vibrio* genus consists of about 150 species which can be further categorized into halophilic and nonhalophilic *Vibrio* spp based on their sodium requirement. Although nonhalophilic *Vibrio* spp in humans usually cause moderate infection, they are also known to cause outbreaks in humans, fish, and other aquatic animals with undesirable economical and public health implications (Deen *et al.,* 2019).

Human vibriosis can be classified into two major groups: cholera and non-cholera. (Deen *et al.,* 2019) *Vibrio cholerae* is an important human pathogen responsible for cholera. Cholera is both waterborne and foodborne causing approximately 1.3 to 5 million cases reported annually with the death toll of 21,000–143,000 lives/year (Ali *et al.,* 2015) *V. cholerae* serotypes O1 and O139 are exclusively accountable for cholera epidemics. Upon entry into the human host, *V. cholerae* adhere to intestinal epithelium followed by secretion and accumulation of cholera toxin (CT), which is responsible for intense watery diarrhea that potentially lead to fatality. Virulence genes located in the ToxR regulon within *V. cholerae* genome are in particular responsible for severe symptoms of cholera (Ali *et al*., 2015). Meanwhile, *V. cholerae* serotypes non-O1 and non-O139 are responsible for sporadic cholera-like infections, bacteremia, and septicemia (Zhang *et al*., 2024). The *V. cholerae* non-O1/non-O139 are categorized as noncholera *Vibrio cholerae*, as well as *V. cholerae* serotypes O1 and O139 that do not produce cholera toxin. Such serotypes have been associated with clonal outbreak among diarrheal patients in Kolkata (Wu *et al.,* 2022).

Other *Vibrio* spp., such as *V. mimicus*, *V. metschnikovii*, *V. vulnificus*, *V. alginolyticus*, *V. parahaemolyticus*, and *V. furnissii*, can also cause illnesses in humans known as vibriosis, which commonly is presented with gastrointestinal infection, wound infections, and septicemia (Baker, 2016). Certain species such as *V. metschnikovii* have been isolated from a range of conditions such as septicemia and wound (Baker, 2016). Differently, cholera epidemics are highly associated with sanitary quality and socioeconomic status (typically transmitted from human to human or contaminated water). However, vibriosis caused by other *Vibrio* spp. is closely related to the consumption of contaminated shellfish or contaminated water and is independent of the sanitation status (Baker, 2016). The presence and compositions of *Vibrio* spp. in aquatic habitats are dynamic and influenced by geographical factors of climate, and salinity (Davis *et al*., 2023). Therefore, analysis of local clinical and environmental data is necessary to develop an understanding of occurrences of vibriosis as a result of interaction between human health and other domains over time.

Vibrio species are autochthonous bacteria that are natural inhabitants in estuarine and marine environments worldwide (Naidoo, *et al*, 2025). Approximately 12 *Vibrio* spp. can cause human illness, known as vibriosis, and have emerged as a severe threat to human health worldwide (Park *et al*., 2024). The major species causing human illness are *Vibrio parahaemolyticus*, *V. vulnificus*, and *V. cholerae* (Baker-Austin *et al.,* 2024; Silva *et al*., 2016). *Vibrio parahaemolyticus* is the most common pathogen causing seafood-borne illnesses in many countries, due to the consumption of raw or undercooked seafood, especially bivalve shellfish such as oysters (Fearnley *et al*., 2024). In the USA, the most common *Vibrio* species causing human illness is also *V. parahaemolyticus*, which is estimated to cause 45,000 illnesses annually; moreover, most people become infected by eating raw or undercooked shellfish, particularly oysters (Osunla and Okoh. 2017).

Although not all *V. parahaemolyticus* strains are pathogenic in humans (Osunla and Okoh. 2017).The virulence of *V. parahaemolyticus* is mainly attributed to the presence of two major genes: *tdh* (encoding thermo–stable direct hemolysin) and *trh* (encoding *tdh*-related hemolysin) (Osunla and Okoh. 2017). Thus, the presence of *tdh*- and/or *trh*-positive *V. parahaemolyticus* strains in marine food sources, particularly oysters, is considered a major public health risk (Osunla and Okoh. 2017).

Another problem is the occurrence and prevalence of antimicrobial resistant *V. parahaemolyticus* in marine environments. Since the discovery of penicillin in the 1920s, antimicrobials have been used for the treatment of infectious diseases (Zhou *et al*., 2007). Antimicrobial resistance has emerged in a wide range of infectious agents and has evolved in a variety of bacteria, including *V. parahaemolyticus* (Zhou *et al*., 2007). This emergence may be related to the misuse of antimicrobials to prevent and treat bacterial infections in aquaculture systems as well as in humans and agriculture (Letchumanan *et al*., 2015). In particular, *V. parahaemolyticus* strains with multiple antimicrobial resistance may pose a severe threat to public human health and the commercial aquaculture industry (Onifade *et al*., 2011).

2.17.1 Sea food and *Vibrio*

Seafood is a nutritious food that constitutes one of the desirable components of a healthy diet. Nevertheless, there is health risks associated with the consumption of seafood. One of the major risks involves the consumption of raw or undercooked seafood that may be naturally contaminated by foodborne pathogens present in the marine environment. Such risk is further increased if the food is mishandled during processing where pathogens could multiply exponentially under favourable conditions. In contrast to most other foodborne pathogens, *Vibrio* spp. have the aquatic habitat as their natural niche. As a result, vibrios are most

commonly associated with seafood as natural contaminants. Foodborne infections with *Vibrio* spp. are common in Asia, including Hong Kong (Scallan *et al*., 2011).

Vibrios are associated with live seafood as they form part of the indigenous microflora of the marine. Seafood products are widely consumed all around the world and play a significant role on the economic market. Bacteria of the *Vibrio* genus can contaminate seafood and thus pose a risk to human health. Three main *Vibrio* species, *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus*, are potentially pathogenic to humans. These species are responsible for a dramatic increase of seafood-borne infections worldwide (Scallan *et al*., 2011).

Vibrio species are the primary indigenous pathogens and account for 75% of seafood related infectious disease (Scallan *et al*., 2011). *Vibrios* are present ubiquitously in Coastal habitats and comprise the natural microflora of shellfish. Risk of Vibrio-related diseases has been shown to increase greatly with warming water temperature, as cases are more prevalent in summer months. *Vibrio paraheamolyticus* is responsible for the estimated 80,000 cases/year of *Vibrio* infections in the United States of which 65% are thought to be foodborne (Scallan *et al*., 2011). Disease incidence varies from year to year but is most common in the Pacific Northwest and North Atlantic states, *V. parahaemolyticus* monitoring programs have been used for disease prediction and guidance for harvest closure in some areas. The second most common cause of vibriosis in the United States is *Vibrio vulnificus*, which produces a much more serious disease Also, warming of Coastal waters due to climate change is likely to contribute to increased disease incidence (Vezzulli *et al*., 2016). The majority of foodborne vibriosis in the United States is attributed to *Vibrio parahaemolyticus*, accounting for 45% or 54% of cases from 1996 to 2010 (Baker-Austin *et al*., 2024). This bacterium generally causes mild diarrhea but is frequently associated with large outbreaks of disease. Although this organism is responsible for only 19% or 13% of infections, depending on the reporting source, it causes greater than 80% of mortality associated with seafood (Onifade and Aiyenuro, 2018).

Diarrheal symptoms are rare, but overwhelming sepsis may follow ingestion of seafood or wound infections that can be fatal within 48 h. The vast majority of persons with foodborne *V. vulnificus* disease have ingested raw oysters and have some type of underlying condition, such as hemochromatosis (iron overload), liver disease, diabetes, and AIDS (Onifade *et al*., 2011). These persons should avoid consumption of raw oysters and also exposure of open wounds to seawater, fish, or shellfish, as their fatality rate exceeds 50% for infected individuals. *Vibrio paraheamolyticus* is the third most common cause of vibriosis in the United States. Although this species is considered an emerging foodborne pathogen, it is more commonly implicated in wound and ear infections. Symptoms include gastroenteritis and occasionally septic shock. Other *Vibrio* spp. associated with consumption of raw shellfish include *Vibrio cholerae,* but cases are rare and mostly sporadic, although a small outbreak of cholera attributed to consumption of raw oysters was reported (Onifade *et al*., 2011).

2.18 *Listeria*

Listeria is Gram-positive, non-spore forming, facultative anaerobic and psychrotrophic bacteria that are widely distributed in nature. The genus *Listeria* has ten species viz*., L. monocytogenes*, *L. innocua, L. welshimeri*, *L. seeligeri, L. ivanovii, L. grayi, L. marthii, L. rocourtiae* (*L. weihenstephanensis and L. fleischmanii* (Jenney *et al*., 2015). (*L. monocytogenes is* widely recognised as a principal human pathogen that causes serious diseases, such as septicemia, meningitis, meningoencephalitis in immunocompromised individuals, newborns and the elderly; and abortion and stillbirth in pregnant women (Welekidan *et al*., 2019). Although rare, listeriosis manifests as a severe illness with an exceptionally high level of mortality (20–40%), particularly of those who are most vulnerable, for both epidemic and sporadic cases. More recently, an increasing number of cases have been associated with febrile gastroenteritis (Rocourt *et al*., 2000). *Listeria* spp. are indigenous to the marine and estuarine environments (Rocout *et al*., 2000). *L. monocytogenes* and other

Listeria species, isolated from a variety of raw and processed foods like milk and salads, seafood and seafood products as well as from ready-to-eat foods, have been responsible for several epidemics and sporadic outbreaks (Kalorey *et al*., 2008). There are 13 recognised serotypes of *L. monocytogenes*, out of which only 3 serotypes i.e., 1/2a, 1/2b and 4b are largely involved in causing human listeriosis (Doumith *et al*., 2004). Because of the importance of *L. monocytogenes* strain characterisation for epidemiological investigations, a number of discriminatory subtyping methods have been described (Tchatchouang *et al*., 2020). However, most of these methods are not always convenient for routine use in clinical laboratories and require special instruments and skilled personnel. Multiplex-PCR (MPCR) serogrouping is currently used by several public and private laboratories for the characterisation of *L. monocytogenes* (Doumith *et al*., 2004). In India, *L. monocytogenes* has been isolated from various foods (Suryawanshi *et al.,* 2023). The organism has been isolated from fish and fishery products from different parts and interestingly the incidence rate reported from tropical fish is rather low (Vijay *et al*., 2019).

The bacteria *Listeria* spp. have become a significant subject in biomedical research due to its central role in Medical microbiology and food microbiology. The presence of any *Listeria* species in food may be an indicator of poor hygiene. However, since *L. monocytogenes* is the major human pathogen, there is widespread agreement that the goal should be to exclude this organism from the food chain wherever possible, and to maintain conditions that will inhibit its multiplication in foods in which this bacterium can grow (Manyi-Loh and Lues, 2025). Listeriosis is a severe and often fatal illness with clinical manifestations resembling sepsis or meningitis in immunocompromised patients and neonatal babies, and flu-like illness or abortion during pregnancy in women (Delgado, 2008). The case-fatality rate from listeriosis is generally about 20–30% (Sibanda, 2023).

Major outbreaks of listeriosis have been associated with the consumption of foods of animal origin (Manyi-Loh *et al*. 2025), especially sea foods such as shrimp, mussels and undercooked fish (Brett *et al.,* 1998). Since fish and fishery products may be a vehicle for *L. monocytogenes,* it is important to have information on the incidence of this pathogen. This organism has been isolated frequently from fish and fish products from different parts of the world (Rocout *et al.,* 2000).

2.19 Epidemiology of *Listeria*

The epidemiology of *Listeria* in Africa is often marked by sporadic cases or major outbreaks. The 2017/2018 South African listeriosis outbreak awakened the world to the possible wide-spread of the disease in Africa (Thomas *et al*., 2020). Humans risk multiple exposures to infection via contact with carriers of *Listeria*. *Listeria*, the causative agent for listeriosis, can colonize up to 5% of healthy adults (Sibanda and Manyi-Loh, 2023). Adults with invasive listeriosis can present symptoms such as fever, stillbirth, and convulsion among others. The risk of infection is further exacerbated by the ubiquitous nature of the pathogen, making it difficult to get rid of listeriosis (Manyi-Loh, 2025). In Africa, *Listeria* spp., particularly *L. monocytogenes*, has been isolated from various food, animal and environmental sources in countries such as Nigeria, South Africa, Ghana, Ethiopia, Egypt and Botswana (Sibanda and Manyi-Loh, 2023). To date, the listeriosis outbreak in South Africa remains the largest in the world with over 1000 laboratory-confirmed cases and over 200 fatalities (Manyi-Loh, 2025). This unprecedented outbreak has been attributed to changes in food production and distribution processes in South Africa (Dufailu, 2021). Before this outbreak, a marked increase in listeriosis cases was recorded in June 2017 with the source of infection being ready to eat (RTE) processed meat product (Dufailu, 2021). Following identification of the source of the outbreak, recalls of the affected products were made in South Africa and 15 other African countries (Thomas *et al*., 2020).

Other reports from South Africa described the presence of *L. monocytogenes* in various food items (Thomas *et al*., 2020) .In 2015, *L. monocytogenes* ST6 affecting humans were reported in Western Cape Province of South Africa (Dufailu, 2021). From 2014 to 2016, the overall prevalence of *L. monocytogenes* in meat and meat products in South Africa was reported to be 14.7% (296/2017), with meats from local markets and ports of entry recording prevalence rates of 15.0% (264/1758) and 12.4% (32/259), respectively (Smith, 2019). On the other hand, the prevalence of *L. monocytogenes* recovered from irrigation water and agricultural soil samples in South Africa were 6.8% (8/117) and 6.6% (12/183), respectively (Smith, 2019). Other studies have also reported that municipal wastewater effluent is a possible reservoir and transporter of pathogenic *Listeria* in South Africa and this is of public health concern (Thomas *et al*., 2020). Taking into account that approximately 77% of South Africans depend on surface water for their daily activities, the reported prevalence of *Listeria* in irrigation water and agricultural soil could be potential source for infections (Luyt *et al*., 2012).

From available data, the calculated mean prevalence of *L. monocytogenes* in South African is 17.9%. Despite the few recent reports on the prevalence of listeriosis in South Africa, comprehensive historic data on prevalence, epidemiology and outbreaks associated with *L. monocytogenes* is still lacking (Dufailu, 2021). In Northern Africa, *Listeria* spp. was detected in 32% of luncheon, 32% of minced frozen beef, 52% of frozen chicken leg and 56% of frozen chicken fillet in Assiut, Egypt (Kayed *et al.,* 2024). Additionally, the incidence of *Listeria* spp. stool samples of children with underlying health conditions at Assiut University hospital was reported to be 7.14% (2/28) (Kayed *et al.,* 2024). The data demonstrate a potential infection linked to consumption of listeria-containing meat and chicken products in Assiut city, Egypt (Kayed *et al.,* 2024). In East Africa, the prevalence rate of *L. monocytogenes* among pregnant women in northern Ethiopia was reported to be 8.5% (Amira *et al.,* 2024 Walekidin *et al*., 2019). Also, 4.4% prevalence of *L. monocytogenes* was reported

in beef and fomites (Amira *et al.,* 2024). In another study, a 26.1% prevalence of *Listeria* spp. was reported in food of animal origin (Meshref *et al.,* 2024). Furthermore, a prevalence of 25 and 6.25% for *Listeria species* and *L. monocytogenes*, respectively, was reported from ready-to eat food of animal origin in Ethiopia (Morshdy *et al.,* 2023). In an investigation to assess the occurrence of *L. monocytogenes* in Gaborone, Botswana in Central Africa (Gana *et al.,* 2024) analyzed food samples collected from five geographical areas. In their report, the overall prevalence of *L. monocytogenes* was 4.3% (57/1324), out of the 57 *L. monocytogenes* isolates, 12.3% were derived from cheese while the highest *L. monocytogenes* prevalence of 33.3% was recorded in Gaborone, South Africa (Morobe *et al.,* 2009).

In West Africa, the Nigerian meat industry has been implicated in the transmission of *Listeria* spp (Koladaisi and Balogun, 2023). In Rivers State, South-South Nigeria, 7% prevalence of L. monocytogenes in raw meat samples was reported (Koladaisi and Balogun, 2023). While 91.8% prevalence of *L. monocytogenes* in chicken flocks and meat was reported in Oyo state, Nigeria (Ishola *et al.,* 2016). In Lafia, Nigeria, they reported prevalence of *Listeria* spp. In beef and chevon were 58.2% (78/134) and 41.8% (56/ 134), respectively. Of these isolates, 64.4% (67/104) were confirmed to be *L. monocytogenes* (Chuku *et al.,* 2020). In Enugu state, Nigeria, the frequency of *Listeria* spp isolated from chicken meat, pork, and beef samples was 27.1, 13.2 and 45.8 %respectively (Okore –kanu *et al*., 2014) While in Zaira, Nigeria ,the prevalence of*L. monocytogenes* in raw meat and meat products was 4.0% (Ndahi *et al*., 2014). Other studies in Nigeria reported the prevalence of *L. monocytogenes* in vegetables including in cabbage, carrot, cucumber, lettuce, and tomatoes to be, 28.28, 9.02, 23.36, 19.67, and 19.67%, respectively (Ajayeoba *et al.,* 2016; Odetokun and, Adetunji 2016).

Additionally, the prevalence of *Listeria* spp in locally made soft cheeses (wara) was 78%. Out of which 12.4% were identified as L. monocytogenes (Oyinloye *et al.,* 2016). From this

review, the calculated mean average prevalence of L. *monocytogenes* for Nigeria is 43.5%. In Ghana, data on the prevalence of *L. monocytogenes* remains scarce. However, 5.5% (14/254) prevalence of *L. monocytogenes* in traditional milk products was reported *Listeria* spp., particularly *L. monocytogenes,* is generally reported to have a low prevalence rate but a high fatality rate. The possible explanations for the epidemiological differences of *Listeria* spp. across Africa could be attributed to the variation in study groups, sampling source, microbial diversity, and geographic location.

2.20 Antimicrobial resistance in *listeria*

Antimicrobial resistance has become a global public health issue. Several studies across Africa have reported the antimicrobial resistance profiles of *Listeria* species, specifically *L. monocytogenes*. In Ethiopia, resistance of *L. monocytogenes* was reported for the following antibiotics: penicillin 66.7% (16/ 24), nalidixic acid 50% (12/24), tetracycline 37.5% (9/24) and chloramphenicol 16.6% (4/24). (Gana *et al.,* 2024) Furthermore 16.6% were found to be multi-drug resistant (Meshref *et al.,* 2024). In a different study, *L. monocytogenes* isolated from pregnant women in Ethiopia showed that isolates were resistant to clindamycin (66.7%), penicillin G (66.7%), vancomycin (50%) and amoxicillin (50%) (Welekidan *et al*., 2019) However, isolates were sensitive to erythromycin (75%), ciprofloxacin (75%), trimethoprim/sulphamethaxazole (66.7%) and chloramphenicol (60%) (Walekidan *et al.,* 2019). Whereas, *Listeria* spp. isolated from wastewater treatment plants in Durban, South Africa, demonstrated 100% resistance to penicillin, nalidixic acid and erythromycin. (67.95%), nitrofurantoin (64.10%) and cephalosporin (60.26%) (Olaniran *et al*., 2015). Similarly*, L. monocytogenes* isolates recovered from irrigation water and agricultural soil from Eastern Cape Province, South Africa, were resistant to tetracycline (90%), doxycycline (85%), penicillin (80%), cefotaxime (80%), chloramphenicol (70%), linezolid (65%), erythromycin (60%) and trimethoprim/sulfamethoxazole (55%) (Olaniran *et al*., 2015) .The

isolates were also reported to harbour tetA, tetB, tetC, sulI, sulII, aadA, aac (3)-IIa resistance genes and extended-spectrum beta-lactamase (ESBLs) including blaTEM, blaCTX-M group 9, blaVEB as well as ampC. (Olaniran *et al*., 2015).

However, none of the isolates carried the carbapenemase resistance genes, In South Africa, another study reported that 1.7% *L. monocytogenes* isolated from meat showed multiple resistance to 13 of the 19 test antibiotics. (Olaniran *et al*., 2015). Resistance was recorded for streptomycin (99.0%), clindamycin (97.3%), fusidic acids (95.6%), nitrofurantoin (79.7%), and gentamycin (74.4%). However, the isolates were sensitive to ampicillin (85.6%), kanamycin (84.6%), amikacin (77.6%), vancomycin (74.2%), and tetracycline (62.5%) (Smith, 2019). In Botswana, *L. monocytogenes* isolated from RTE/ meat showed multiple resistance against penicillin G (42.1%), sulphamethaxozole/trimethoprim (29.8%), chloramphenicol (28.3%), and tetracycline (22.8%). Resistance against penicillin G and tetracycline was a common pattern in all *L. monocytogenes* isolated from food products.

However, the isolates were generally susceptible to fusidic acid, erythromycin, methicillin, ampicillin and cephalothin (Morobe *et al.,* 2009). In Enugu State, South-East Nigeria, *L. monocytogenes* isolated from beef, chicken, and pork were examined against penicillin, cephalothin, amoxicillin, ampicillin, nitrofurantoin, vancomycin, tetracycline, gentamicin (aminoglycosides), gentamicin (macrolides), ciprofloxacin, sulphamethoxazole/trimethoprim and rifampicin. All *L. monocytogenes* showed 100% resistance against penicillin. Interestingly, only *L. monocytogenes* recovered from pork showed 100% resistance to more than one *antibiotic,* that is *penicillin, cephalothin, sulphamethoxazole/ trimethoprim and* ciprofloxacin (Okorie-kanu *et al.,* 2020). The high and multidrug resistance of isolates from pork is a public health concern and could be attributed to the misuse of antibiotics in pig farming. Also*, L. monocytogenes* isolated from beef, pork and chicken recorded resistance to amoxicillin, tetracycline, augmentin and cloxacillin but sensitivity to erythromycin and

gentamicin (Peter *et al*., 2016). In Ekiti, South-West Nigeria, *Listeria spp*. isolated from soft cheese (wara) showed 90 and 89% resistance against ampiclox (ampicillin/cloxacillin) and amoxicillin, respectively. However, the isolates were susceptible to ciprofloxacin and septrin (co-trimoxazole) (Oyinloye *et al.,* 2016). *L. monocytogenes* isolated from beef and chevon in North Central Nigeria were reported to be resistant to streptomycin (58.2%), sparfloxacin (55.2%), ampicillin (34.3%), and gentamicin (20.9%) (Chuku *et al*., 2020). *L. monocytogenes* has also been reported to be resistant to some antibiotics and susceptible to few vantibiotics In another study, *L. monocytogenes* strains were resistant (100%) to some antibiotics such as ampicillin-cloxacillin and cefuroxime but susceptible to amoxicillin clavulanate (86.1%) ciprofloxacin (43.8%), cloxacillin (36.1%), ceftriaxone (32.5%), gentamicin sulphate (27.8%), streptomycin sulphate (25.0%), pefloxacin (17.5%), erythromycin 5 /µg (16.7%), co-trimoxazole (12.5%), erythromycin 10 µg (12.5%), and amoxicillin (6.3%) (Ishola *et al.,* 2016).

Similarly, 100% resistance to augmentin, erythromycin, tetracycline, rifampicin, and cloxacillin was recorded, with some isolates demonstrating a varying degree of resistance to norfloxacin (57.2%), levofloxacin (71.4%), and ciprofloxacin (71.4%). On the contrary, all *L. monocytogenes* isolates from retail meats were (100%) susceptible to chloramphenicol, gentamicin, ampiclox, clotrimoxazole, and streptomycin (Morshdy *et al.,* 2023). In Ghana*, L. monocytogenes* had shown resistance against some antibiotics such as neomycin (61.3%) and tetracycline (24.2%) (Ishola *et al*. 2016). However,intermediate susceptibilities were recorded for chloramphenicol, ciprofloxacin, clindamycin, doxycycline, kanamycin, neomycin, streptomycin, and tetracycline, general susceptibility (100%) to amoxicillin, ampicillin, erythromycin, gentamycin, penicillin, rifampicin, and vancomycin (Penduka *et* al.,. 2014).

Although antibiotics remain the conventional protocol for the treatment of listeriosis, some studies have also shown the potential of plant extracts in listeriosis chemotherapy (Penduka *et al*., 2014). The effect of plant triterpenes: 3βhydroxylanosta-9,24-dien-21-oic acid, methyl-3βhydroxylanosta-9,24-dien-21-oate and 3β-acetylursolic acid, against *L. monocytogenes, L. ivanovii* and *L. grayi* species was investigated by Penduka *et al*.(2014) . The triterpenes' minimum inhibitory concentration (MIC) values ranged from 0.185 to 1.67 mg/ml while the minimum bactericidal concentration (MBC) determination assay revealed that the triterpenes were bacteriostatic against *Listeria* spp (Penduka *et al*., 2014). In summary, *Listeria* isolates from Africa are generally susceptible to ampicillin and ciprofloxacin. However, the emergence of multidrug resistant strains is of serious public health concern in Africa.

2.21 Molecular characterization of *Listeria*

Genomic studies have been employed to elucidate the global circulation of *Listeria monocytogenes* (Nwaiwu *et al*., 2017). In Africa, the serotypes of circulating strains of *L. monocytogenes* are largely unknown (Odetokun *et al.,* 2016). In epidemiological studies, rapid detection of listeriosis outbreaks is often by phenotypic and molecular characterizations (Meshref *et al*., 2024). Subtyping of *L. monocytogenes* isolates is essential for epidemiological investigation and for identification of the source of contamination. Multilocus Sequence Typing (MLST) has been used to prove that the 2015 and 2017 listeriosis outbreaks in South Africa were mainly due to contamination of meat products by *Listeria. monocytogenes* ST6 . *L. monocytogenes* ST6 is often associated with high fatality cases. Whole-genome sequencing approach, MLST, was used to demonstrate that *L. monocytogenes* ST6 was the most common serotype of *L. monocytogenes* detected in human listeriosis cases in Western Cape Province in South Africa (Silbernagel, 2024). Four different sequence typing(STs) (ST1, ST121, ST204, and ST876) belonging to lineage I (serogroup 4b)

and lineage II (1/ 2a) were identified using whole genome sequencing (WGS) to characterize six L. *monocytogenes* isolated from RTE meat products in South Africa (Matle *al.,* 2019).

2.22 Depuration of Bivalve Mollusc

Depuration is a technique used to reduce microbial contamination of filtering molluscs, to levels acceptable by legislation for human consumption, by keeping the animals in tanks with clean water (Silvestre *et al.,* 2021). Since depuration is a way to ensure product quality, protocols applicable to the local product are needed.

The recommended minimum water flow rate in the United States and New Zealand is 107 liters/minute per cubic meter of animals. In Japan, the minimum flow rate described is 12 liters/ minute per 1,000 oysters (Souza *et al*., 2021). Marine water of good quality must be available to ensure that the depuration process reduces the contamination of bivalve molluscs. However, seawater with adequate composition and highly transparent (with low turbidity) is required, especially in establishments that use ultraviolet light disinfection (Chinnadurai *et al.,* 2023). However, when the coastalwater available in the region does not have adequate transparency and salinity, or when the purification establishment is far from the sea, artificial seawater can be used (Souza *et al.,* 2021). According to Rodrick and Schneider (2003), salinity, water temperature, dissolved oxygen content, turbidity and phytoplankton concentration can impact the process of elimination of pathogens. These factors must be controlled, as the filtration rate, physiological activity and behavioral responses of molluscs may vary depending on the depuration environment. The process of depuration depends on the level of risks related to fecal pollution that affects the areas of cultivation or extraction of bivalve molluscs. This risk is determined through the National Programme for Hygiene and Sanitary Control of Bivalve Molluscs (PNCMB), which aims to establish the minimum requirements necessary to guarantee the safety and quality of bivalve molluscs for human

consumption (Silvestre *et al.,* 2021). As for the depuration time, it must be long enough for the molluscs to release pathogens from the intestinal tract.

However, relevant international legislation is extremely strict, given the high number of cases of diseases associated with the consumption of contaminated seafood (Richards, 2003; Younger *et al.,* 2003). Most countries that produce foods of marine origin have their own legislation based on regulations from large markets such as the United States and the European Union. The European Directive 91/492/EEC, of July 15, 1991 (European Communities, 1991) sets out hygiene rules for the production and placing on the European Common Market of live bivalve molluscs (Younger *et al*., 2003) The areas destined for cultivation are classified according to the microbiological quality of the meat of molluscs produced in these waters. The main strategy of depuration is to control the risks of shellfish sold to final consumers and consumed live, such as oysters. Cockles or mussels that are purchased alive and eaten cooked by the consumers must also be submitted to the depuration process, as commercialization does not guarantee the elimination of the causative agents of diseases (Souza *et al*., 2021). However, the United States counts on a National Shellfish Sanitation Program, with regulations based on interstate commercial agreements recognized by the FDA (National Shellfish Sanitation Program, 2007). This program aims to promote and value the production of shellfish in the country, standardizing regulations among the American states (Rodrick *et al*., 2003).

2.23 Depuration Systems

There are different types of depuration systems, as follows: the open system - with constant flow of water; the closed system - a closed recirculated seawater system or the “Batch-process” system where water is replaced at regular intervals. The closed recirculation system is currently the most used because it uses less water (Corrêa *et al.,* 2007). According to the

Brazilian legislation, a depuration plant is the establishment intended for the reception, purification, packaging, labeling, storage and shipping of bivalve molluscs (Silvestre *et al.,* 2021). The seafood processing sector is responsible for the depuration procedure of bivalve molluscs before they are taken to restaurants and fish markets. Consequently, mariculturers are not responsible for the referred procedure (Silvestre *et al.,* 2021).

Ozone gas can be produced by an ozone generator or purchased in cylinders. When diffusers are added to Coastal water. Then bacteria and viruses are inactivated. The gas is mostly used in depuration centers for large quantities of bivalve molluscs, and very strict safety protocols are adopted (Souza *et al.,* 2021). As an antimicrobial agent, ozone acts directly on the cell wall, causing its rupture and destruction, at shorter time contact (Silva *et al.,* 2011). Studies have shown the bactericidal effect of ozone on a wide variety of microorganisms, including Gram-negative and Gram-positive bacteria. Gram-negative bacteria are more sensitive to ozone compared to Gram-positive bacteria, as they have less peptide glycan in their cell wall (Silva *et al*., 2011). The chemical water disinfection method for the depuration of molluscs uses chlorination, due to its disinfectant capacity and easy handling. However, chlorine impairs molluscs' filtering ability and may modify the appearance and taste of seafood (Souza *et al.,* 2021). Thus, alternative methods of water purification should be explored, to minimize sensory alterations of molluscs at the end of the process (Suplicy, 1998). Chlorine acts by diffusion, passing through cell membranes. Once inside the cell, it disrupts its vital elements, such as enzymes, proteins, DNA and RNA (Silva *et al.,* 2011).

Given the fast growth of malacocultures, it is necessary to ensure that the areas of cultivation and production of bivalve molluscs have microbiological quality. Thus, consumer safety will depend on the quality of the product, which is related to the physical, chemical and microbiological conditions of the original environment. To ensure that the export of bivalve molluscs is competitive in the international market, all guidelines must also comply with the

applicable legislation of trading partners. The main markets for bivalve molluscs are the United States and the European Union, and depuration of molluscs in these countries guarantees a final product with high commercial value and in good sanitary conditions. Nonetheless, the depuration process has the main purpose of controlling the risks to bivalve molluscs commercialized and sold to consumers, eliminating pathogenic etiological agents that cause diseases (Souza *et al.,* 2021). Therefore, the three main water disinfection methods used in depuration are ozone, ultraviolet light and chlorine-based compounds. Shellfish business operators are responsible for the depuration procedure of bivalve molluscs before they are taken to restaurants, fish markets and the final consumers.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Media, Materials, Chemicals and Reagents

All media used were prepared according to the manufacturer instructions and these included nutrient agar, thiosulphate citrate bile salt sucrose agar and University of Vermor broth (UVM1)(UVM11), Polymyxin Acraflavin Lithium Chloride CeflaxidimeAsculin Mannitol (PALCAM) media tryptone soy yeast agar and agar.

All glass ware were washed with detergent and rinsed with water, allowed to dry, wrapped in aluminium foil and sterilized in a hot air oven at 160ºC for 3 h. All chemicals and reagents used for polycyclic aromatic hydrocarbons in this study were of high purity quality and were of analytical grade. Dichloromethane used for the extraction was obtained from Fischer Scientific, New Jersey. Silica gel used in the cleaning up of the extract was supplied by BDH Labs (UK) and anhydrous sodium sulphate was purchased from Sigma-Aldrich, Germany. Acetone (99.5%) was purchased from BDH, England.

3.2 Sample preparation and laboratory analysis of PAHs.

The bivalve flesh (*Ergeria radiate* flesh) were oven dried and blended to powdered using an electric blender (Kenwood 1Litre BlenderBL335 350 Watts, China). A PAH standard mixture (NIST, Baltimore, MD, USA) containing naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, benzo[ghi]perylenedibenz[a,h]anthracene and indeno[1,2,3-cd]pyrene was used in this study. A mixture containing four isotopically labeled PAHs (ChemService, West Chester, PA, USA), namely (2H10) acenaphthene (acenaphthene-d10), (2H12) chrysene(chrysened12),

(2H10)phenanthrene (phenanthrene-d10) and (2H12)perylene (perylene-d12), was used as an internal standard (REF).

3.3 Study Area

The study was carried out in Ekowe community, Southern Ijaw Local Government Area in Bayelsa State, Nigeria. The city is located in the Niger Delta, South-South region of Nigeria with the waters of the Atlantic Ocean dominating the southern borders. Map of the study area is shown in Figure 3.1.

3.4 Sample Collection

A total of three hundred (300) edible bivalves species were collected from March 2023 to February 2024. The collection of samples was seasonal namely; wet and dry season. The species were randomly collected from different locations in Ekowe community Coastal waters and from their wet markets (Swali and Opolo) in sterile containers. Samples of edible bivalves species were identified at the Department of Animal and Environmental Biology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria before transporting them to the laboratory for microbiological, chemical and physiochemical analyses.

3.5 Microbiological Analyses of Samples

3.5.1 Microbial isolation

The outer shell of bivalve was washed in running tap water and rinsed in several changes of distilled water. The shells were then disinfected using cotton wool moistened in 70 % alcohol. Thereafter, the bivalve was deshelled under aseptic condition. The soft body part was separated from the shell using a dissecting kit (name of manufacturer, model). The soft body

part was crushed in a mortar and 10 g of the crushed part was taken for bacterial analysis. Standard pour plate were prepared from 10 fold serial dilutions into nutrient agar medium for total heterotrophic bacterial counts, Thiosulphate citrate bile salt sucrose agar for total *Vibrio* counts. The bacterial plates were incubated at 37 ^{o}C for 24 – 48 h (Adebayo Tayo *et al*., 2006).

The modification of the United States Department of Agriculture (USDA) and Food and Drug Administration (FDA) protocols was used for the isolation of *Listeria* spp. It included two-step enrichment of the sample in University of Vermont broth (UVM I) for 24 h at 37°C followed by inoculation in UVM II broth for 48 h at 37°C. The selective plating after enrichment was done in Polymyxin-Acriflavine-Lithium Chloride-Ceftazidime-Aesculin-Mannitol (PALCAM) (HiMedia) agar. Twenty-five grams (25 g) of each sample was homogenized in 225 ml *Listeria* enrichment broth (UVM I) (Merck, Darmstadt, Germany) and incubated at 37°C for 24 h. A 1 ml sample of this primary enrichment was transferred to 9 ml UVM II (Frazer broth) (Merck) and incubated at 37°C for 24 h. Secondary enrichments were streaked onto Palcam agar (Merck) and incubated at 35°C for 48 h. The plates were examined for *Listeria* colonies (black colonies with black sunken center). At least three suspected colonies were subcultured onto tryptone soy agar supplemented with 0.6% yeast extract (TSAYE) (Merck) and incubated at 37°C for 24 h. All isolates were subjected to standard biochemical tests (Aygun and Pehlivanlar 2006; Ukwo *et al*., 2019).

3.5.2 Bacterial counts

Discrete colony on the bacterial plate were counted after incubation and recorded as colony forming unit per gram (cfu/g) (Holt *et al*., 1994).

3.5.3 Cultural and morphological characterization of bacterial isolates

Colonial characteristics of bacterial isolates were determined using parameters such as size, elevation, pigment, margin and shape. Morphological characteristics of the isolates were determined by the Gram's staining technique (Olutiola *et al.,* 2000).

3.5. 4 Morphological and biochemical characterization of bacterial isolates

a. Gram Staining Test

The Gram staining technique was done on the basis of the component of the cell wall. A drop of peptone water was placed on a clean greasefree slide. The inoculating wire loop was flamed until red hot. The loop was allowed to cool and a small portion of the organism to be Gram stained was picked and smeared on the drop of water on the slide. The slide was then air dried. It was heatfixed by passing it gently over flame. The smear was stained with 1 % crystal violet for 1 min and washed with distilled water. Gram's iodine was added as a mordant for one minute. This was drained off and 70 % alcohol was added for 30 sec. This acted as a decolorizer. The slide was then rinsed with distilled water. The slide was finally flooded with counter stain, safranin for 1 min and washed off with distilled water and air dried. The slide was observed under the microscope using the oil immersion (x100) objective lens. The Gram positive organisms appeared purple while the Gram negative organisms appeared red (Olutiola *et al.,* 2000).

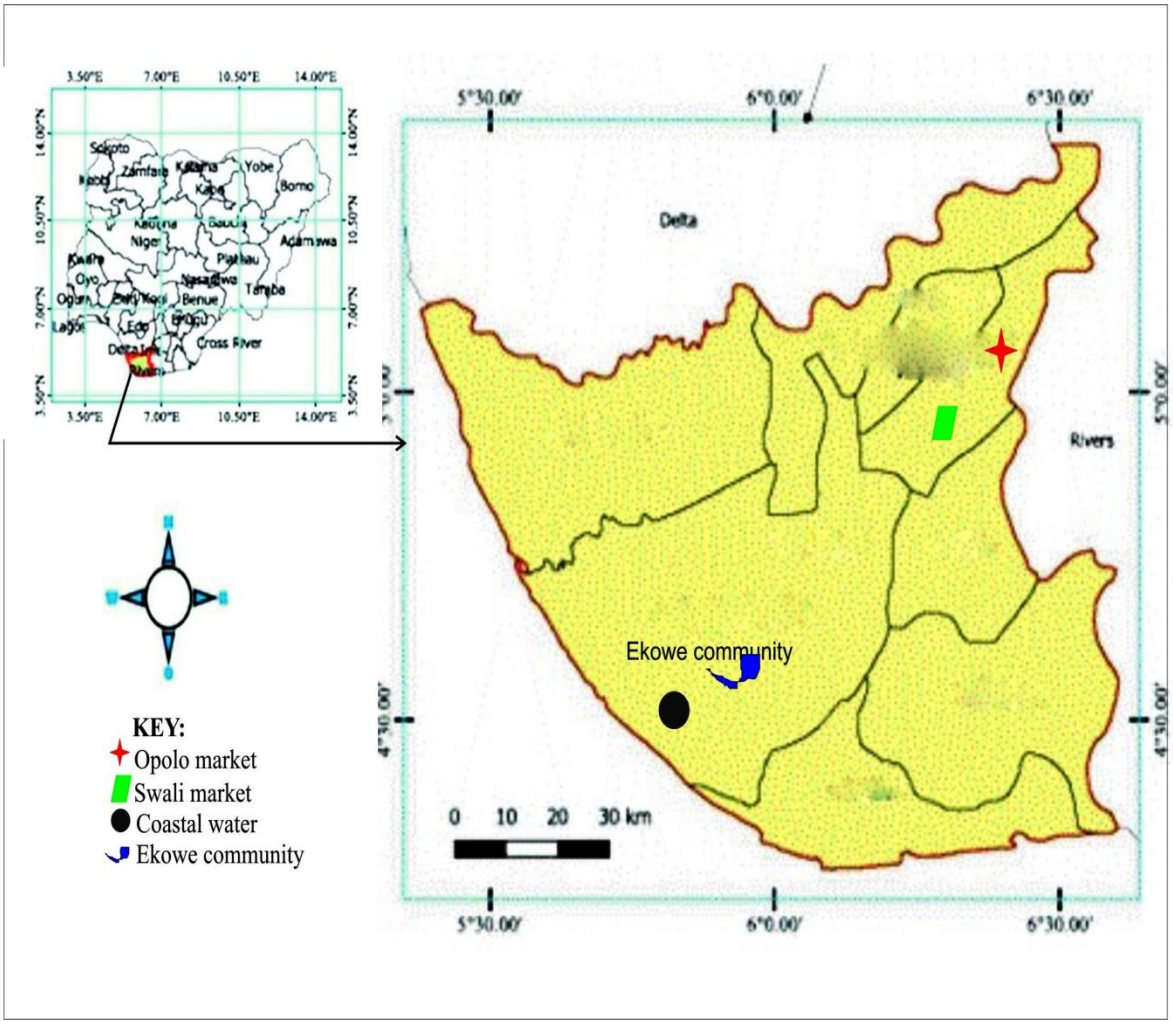

Figure 3.1: Map of study area

b. Motility Test:

A sterile needle was used to pick a loop of a 24 h old culture and was stabbed onto semi solid nutrient agar in glass vials. The vials were incubated at 37°C and at (room temperature ; 20-28°C for *Listeria*) for 24 - 48 h. Non-motile bacteria had growth confined to the stab line with definite margins without spreading to surroundings area, while motile bacteria gave diffused growth extending from the surface (Singleton, 1999).

c. Oxidase Test

This test was carried out to detect the production of the enzyme (oxidase) by the bacteria isolates. A piece of filter paper was soaked in a few drops of oxidase reagent (Tetramethyl- p-phenyl-diamine-dihydrochloride). A colony of the test organism was then smeared on the soaked filter paper. An oxidase producing organisms on the filter paper oxidized the phenyl-diamine in the reagent to deep purple colour. This change in colour to deep purple within 10 sec indicated a positive result, while the absent of deep purple colour with the time frame indicate a negative result (Olutiola, 2000).

d. Catalase Test

This test was used to demonstrate the presence of the enzyme (catalase) which catalyzes the release of oxygen from hydrogen peroxide. A smear of 24 h old subculture of each of the test organisms was placed on different clean grease free slides and each slide was flooded with 3 % hydrogen peroxide. The production of gas bubble from the surface indicated a positive result. The absent of gas bubble production from the surface indicated a negative result (Singleton, 1999; Olutiola., 2000).

e. Coagulase Test

This test was carried out to determine the enzyme (coagulase). A colony of the test organisms was emulsified with sterile normal saline solution on a clean slide using a sterile wire loop. A drop of human plasma was added and mixed gently. After about 10 sec the positive coagulase organisms showed clumping, while negative coagulase organisms showed no clumping (Olutiola, 2000).

f. Indole test

Tryptone broth (5 ml) was placed into different test tubes after which a loopful of the bacterial isolates was inoculated into the test tubes, leaving one of the test tubes uninoculated to serve as control. The test tubes were then incubated at 37 °C for 48 h. After incubation, 0.5 mL of Kovac's reagent was added and shaken gently; it was allowed to stand for 20 min to permit the reagent to rise. A red or red-violet colour at the top surface of the tube indicated a positive result, while yellow colouration indicated a negative result (Singleton, 1999; Olutiola, 2000)

g. Urease Test

This test was aimed at identifying organisms that produce urease enzyme which hydrolyze urea to give ammonia and carbon dioxide. The medium employed was urea agar base. Autoclaving was carried out on the urea medium and was allowed to cool to 50^{o}C and 5 ml of 40 % urea solution was added to the prepared medium. The different bacterial isolates were inoculated into different tubes containing the urease reagent and incubated at 37^{o}C for 24 h thereafter a pink colour in the medium showed a positive test. The absent of a pink colour in the medium indicated a negative test (Singleton, 1999; Olutiola., 2000).

h. Citrate Test

This test was based on the ability of an organism to use citrate as its source of carbon. Simon's citrate agar medium was prepared in a slant Bijou bottle, then using a sterile wire loop, a colony of the organism was inoculated onto the slant medium and incubated at 37°C for 48 h after which it was examined for colour formation. A bright blue color in the medium gave a positive citrate test (Olutiola, 2000).

i. Sugar Fermentation Test

This test is used to determine the ability of bacteria to utilize different sugars as its carbon source. The sugars ued for this test were glucose, maltose lactose and sucrose. The different fermentation media were prepared by the addition of 0.1 g of peptone, 0.1 g of sodium chloride and 0.1 g of fermentable sugar (glucose, maltose, sucrose and lactose) in 10 ml of distilled water. A measured 4 ml of the medium was pipetted into Bijou bottles containing Durham tubes after which 1 ml of phenol red indicator was added to the already sterilized tubes. The Bijou bottles containing the sugar solution were inoculated with the test bacterial isolates and incubated at 37^{o}C for 24 - 48 h. A change in colour from red to yellow showed acid production and the presence of gas in the inverted Durham tubes was indicative of gas production which was a positive test (Singleton, 1999; Olutiola, 2000).

3.6 Molecular identification of isolates.

3.6.1 Bacteria DNA extraction

DNA was extracted using standard protocol. Briefly, Single colonies grown on medium were transferred to 1.5 ml of liquid medium and cultures were grown on a shaker for 48 h at 28ºC. After this period, cultures were centrifuged at 4600 g for 5 min. The resulting pellets were resuspended in 520 µl of TE buffer (10 mMTris-HCl, 1mM EDTA, pH 8.0). Fifteen

microliters of 20 % Sodium dodecyl sulfate (SDS) and 3 µl of Proteinase K (20 mg/ml) were then added. The mixture was incubated for 1 hour at 37ºC, then 100 µl of 5 M NaCl and 80 µL of a 10% CTAB solution in 0.7 M NaCl were added and votexed. The suspension was incubated for 10 min at 65ºC and kept on ice for 15 min. An equal volume of chloroform: isoamyl alcohol (24:1) was added, followed by incubation on ice for 5 min and centrifugation at 7200 g for 20 min. The aqueous phase was then transferred to a new tube and isopropanol (1: 0.6) was added and DNA precipitated at –20 ºC for 16 h. The DNA was collected by centrifugation at 13000 g for 10 min, washed with 500 µl of 70% ethanol, air-dried at room temperature for approximately three hours and finally dissolved in 50 µl of TE buffer.

3.7 PCR Analysis

3.7.1 Bacteria PCR

Polymerase chain reaction (PCR) sequencing preparation cocktail consisted of 10 µl of 5x GoTaq colourless reaction, 3 µl of 25 mM $MgCl_2$, 1 µl of 10 mM of dNTPs mix, 1 µl of 10 pmol each 27F 5'- AGA GTT TGA TCM TGG CTC AG-3' and - 1525R, 5′-AAGGAGGTGATCCAGCC-3′ primers and 0.3units of Taq DNA polymerase (Promega, USA) made up to 42 µl with sterile distilled water and 8µl DNA template. PCR was carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA) with a Pcr profile consisting of an initial denaturation at 94°C for 5 min; followed by a 30 cycles consisting of 94°C for 30 s, 50°C for 60 s and 72°C for 90 secs ; and a final termination at 72°C for 10 mins. And chill at 4 °C GEL

3.7.2 Integrity

The integrity of the amplified DNA about 1.5 Mb gene fragment was checked on a 1% agarose gel ran to confirm amplification. The buffer (1XTAE buffer) was prepared and subsequently used to prepare 1.5% agarose gel. The suspension was boiled in a microwave for 5 mins. The molten agarose was allowed to cool to 60°C and stained with 3 µl of 0.5 g/ml

ethidium bromide (which absorbs invisible UV light and transmits the energy as visible orange light). A comb was inserted into the slots of the casting tray and the molten agarose was poured into the tray. The gel was allowed to solidify for 20 minutes to form the wells. The 1XTAE buffer was poured into the gel tank to barely submerge the gel. Two microliter (2 µl) of 10X blue gel loading dye (which gives colour and density to the samples to make it easy to load into the wells and monitor the progress of the gel) was added to 4 µl of each PCR product and loaded into the wells after the 100 bp DNA ladder was loaded into well 1. The gel was electrophoresed at 120 V for 45 minutes, visualized by ultraviolet transillumination and photographed. The sizes of the PCR products were estimated by comparison with the mobility of a 100 bp molecular weight ladder that was ran alongside experimental samples in the gel (refereces).

3.8 Purification of amplified product

After gel integrity, the amplified fragments were ethanol purified in order to remove the PCR reagents. Briefly, 7.6 µl of Na acetate(3 M) and 240 µl of 95% ethanol were added to each PCR amplified product (40 µl) in a new sterile 1.5 µl tube eppendorf, mixed thoroughly by vortexing and kept at 20°C for at least 30 mins. Centrifugation was for 10 min at 13000 g and 4°C followed by the removal of supernatant (invert tube on trash once) after which the pellet were washed by adding 150 µl of 70% ethanol, mixed and centrifuged for 15 min at 7500 g and 4°C. Again all supernatant were removed (invert tube on trash) and invert tube on paper tissue and was allowed to dry in the fume hood at room temperature for 1015 mins. It was resuspend with 20 µl of sterile distilled water and kept in 20ºC prior to sequencing. The purified fragment was checked on a 1.5% agarose gel ran on a voltage of 110 V for about 1 hr as previous, to confirm the presence of the purified product and was quantified using a nanodrop of model 2000 from thermo scientific (refereces).

3.8.1 Sequencing

The amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer from Applied Biosystems using manufacturers' manual, while the sequencing kit used was that of BigDye terminator v3.1 cycle sequencing kit. Bio Edit software and MEGA 6 were used for all genetic analysis.

3.9 Antimicrobial Susceptibility Testing of Bacterial Isolates

Antimicrobial susceptibility testing of isolates was carried out using Kirby-Bauer disc diffusion method and readings interpreted by adopting the breakpoints of Clinical and Laboratory Standard Institute (CLSI) (Bauer *et al.*, 1996; CLSI, 2017).

3.9.1 Antibiotic Susceptibility by Disc Diffusion Method

The antimicrobial susceptibility pattern of the strains was studied by the disc diffusion method (Bauer *et al*., 1996). To perform the disc diffusion test, each culture was grown in 5 ml of Muller-Hinton broth until the turbidity corresponded to 0.5 MacFarland standard tubes ($1.5x10^8$ cells ml^{-1}). The suspension was spread and inoculated using sterile cotton swab onto Muller-Hinton agar plate and various antibiotic discs were placed on it. After incubating the plates at 37°C under microaerophilic conditions for 48 h the zones of inhibition were recorded. Results were interpreted as susceptible (S) or intermediate (I) or resistant (R) according to standardized CLSI chart (CLSI, 2017). The antibiotic discs (OXOID) used were, Septrin (SXT) 30 µg, Sparfloxacin (SP) 10 µg, Ciprofloxacin (CPX) 10 µg, Amoxacillin (AM) 30 µg, Augmentin (AU) 30 µg, Pefloxacin (PEF)10 µg, Ofloxacin (10 µg), Streptomycin (ST) 10 µg, Gentamycin (CN) 10 µg, Rocephin(RX) 30 µg, Zinnacef (Z) 20 µg, Erythromycin (E) 19 µg, Ampicillin (25 µg), Chloraphenicol (CH) 30 µg and Cotrimoxazole (25 µg).

3.10 Plasmid Isolation

Five militres (5 ml) of bacterial culture was centrifuged at full speed for 15-20 sec.The supernatant was discarded and 200 µl of Pl buffer (red) was added to the tube and pellet was resuspended completely by vortexing. Buffer P2(green) of 200 µl was added and mixed by inverting the ube 2-3 times. Cells were completely lyzed when the solution appeared, purple and viscous. Buffer P3 (yellow) of 400 µl was added and mixed gently without vortexing. The sample turned yellow when neutralization was completed. The lysate was incubated at room temperature for 1-2 minutes. Sample was centrifuged for 2 min. and the supernatant was transferred into a zymo-spin IIN column in a collection tube. The zymo-spin IIN column/collection tube assembly was centrifuged for 30sec. The flow through in the collection tube was discarded and the zymo-spin IIN column returned to the collection tube, then 200 µl of Endo-wash buffer (p4) was added to the column and centrifuged for 30 sec after which, 200 µl of plasmid wash buffer was added to the column and centrifuged for 1min. The column was then transferred into a clean 1.5 ml microcentrifuge tube and then 300 µl of DNA eluting buffer was added to the column for 30 sec to elute the plasmid DNA. Plasmid DNA was analysed by electrophoresis through 0.8% agarose gel, visualized under UV transilluminator, and photographed and recorded using the Gel Documentation system (Model G:BOX, Syngene) (Reference).

3.10.1 Plasmid Profiling of the Isolates

Plasmid isolation was done using TENS- prep according to the manufacturer's instruction. agarose gel electrophoresis was carried out on the plasmid DNA at 90 V for 10 min and viewed under UV Trans-illumination for the band harbouring plasmid (Zhou *et al*. 1990 ; Ojo and Oso (2009)

Plasmid Curing of isolates

Isolates that showed resistance to any of the antibiotics were subjected to the curing experiment to determine if the gene responsible for such antibiotic resistance was resident on plasmid or not. The plasmid was cured by treatment with acridine orange. Nutrient broth was prepared and then supplemented with 0.1 mg/ml acridine orange. Twenty microlitre (20 µl) of overnight culture of the bacteria was subcultured into 5 ml of the nutrient broth containing acridine orange. The samples were then incubated at 37°C for 72 h. After incubation, the isolates were subcultured onto Muller Hinton agar and antibiotic sensitivity repeated to verify if the plasmids were successfully cured (Peter *et al.* 1989; Fortina and Silva, 1996).

3.11.0 Detection of antimicrobial resistant genes

Following antibiotics susceptibility testing, genes for resistance were assayed for in the isolates. Genes coding for resistance to different antibiotic classes were evaluated. Genes such as penicillin binding protein (*pbp* genes) for *Listeria* and *aph(2")-If* gene for *Vibrio* were evaluated. (Ferreira *et al.* 2014; Kwan and Bolch 2015)

3.11.1 Penicillin-Binding Protein Gene Amplification and Sequencing

The isolates were cultured on blood agar plates and incubated overnight at 35°C in a 5% CO_2 atmosphere. The DNA was extracted using the AxyGen amp DNA Mini Extraction Kit (Axygen, United States) according to the manufacturer's instructions. The final pure DNA was stored at −20°C until use. The nucleotide sequence of an around 1-kb region encoding the penicillin-binding domain of *pbp2b* gene was amplified using polymerase chain reaction (Chu *et al*., 2018).

3.11.2 Detection of *aph*(2")-If Resistance Genes in Bacteria Isolates.

The known gentamicin resistance genes, including *aph(2")-If* was detected in *bacteria* isolates by PCR method according to the PCR mixture composed of 12.5 µl of *Ex-Taq* (*TaKaRa*, Dalian, China), 0.5 µl of each primer, 0.5 µl of chromosomal DNA template prepared by boiling as described previously, and 11 µl of sterile distilled water. The PCR was conducted in a Veriti 96-well Thermal Cycle. (Yao *et al*., 2017).

Detection of virulence genes

Following DNA extraction, the presence of the virulence gene (Cholera toxin (CtXA)), in all *Vibrio* species was determined by polymerase chain reaction. The presence of the *hylA* gene was analyzed for, in *Listeria* species (Kwan and Bolch, 2015) ***hylA* gene**

The genomic DNA of all the isolates was extracted and was subjected to PCR amplification for detection of hlyA gene .The primers employed were, forward 5'-GCA GTT GCA AGC GCT TGG AGT GAA-3' and reverse 5'-GCA ACG TAT CCT CCA GAG TGA TCG-3' .The reaction was performed in Mastercycler epGradient (Eppendorf, Germany) with a preheated lid. The reaction mixture was subjected to an initial denaturation at 95ºC for 2 min followed by 35 cycles each of 15 sec denaturation at 95ºC, 30 s annealing at 60ºC and 1 min 30 sec extension at 72 ºC. It was followed by final extension of 10 mins at 72ºC and held at 4°C. Standard strain of *Listeria*, procured from IMTECH, Chandigarh was used as standard for PCR optimization. The isolates were serotyped using commercial *Listeria* antisera (Denka Seiken, Japan), in accordance with the manufacturer's instructions. Multiplex serotyping PCR was performed in a Master Cycler Gradient Thermocycler (Eppendorf, Germany). Pulsed field gel electrophoresis (PFGE) was performed according to the Pulse Net standardised protocol. Briefly, sample plugs were prepared by mixing 240 µl of a standardised cell suspension and 60 µl of a 10 mg ml^{-1} lysozyme solution (Sigma, St. Louis,

MO) Sample plugs were digested with 25 U of AscI (Fermentas, MY, USA) at 37°C for 3 h or 160 to 200 U of ApaI (Fermentas, MY, USA) at 30°C for 5 h. Plugs were then loaded on 1% agarose gel in 0.5x TBE (45 mM Tris, 45 mM borate, 1 mM EDTA) buffer and electrophoresed on a CHEF-DR II apparatus (Bio-Rad, USA). Gels were stained with ethidium bromide and visualised in a UV transilluminator (Grave and Swaminathan 20001 ; Doumith *et al*. 2004).

CTXA gene

The genomic DNA of all the isolates was extracted and was subjected to PCR amplification for detection of CTXA gene. The primers employed were 5′ ACA GAG TGA GTA CTT TGA CC 3′ 5′ ATA CCA TCC ATA TAT TTG GGA G 3′. For each PCR, 1 to 5 µl of sample DNA was added to a 20- to 24 µl master mix prepared for each target by using a TaqMaster kit from Eppendorf (Hamburg, Germany). All reaction mixtures included 0.2 mM (each) deoxynucleoside triphosphates, 1.25 µM (each) primers, 1× PCR buffer providing 1.5 mM $MgCl_2$, 0.5× TaqMaster additive, and 0.625 U of *Taq* polymerase. For each sample, at least three dilutions were tested (undiluted, 1:20, and 1:100) targeted to a ~300-bp region of the 16S-23S intergenic spacer region Two separate primer sets were used to target the *ctxA* gene of the CTX element and *ctxA* targets, where two primer sets were employed, when either set resulted in a positive signal the sample was considered positive for the respective target. Universal primers for the 16S rRNA gene were used as a control test for inhibition in all samples (Rivera *et al*. 2001; Singh *et al*., 2002)

3.12 Proximate analysis of bivalve (*Ergeria radiata)*

3.12.1 Determination of Moisture Content

The moisture content of the sample was determined according to the standard of AOAC (2016). The crucibles were washed and dried in an oven at 100 ºC for 1 hour. The

weight was noted as W1. Two (2) g of each sample was separately weighed into the crucibles and their weights were taken and noted down as (W_2) before and after drying at 100ºC to constant weight (W_3).

$$\text{Moisture content} = \frac{w_2 - w3}{w_1} x\ 100 = \frac{weight\ of\ moisture}{weight\ of\ sample} x\ 100 \quad \text{Equation 1}$$

Where

W_1 = weight of empty crucible

W_2 =weight of crucible and sample before drying

W_3= weight of sample after drying to a constant weight

3.12.2 Determination of Crude Fibre Content

Percentage of crude fibre was determined by the method of AOAC (2016), in which 2 g of ground sample was weighed (W_0) into a 1 dm^3 conical flask. Water (100 cm^3) and 100 cm^3 of 1.25 % H_2SO_4 were added and boiled gently for 30 min. The content was filtered through Whatmann No. 1 filter paper. The residue was scrapped back into the flask with a spatula and 100 cm^3 of 1.25% NaOH were added and allowed to boil gently for 30 mins. The content was filtered and the residue was washed thoroughly with hot distilled water, and then rinsed once with 1:1 ethanol and acetone to neutrality. It was allowed to dry and scrapped into the crucible and dried to constant weight at 105^0C in a hot oven. It was then removed and cooled in a desiccator. The sample was weighed (W_1) and ashed at 300ºC for 90 min in a Lenton muffle furnace. It was finally cooled in a dessicator and weighed again (W_2).

calculation

$$\% \text{ crude fibre} = \frac{w_1 - w_2}{w_0} x\ 100 \qquad \text{Equation 2}$$

Where

W_1 = weight of sample after oven drying

W_2 = weight of sample after ashing

W_0 = weight of sample

3.12.3 Determination of Crude Protein Content

The crude protein of the sample was determined using the micro – Kjeldahl method described by AOAC (12016). The sample (0.5 g) was weighed into a micro – Kjeldahl digestion flask of foss automatic digester block system. It was shaken and allowed to stand for sometime. One tablet of selenium catalyst with a mixture of 2:1 copper sulphate and potassium sulphate was added followed by the addition of 20 cm^3 concentrated sulphuric acid. The flask was heated on the digestion block at 450 ^oC for 1 h until the digest became clear. The flask was removed from the block and allowed to cool. The content was transferred into 100 cm^3 volumetric flask and diluted to the mark with water.An aliquot of the digest (10 cm^3) was transferred into another micro-Kjeldahl flask along with 20 cm^3 of distilled water, and placed in the distilling outlet of the micro – Kjeldahl distillation unit. A conical flask containing 20 cm^3 of boric acid indicator was placed under the condenser outlet. Sodium hydroxide solution (20 cm^3, 40 %) was added to the content in the Kjeldahl flask by opening the funnel stopcock. It was distilled and the heat supplied were regulated to avoid sucking back. When all the available distillate was collected in 5 cm^3 of boric acid mix indicator, the distillation was stopped. The nitrogen in the distillate was determined by titrating with 0.1N of HCl; the end

point was obtained when the colour of the distillate changed from green to pink. Crude protein is a measure of nitrogen in the sample. It was calculated by multiplying the total nitrogen content by a constant, 6.25. This is based on the assumption that, proteins contain about 16 % N which includes both true protein and non – protein N and does not make a distinction between available or unavailable protein (AOAC, 2016).

Calculation;

$$\% \text{ Crude Protein} = \frac{S \times N \times 0.014 \times D \times 100}{\text{Weight of sample} \times V} \times 6.25$$

Where

S = sample titration reading

N = Normality of HCl

D = dilution of sample after digestion

V = volume taken for distillation

0.014 = milliequivalent weight of Nitrogen

3.12.4 Determination of Fat Content

The fat content of the sample was determined using the standard AOAC (2016) method

Procedure:

The already dried bivalve (*Ergeria radiata*) was crushed to powder form to facilitate entry of organic solvent. A filter paper was weighed and recorded as W_1, The reading was taken to zero and 1 g of bivalve (*Ergeria radiata*) was weighed on filter paper (weight of filter paper and bivalve (*Ergeria radiata*) was recorded as W_2). The conical flask of the soxhlet extractor was half filled with petroleum ether. The bivalve (*Ergeria radiata*) (with the filter paper was transferred into the sample holder of the soxhlet extractor, the extractor was fitted with reflux condenser. The heat source was adjusted and was allowed to boil. It was allowed to siphon

round the barrel for over 5 hr (condensation rate of 5-6 drops per second).The condenser was detached and the filter paper containing the sample was removed. The filter paper containing the bivalve (*Ergeria radiata*) was dried in an oven at 105^0C for 1 – 2 hrs. It was dried till it attained a constant weight W_3.

Calculation

Fat (%) content = _

$$\frac{w_2 - w3}{w1} x\ 100$$

Equation 4

Where

W_1 = weight of empty extracting flask

W_2= weight of flask and extracted oil W3= costant weight of dried sample

3.12.5 Determination of Ash Content

The crucibles were pre dried, weighed and recorded (2 for each sample) as W1 the crucible was put on the weigh and was put on zero . Then 1g of sample was into each crucible as W2, the weight of crucible and sample was recorded. It was kept in a muffle furnace and left for 3 hours. It was weighed and kept back in the oven and then reweighed till it attained a constant weight W3.

Percentage ash was then calculated aand average taken.

Calculation:

$$\text{Ash content} = \frac{W_3 - W_1}{W_2 - W_1} \times 100$$

Equation 5

Where W_1= weight of empty crucible

W_2= weight of crucible + weight of sample

W_3= weight of crucible + weight of sample after ashing

3.12.6 Dry Matter Determination

% dry matter = 100 - % moisture content

3.12.7 Determination of Carbohydrates

The carbohydrate content was determined by AOAC (2016) method as follows:

Total carbohydrate contents = 100 - (% moisture +% ash + % protein + % fibre + % fat).

3.12.8 Determination of Mineral Content and Heavy Metal

The different mineral element Na and K were determined using Jenway Digital Flame Photometer (PFP 7 Model), while Ca and heavy metals such as Fe, Zn, Cu, Mg, Pb, Hg and As were analyzed using Atomic Absorption Spectrophometer (ASS) according to the methods of AOAC (2016)

3.12.9 Wet Digestion of Sample

For wet digestion of sample, exactly (1.00 g) of the powdered bivalve (clam) was taken in digesting glass tube. Twelve millilitres (12 ml) of HNO_3 was added to the bivalve (clam) and the mixture was kept for overnight at room temperature. Then 4.0 ml perchloric acid (HClO) was added to this mixture and was kept in the fumes block for digestion. The temperature was increased gradually, starting from 50ºC and increasing up to 250-300ºC. The digestion was completed in about 70-85 min as indicated by the appearance of white fumes. The mixture was left to cool and the contents of the tubes were transferred to 100 ml volumetric flasks The volumes of the contents were made to 100 ml with distilled water. The wet digested solution was transferred to plastic bottles labeled accurately. The digest was stored for mineral (Na , K) determination.

3.12.10 Determination of Sodium (Na) and Potassium (K) by flame photometer

Principle:

This was conducted to determine the concentration of potassium (K) and sodium (Na) in a sample using flame photometry. In this case, the standard solutions of potassium (KCl) and sodium (NaCl) were prepared with concentrations ranging from 0.1 to 10 ppm. The sample solutions were prepared according to specific requirements and diluted with distilled water to bring the concentration within the range of the working standard solutions (APHA, 2017). The flame photometer was set up according to the manufacturer's instructions, and the K or Na filter was selected depending on the element being analyzed. The working standard solutions were aspirated into the flame photometer, and the emission intensity readings were recorded. A calibration curve of emission intensity vs. concentration was plotted for K and Na (Skoog *et al*., 2017). The sample solutions were then aspirated into the flame photometer, and the emission intensity readings were recorded. The concentration of K and Na in the sample solutions was determined using the calibration curve. The results showed that the concentration of K and Na in the sample solution was [K] ppm and [Na] ppm, respectively. The precision and accuracy of the flame photometry method were evaluated by analyzing replicate samples and standard solutions (Miller and Miller, 2010, Dean, 2015).

3.13 Heavy Metal and Macronutrient (Ca and Mg) Determination

Ashed sample of 0.1 g was weighed into a clean borosilicate 250 ml capacity beaker for digestion. Nitric acid of 20 ml was added to the weighed sample in the beaker. The sample with the digesting solvent was placed on the hot plate for digestion in the fume cupboard. The beaker and its contents after the digestion were allowed to cool. Another 20 ml of the digesting solvent was added and digested further in the fume cupboard and the mixture was allowed to cool to room temperature. The mixture was filtered into the 250 ml volumetric

container. The filtrate was made up to the mark with deionised water. The digested samples were sub-sampled into pre-cleaned borosilicate glass containers and then analyzed for its elemental composition using UNICAM 929 London atomic absorption spectrophotometer powered by the SOLAAR software (AOAC, 2016).

3.14 Determination of Polycyclic Aromatic Hydrocarbon (PAH)

3.14.1 Soxhlet extraction and Clean-up ofbivalve (*Egeria radiata*) samples

Prior to extraction, an aliquot of 10 g of blended clam was weighed using a Whattman filter paper on a weighing balance and transferred into a semi-permeable membrane material and placed inside the Soxhlet extraction thimble for 3 h via a soxhlet heating mantle. Extraction was carried out with dichloromethane (200 ml) in a cold extraction mode at temperature of 60 ºC. The extracted solvent was evaporated using a rotary evaporator (name of manufacturer and model) under reduced pressure of 40 ºC (Hong *et al*., 2006; Adeyemi *et al*., 2008; Ukwo *et al*., 2022). Clean-up was carried out using a clean grease free beaker and a separating glass tube. Seven grams (7 g) analytical grade silica gel (160-200 mesh size) was packed unto a clean cotton wool with additional 1 cm of anhydrous sodium sulphate (Na_2SO_4) was added to the column to absorb water Column chromatographic method (15 cm x 9 mm I.D film thickness) was used to elute solvents. The eluents were allowed to air-dry and 0.5 ml of dichloromethane was used to aid transfer into eppendorf tubes (Ize-Iyamu *et al*., 2007).

3.14.2 Preparation of Calibration Standards

Five standard solutions each containing 16 target compounds were prepared by diluting the standard mix (1647 mix from NIST) to desired concentrations with dichloromethane (Guillen *et al*., 1997). Quantification of polycyclic aromatic hydrocarbon (PAH) was made on a Gas Chromatograph (Hewlett-Packard 5890 Series 11) tandem Mass spectrometer. (GC/MS) One µL of sample solution was injected in the pulsed splitless mode onto a 330 m and 0.322 mm

i.d. fused capillary column with a film thickness of 0.25 µm. Helium gas was used a carrier gas. An injection temperature was set at 300^{o}C. The colunm temperature was initially 80^{o}C for 60 sec. and ramped to 320^{o}C at a rate of 20^{o}C /min and then 320^{o}C was held for 20 min. Identification of organic pollutants in the samples was based on comparison of the retention time with those in a standard solution and quantification on the corresponding areas of the respective chromatograms. Procedural blanks were also analyzed and quantified. Mass spectrometer mode was used and all spectra were acquired using a mass range of m/z 50–400 and automatic gain control (AGC) (FEPA, 1991; Adeyemi *et al.,* 2008).

3.15 Physicochemical analysis of the Coastal water, habitat of bivalve (*Ergeria radiata*)

The physicochemical properties of Coastal water samples were determined using standard methods for pH, Total suspended solid (TDS), phosphate (PO_4), nitriate (NO_3). electrical conductivity (EC), alkalinity and total hardness. (Jha *et al*., 2015; A.O.A.C. (2016).

1. Hydrogen Ion Concentration (pH)

This was carried out according to the method described by AOAC (2016). In particular, 20 ml of the water sample was measured using 50 ml capacity measuring cylinder and transferred to a 100 ml beaker. After calibration of the pH meter with pH buffers of 4.0 and 7.0, the pH electrode was dipped into the sample in a beaker and the value was recorded. The measurement was made at ambient temperature. Also the pH metre (model 300408, Denver instrument company, Bohemia New York, USA) was calibrated using millesimal buffers of pH 4.0 and 7.0 before taking measurement.

2. Total Dissolved Solids (mg/l)

This was determined using a HACH CO150 TDS/conductivity/salinity meter. The samples were shaken thoroughly following which the probe of the equipment was dipped in the water sample. The displayed readings were then recorded. It was determined using multiparameter analyzer (Hach model C0150) (Jha *et al.,*2015; AOA.C, 2016).

3. Electrical Conductivity (µS/cm)

This was determined with a HACH CO150 TDS/Conductivity/Salinity meter. The probe was then dipped in the sample after mixing and the values displayed were recorded (Jha *et al.,* 2015; AOA.C 2016).

4. Total Alkalinity (mg/l)

This was estimated by titrimetry using methyl orange as indicator and 0.02 N H_2SO_4 as titrant. Twenty (20) ml of the sample was measured followed by the addition of 3 drops of methyl orange. This resulting pale yellow solution was then titrated to a light pink coloured end point (jha *et al.,* 2015; AOA.C 2016).

5. Total Hardness (mg/l)

One (1) ml of ammonium buffer solution was added to 50 ml of the sample followed immediately by the addition of a pinch of ManVer 2 hardness indicator. The resulting mixture was titrated against 0.8 M EDTA solution till a blue coloured end point was observed.

Calculated total hardness values were obtained by multiplying the number of digits on the digital titrator with the digit multiplier (2.0) (Jha *et al.,* 2015; AOA.C 2016).

5. Water temperature

Surface water temperature was measured by dipping a standard mercury in-glass thermometer half way into the water for 2 minutes and then reading off the level of mercury

in situ. This was done twice backing the sun to make for fairly accurate reading and the average of the two reading was recorded Jha *et al.,* 2015; AOA.C 2016).

6. Dissolved Oxygen (DO) (mg/L)

Azide modification of Winkler method was used to determine dissolved oxygen content of the water samples. The fixed samples were dissolved in the laboratory by adding 2 ml of concentrated sulphuric acid (H_2SO_4). Fifty (50) ml of this solution was then poured in a conical flask and titrated with 0.2 N sodium thiosulphate until a pale yellow colour was observed. Three (3) drops of 1% aqueous starch solution indicator was then added to the sample and the resulting blue-black colour was finally titrated till a colourless end point was reached. Dissolved oxygen was calculated as follows;

DO (mg/l) = number of digits on digital titrator x digit multiplier (0.04)

7. Biochemical Oxygen Demand (BOD) (mg/l)

The water sample was incubated for 5 days in the dark, using the bottle incubation method. The reduction in dissolved oxygen concentration during the incubation period yielded a measure of the biological oxygen demand. BOD = D1 – D5, where D1 = initial DO of the sample, D5 = final DO of the sample after 5 days incubation (AOAC, 2016).

8. Turbidity (NTU)

This was done using a HACH DR 2000 spectrophotometer. The sample was homogenized thoroughly. Twenty five (25) ml was poured into a cuvette and placed in the spectrophotometer. The turbidity values was then read at a wavelength of 450 nm and recorded (Jha *et al.,* 2015; AOA.C 2016).

9. Chloride content

The method applied was the Argentometric method. Reagents used were potassium chromate indicator solution, standard silver nitrate ($AgNO_3$) Titrant, 0.0141 N, and standard Sodium chloride. 0.014 N, sodium hydroxide, NaOH (1 N), standard NaCl solution (1000 mg/l), Aluminum hydroxide suspension. By diluting the 70 ml volume of the stock chloride standard to a volume in a 100 ml flask, 100 ml of the standard was taken; 1 ml K_2CrO_4 indicator was added and titrated against standard $AgNO_3$ to a pinkish-yellow end point, a calibration standard covering the range of 100 ppm to 1000 ppm was prepared. By titrating distilled water as described above, a reagent blank value was established. The chloride concentrations in the various standards was estimated as follows:

$$\text{MgCl/L} = \frac{(A-B)\ X\ N\ X\ 354.50}{ml\ of\ sample} \qquad \text{Equation 6}$$

Where, A = Volume of 0.0141 N $AgNO_3$ used for titration.

B = Volume of $AgNO_3$ used for blank titration

N = Normality of $AgNO_3$.

A graph was plotted using the calibrated values against the corresponding concentration. A regression equation was obtained. Sample of 100 ml was taken, 1 ml K_2CrO_4 indicator solution was added and titration was done with $AgNO_3$ to a pinkish end point. As in the standard above, the chloride concentration will be obtained by inserting this value in the regression equation

10 Sulphate SO_4^{2-} (mg/l)

Determination of sulphate was done using a HACH DR 2000 spectrophotometer. Twenty five (25) ml of sample was measured into acid washed glass beakers after rinsing thoroughly with water. The contents of SulfaVer 4 powder pillow was added to the measured sample, swirled

to dissolve and allowed to stand undisturbed for 5 minutes. Another 25 ml of sample was measured and poured into a cuvette and used to zero the spectrophotometer. The earlier sample was then poured into a separate cuvette and read at a wavelength of 450 nm (Jha *et al.,* 2015; AOA.C 2016).

11 Phosphate PO_4^{3-} (mg/l)

This was determined using a HACH DR 2000 spectrophotometer. Twenty five (25) ml of sample was measured into acid washed glass beakers after rinsing thoroughly with water. The contents of PhosVer 3 phosphate powder pillow was added to the measured sample and allowed to stand for 2 minutes, for full colour development. Another 25 ml of the sample was measured and poured into a cuvette and used to zero the equipment. The developed sample was thereafter transferred into a cuvette and read at a wavelength of 890 nm (jha *et al.,* 2015; AOA.C 2016).

12 Nitrate (mg/l)

Nitrate content was determined by measuring ten (10) ml of the sample into a test tube, followed by the addition of 2 ml NaCl solution, this mixture was swirled and 10 ml of H_2SO_4 solution was also added. The resultant solution was swirled and allowed to stand. A sample blank was also prepared. To the first test tube containing the mixture of the sample, NaCl and H_2SO_4, 0.5 ml of brucine –sulphanilic acid reagent was added and the test tube was swirled and left to stand for about 20 minutes. The test tubes were allowed to develop colour and the absorbance reading of the solution was taken using a HACH DR 2000 spectrophotometer at a specified wave length (Ademoroti, 1996; Jha *et al.,* 2015; AOA.C, 2016).

3.17 Statistical Analysis

The data obtained were statistically analyzed using one way analysis of variance (ANOVA) using SPSS version 20.0. Duncan multiple range test was used to separate the means at $p<0.05$ significant differences (Ogbeibu, 2014).

CHAPTER FOUR

4.1 RESULTS

The microbiological analyses of bivale (*Ergeria radiata*) was obtained from three locations; namely, Coastal water, Swali market and opolo market. A total of three hundred samples were analysed for the isolation of *Listeria* species, *Vibrio* species and total heterotrophic bacterial counts fo rboth wet and dry season.

Table 4.1 shows the bacteriological load (×105 cfu/g) of bivalves (Ergeria radiata) during the wet and dry seasons. In the range of 10.88±1.09 cfu/g to 30.48±1.62 cfu/g, the total heterotrophic bacteria of bivalves (Ergeria radiata) were randomly selected from two different markets: Swali market had the highest for the wet season, followed by Opolo market, and the lowest was obtained from Coastal water. Swali market had the most during the dry season, followed by Opolo market, Coastal water had the least.

Total *Vibrio* count of bivalves (*Ergeria radiata*) for wet and dry season was in the range 9.96±0.95 cfu/g -19.90±1.27 cfu/g, but for wet season Swali market had the highest, followed by Opolo market and the least was obtained from Coastal water . During dry season the highest was also obtained from Swal marketi, followed by Opolo and the least was obtained from Coastal water.

Total *Listeria* count of bivalves (*Ergeria radiata*) for wet and dry season was in the range 6.12±0.67 cfu/g – 11.52±0.76 cfu/g. The highest for wet season was obtained from Swali market, followed by Opolo market, while Coastal water the least.

Table 4. 1: Bacteriological load ($\times 10^5$ cfu/g) of bivalves (*Ergeria radiata*) for wet and dry seasons

Seasons	Locations	Number of samples	Media/Organism		
			NA (THBC)	TCBS (*Vibrio*)	PALCAM (*Listeria*)
Wet	Coastal water	50	20.48 ± 1.62^{b}	16.82 ± 1.17^{c}	8.46 ± 0.50^{b}
	Swali market	50	30.06 ± 1.63^{c}	19.90 ± 1.27^{c}	11.52 ± 0.76^{c}
	Opolo market	50	27.08 ± 1.75^{c}	16.94 ± 1.45^{c}	8.08 ± 0.73^{ab}
Dry	Coastal water	50	10.88 ± 1.09^{a}	9.96 ± 0.95^{a}	6.12 ± 0.67^{a}
	Swali market	50	18.18 ± 1.10^{b}	13.58 ± 1.12^{b}	9.72 ± 0.81^{bc}
	Opolo market	50	16.64 ± 1.54^{b}	10.18 ± 0.69^{a}	9.14 ± 0.94^{b}

Note: Similar superscript within column indicates that values are not significantly different ($p > 0.05$)

KEY: NA: Nutrient Agar, THBC: Total heterotrophic bacteria count

TCBS: Thiosulphate citrate bile salt sucrose agar (total *Vibrio* count)

PALCAM**:** Polymyxin-Acriflavine-Lithium Chloride-Ceftazidime-Aesculin-Mannitol Agar

The percentage frequency of *Vibrio* and *Listeria* isolate during the wet and dry season is presented in Figure 4.1 *Vibrio parahaemolyticus* had the highest (17%) occurrence during wet season, and 6% occurrence during dry season. *Vibrio* Cholerae had 13% and 3 % occurrence during wet and dry season respectively. *Vibrio vulnificus* had 10% and 5%, occurrence during wet and dry season respectively and *Vibrio fluvialis* had 5% and 4% during wet and dry seasons respectively. Tthe *Listeria* species, *Listeria monocytogenes* had the highest (t13%) of occurrence during wet season and 2%, occurrence during dry season, while *Listeria innoccua* and *Listeria ivanovii* had 6% and10% respectively during wet season only.

The percentage frequency of heterothrophic bacterial isolates during the wet and dry season is presented in Figure 4.2. Among the heterotrophic bacterial counts, *Escherichia coli* had the highest percentage occurrence(18) during wet season, followed by *Pseudomonas aeruginosa* (10%) during dry season and both isolates had (12% and 4%) of occurrence respectively. *Serretia marcescens* and *Shigella flexneri* had 10% and 12% of occurrence respectively during wet season only, while *Bacillus cereus* and *Salmonella enterica* had 3% and 2% of occurrence respectively during dry season only.

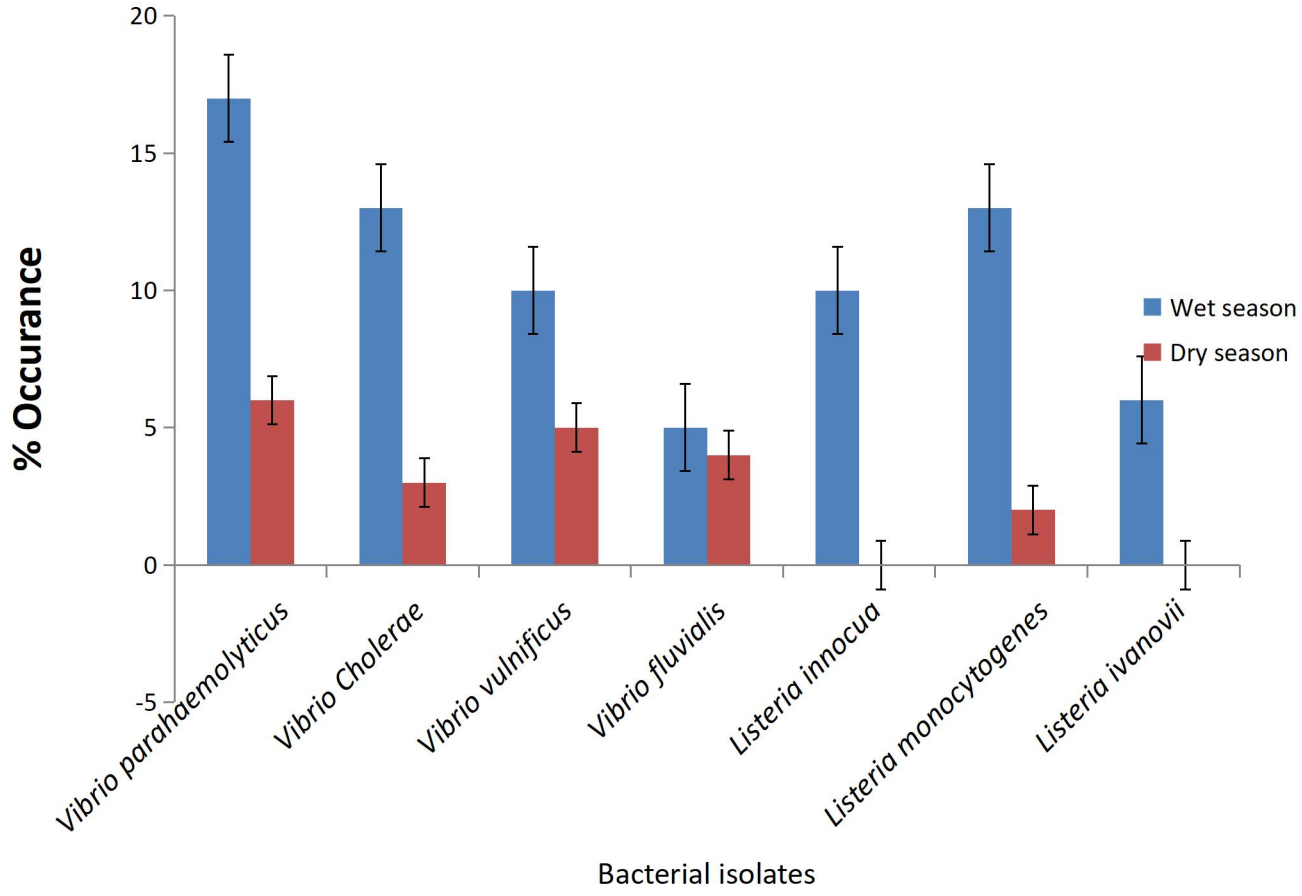

Figure 4.1: Percentage frequency of *Vibrio* and *Listeria* isolates during the wet and dry season

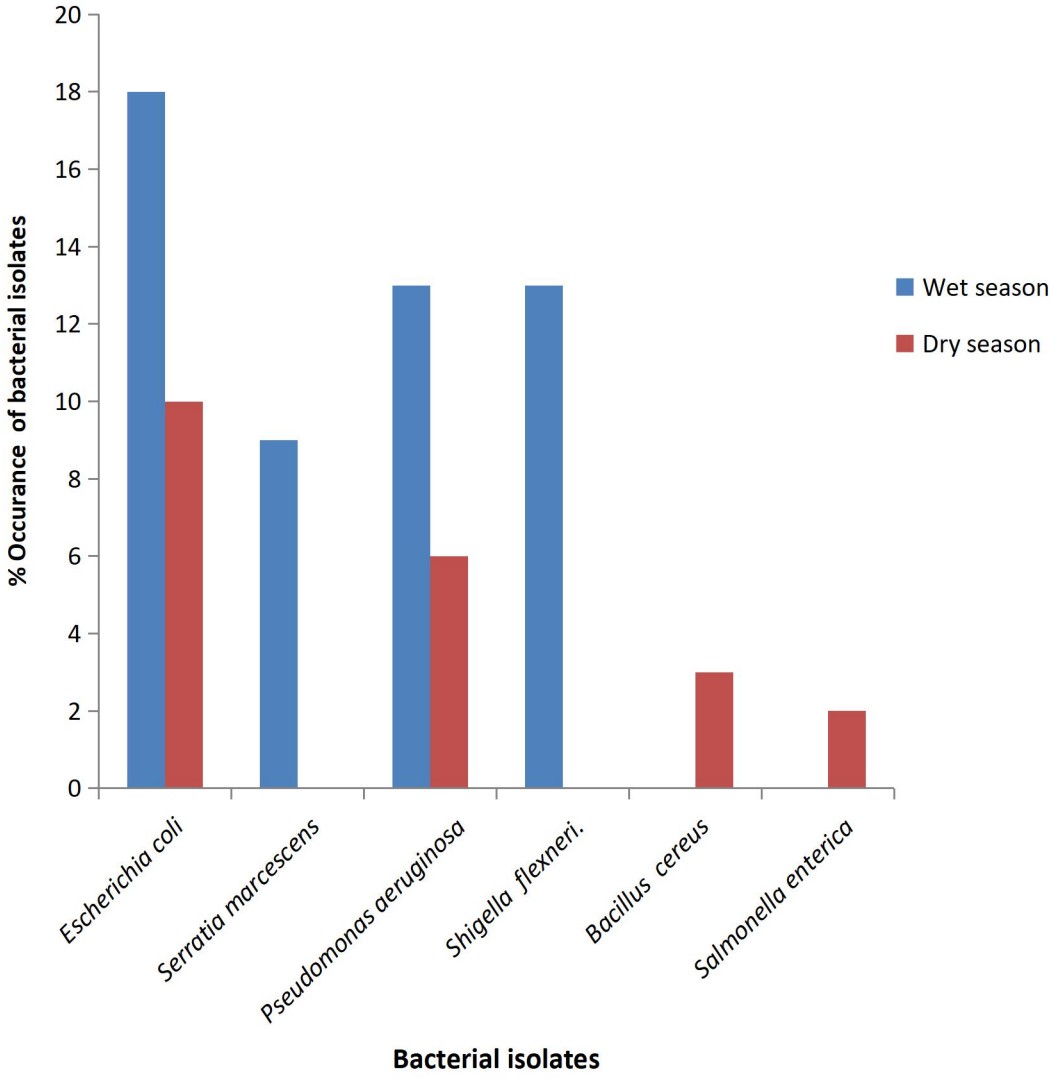

Figure 4.2: Percentage frequency of heterotrophic bacterial isolates during the wet and dry seasons

Agarose gel electrophoresis of the PCR products of 16S rRNA gene amplified from selected *Vibrio* and *Listeria* isolates for wet season is presented in Plate 4.1

The *Vibrio* species identified is presented in Table 4.2. It showed their accession numbers as well as thir percentage identity, the species were, *Vibrio parahaemolyticus*, *Vibrio cholerae*, *Vibrio vulnificus*, and *Vibrio fluvialis* with the accession numbers PP832852, PP382853, PP832854 and PP832855, with percentage identityof 100% and 99% respectively. *Listeria* species isolate identified with accession numbers were, *Listeria innocua* PP832856, *Listeria monocytogenes* PP832857, and *Listeria ivanovii* PP328560.

Molecular characterization of *Vibrio* and *Listeria* isolates for dry is presented in Table 4.3 The *Listeria* and *Vibrio* isolates identified with their accession numbers alongside their sample code were BV1- *Listeria monocytogenes* , BV3- *Vibrio parahaemolyticus*, BV4- *Vibrio cholera,* BV5 –*Vibrio vulnificus,* BV6- *Listeria momocytogenes,* BV8-*Vibrio parahaemolyticus* – BV9- *Listeria monocytoenes* and BV15 –*Vibrio cholerae* with theses accession numbers respectively, PP832845, PP832862, PP832846, PP832847, PP832848, PP832849, PP832850 and PP832851. The percentage identity of the isolates was within the range of 98% and 99%.

Phylogenetic tree of *Vibrio* and *Listeria* isolates for wet and dry seasons is presented in Figure 4.3. From the phylogenetic tree *Vibrio cholerae* is closely related to *Vibrio mimicus*, also *Listeria innocua* was closely related to *Listeria aquuatica, Vibrio vulnificus* was closely related to *Vibrio harveyi*. Also *Vibrio fluvialis, Listeria acquactica* was related to *Listeria ivanovii,* the *Vibrio parahaemolyticus* identified are closely related.

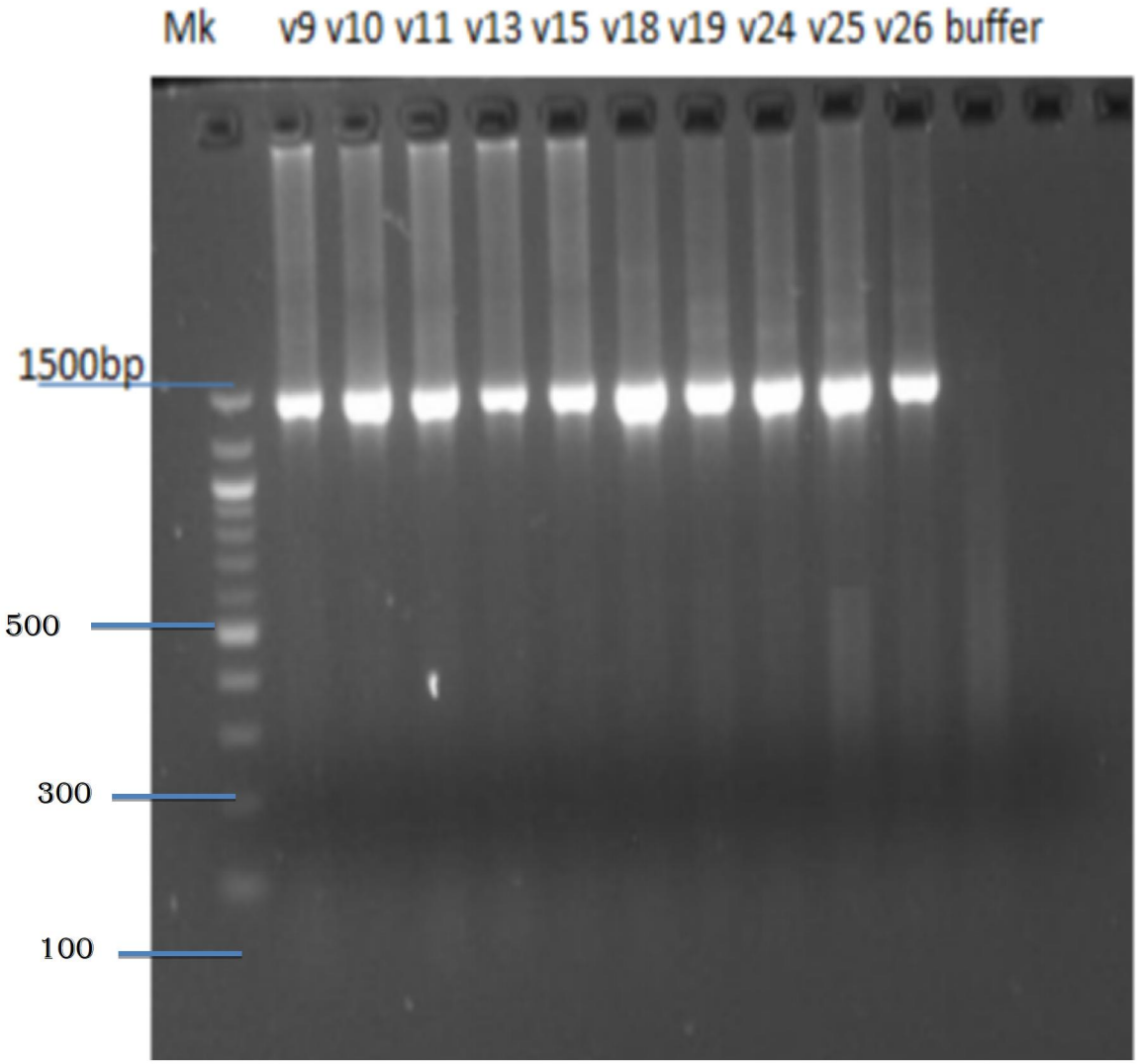

Plate 4.1: Agarose gel electrophoresis of the PCR products of 16S rRNA gene amplified from selected *Vibrio* and *Listeria* isolates for wet season.

Key;

MK: molecular weight marker.

V9 – *Vibrio parahaemolyticus* (1)
V10 - *Vibrio cholera*
V11 – *Vibrio vulnificus*
V13 - *Vibrio parahaemolyticus,*(2*)*
V15 – *Vibrio fluvialis*,V18 – *Listeria innocua*, V19- *Listeria monocytogenes*,(1) V24 - *Listeria monocytogenes*,(2), V25 – *Listeria monocytogenes(3)* V26 - *Listeria ivanovii*

Table 4. 2: Molecular characterization of *Vibrio*and *Listeria* isolates for wet season

sample ID	Scientific Name	Max Score	Total Score	Query Cover (%)	E value	Identity (%)	Accession number
V9	*Vibrio parahaemolyticus*	2656	2656	99	0	99.66	PP832852
V13	*Vibrio parahaemolyticus*	2684	2796	100	0	99.93	PP832861
V10	*Vibrio cholerae*	2623	2623	99	0	100.00	PP832853
V11	*Vibrio vulnificus*	2663	26593	99	0	99.93	PP832854
V15	*Vibrio fluvialis*	2523	2523	99	0	99.85	PP832854
V18	*Listeria innocua*	2593	15551	100	0	100.00	PP832856
V19	*Listeria monocytogenes*	2429	14541	99	0	99.92	PP832857
V24	*Listeria monocytogenes*	2593	2593	99	0	99.93	PP832858
V25	*Listeria monocytogenes*	2595	2595	100	0	99.10	PP832859
V26	*Listeria ivanovii*	2383	2383	99	0	99.85	PP832860

Agarose gel electrophoresis of the PCR products of 16S rRNA gene amplified from selected *Vibrio* and *Listeria* isolates for dry season is presented in Plate 4.2

Antibiotic susceptibility test of *Listeria* isolates for wet season before and after plasmid curing is presented in Table 4.4, Before curing some of the isolates such as *Listeria monocytogenes* showed resistance to the following antibiotics perfloxacin(PEF), gentamycin (CN), ampiclox (APX), amooxillin (AM) with multidrug antibiotics resistane index (MARI) of 0.8 but after curing one of the *Listeria monocytogenes*1 isolate still showed resitance to all the test. However, the other two isolates of *Listeria monocytogenes* showed sensitivity to perfloxacin (PEF) and gentamycin (CN) leading to reduction in the multidrug antibiotics resistance index (MARI) of 0.5, 0.2 and 0.4 after curing.

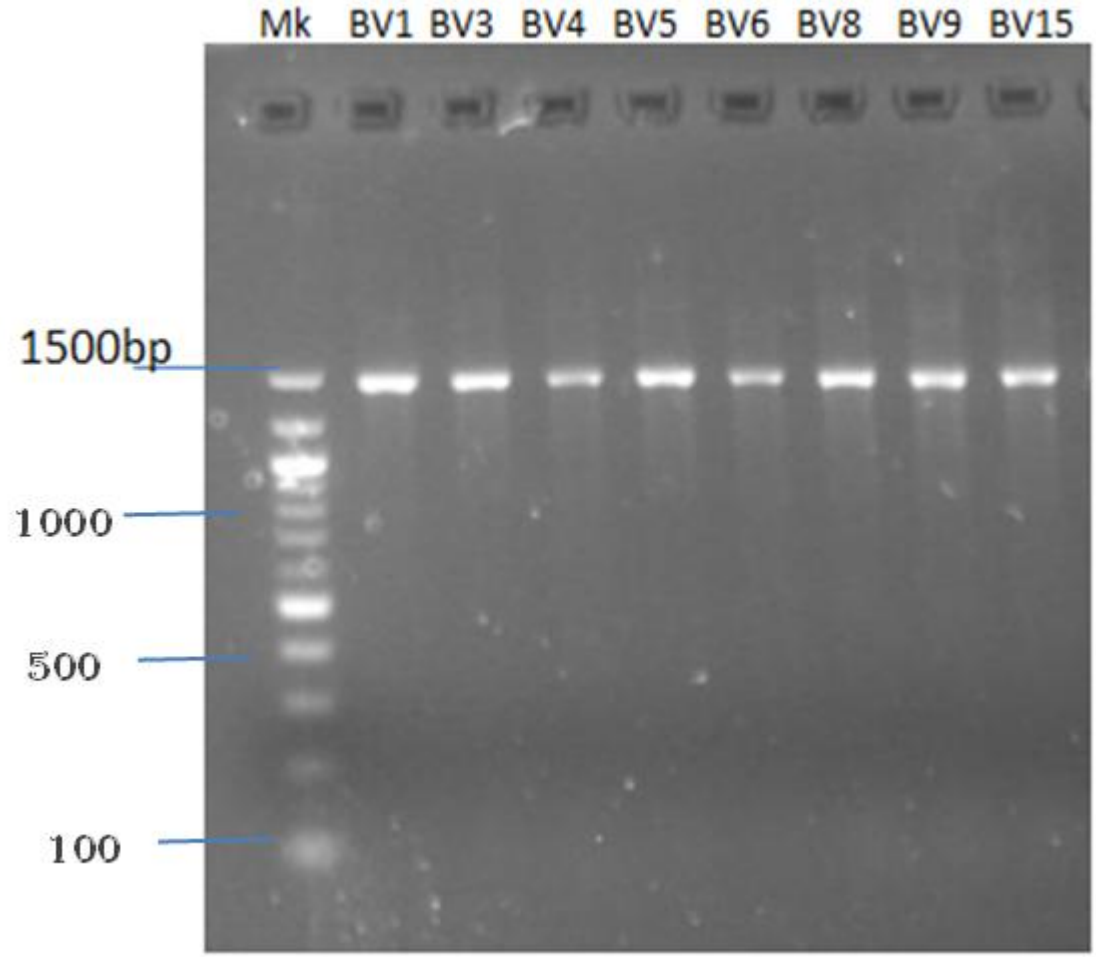

Plate 4.2: Agarose gel electrophoresis of the PCR products of 16S rRNA gene amplified from selected *Vibrio* and *Listeria* isolates for dry season.

Keys;

MK: molecular weight marker.

BV1 – *Listeria monocytogenes* (1)

BV3 – *Vibrio parahaemolyticus* (1)

BV4 – *Vibrio cholerae* (1)

BV5- *Vibrio vulnificus*

BV6 -*Listeria monocytogenes* (2), BV8 – *Vibrio parahaemolyticus (2),* BV9- - *Listeria monocytogene*s (3), BV15 – V*ibrio cholerae* (2)

Table 4. 3: Molecular characterization of *Listeria* and *Vibrio* isolates for dry season

Sample ID	Scientific Name	Max Score	Total Score	Query Cover (%)	E value	Identity (%)	Accession number
BV1	*Listeria monocytogenes*	2394	14331	99	0	99.47	PP832845
BV3	*Vibrio parahaemolyticus*	2663	2759	99	0	99.73	PP832862
BV4	*Vibrio cholerae*	2590	2590	99	0	99.72	PP832846
BV5	*Vibrio vulnificus*	2591	2591	99	0	99.72	PP832847
BV6	*Listeria monocytogenes*	2579	2579	99	0	99.03	PP832848
BV8	*Vibrio parahaemolyticus*	2591	2591	99	0	98.96	PP832849
BV9	*Listeria monocytogenes*	2575	2575	99	0	99.79	PP832850
BV15	*Vibrio cholerae*	2601	2601	99	0	99.72	PP832851

Antibiotic susceptibility profile of *Listeria* isolates for wetseason before and after plasmid curing is presented in Table 4.4. Before curing the isolates , *Listeria monocytogenes* 1 showed resistant to perfloxacin (PEF) while the others where susceptible. After curing the isolates remained the same resistant factor to the antibiotics perfloxacin (PEF) while others showed sensitivity. Also before curing the isolates showed resistant to septrin (SXT) after curing the isolates still showed resistant to it. The *Listeria* isolates showed resistant and sensitivity to some of the antibiotics before and after plasmid curing with was a reduction in the multidrug antibiotics resistance index (MARI) after curing.

Antibiotic susceptibility isolatthees of *Vibrio* isolates for wet season before and after plasmid curing is presented in Table 4.5. Before plasmid curing, all the *Vibrio* isolates showed resistant to septin (SXT) with MARI of 0.7, 0.8 and 0.7. After plasmid curing the isolates showed sensitivity to Septrin (SXT) with a reduction in MARI of 0.3, 0.3 and 0.4. But all the isolates showed resistant to chloramphenicol (CH) before and after plasmid curing all the *Vibrio* isolates showed sensitivity to oflaxacin (OFX) before and after plasmid curing.

Antibiotic susceptibility profile of *listeria* isolates for dry season before and after plasmid curing is presented in Table 4.6. Before curing, the isolates, *Listeria monocytogenes* 1 showed intermediate to perfloxacin (PEF), while the others showed sensitivity. After curing the isolates remained the same intermediate and sensitivity to the antibiotics perfloxacin (PEF). Also, before curing the isolates showed resistant to septrin (SXT) after curing the isolates still showed resistant to test antibiotics. The *Listeria* isolates showed resistant and sensitivity to some of the antibiotics before and after plasmid curing and there was a reduction in the mulidrug antibiotics resistance index (MARI) after curing

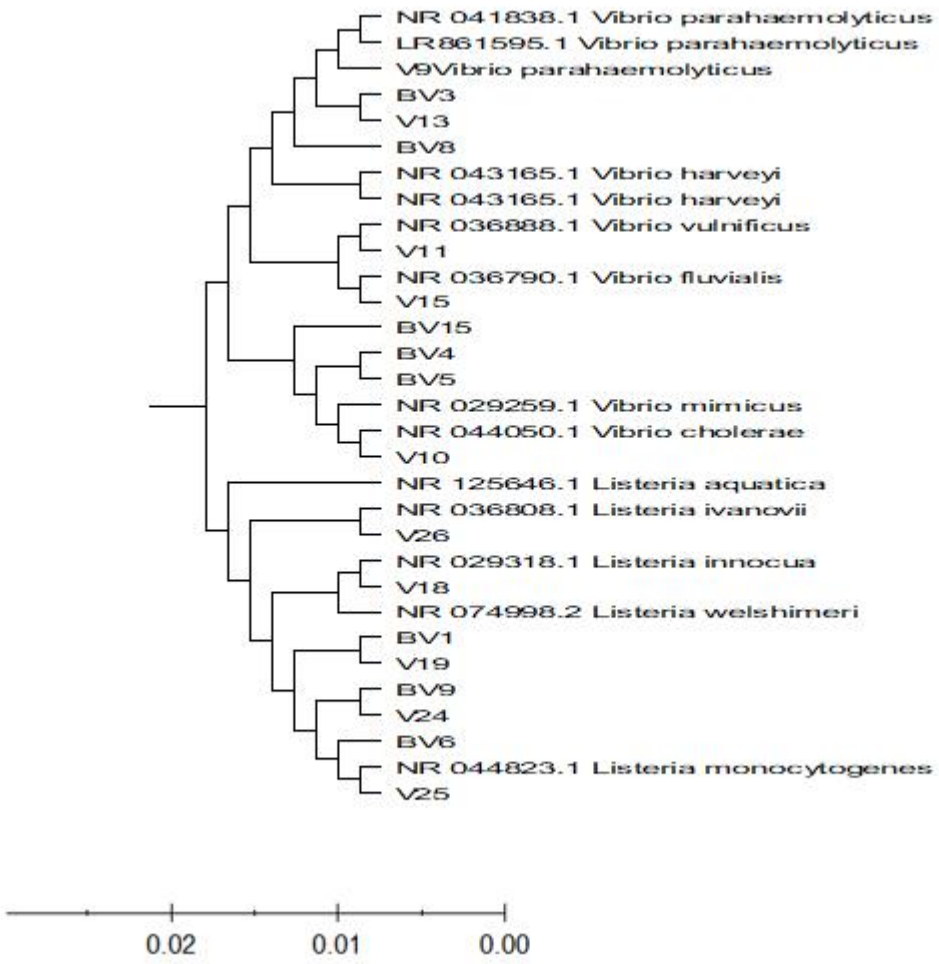

Figure 4. 3: Phylogenetic tree of *Vibrio* and *Listeria* isolates for wet and dry seasons

TABLE 4.4: Antibiotic susceptibility test of *Listeria* isolates for wet season before and after plasmid curing

Before Curing											
Isolates	PEF	CN	APX	Z	AM	R	CPX	S	SXT	E	MARI
Listeria monocytogenes (1)	8(R)	6(R)	0(R)	0(R)	6(R)	20(S)	17(S)	10(R)	8(R)	6(R)	0.8
Listeria monocytogenes (2)	4(R)	0(R)	6(R)	3(R)	0(R)	8(R)	17(S)	6(R)	10(R)	20(S)	0.8
Listeria monocytogenes (3)	4(R)	0(R)	4(R)	0(R)	0(R)	6(R)	18(S)	17(S)	2(R)	2(R)	0.8
Listeria ivanovii	0(R)	0(R)	5(R)	4(R)	5(R)	10(R)	20(S)	5(R)	4(R)	10(R)	0.9
Listeria innocua	4(R)	2(R)	0(R)	0(R)	5(R)	2(R)	14(I)	5(R)	4(R)	10(R)	0.9
After Curing											
Isolate	PEF	CN	APX	Z	AM	R	CPX	S	SXT	E	MARI
Listeria monocytogenes (1)	7(R)	5(R)	0(R)	17(S)	0(R)	20(S)	20(S)	9(R)	18(S)	17(S)	0.5
Listeria monocytogenes (2)	17(S)	17(S)	0(R)	17(S)	17(S)	17(S)	20(S)	17(S)	9(R)	18(S)	0.2
Listeria monocytogenes (3)	17(S)	18(S)	17(S)	4(R)	5(R)	18(S)	17(S)	17(S)	3(R)	3(R)	0.4
Listeria ivanovii	0(R)	17(S)	17(S)	3(R)	2(R)	5(R)	17(S)	4(R)	2(R)	3(R)	0.7
Listeria innocua	17(S)	8(R)	3(R)	2(R)	17(S)	18(S)	17(S)	17(S)	17(S)	2(R)	0.5

Keys: Resistant (R)= 0-10 mm Intermediate (I) = 11-16 mm Sensitive (S) =17 mm and above

MARI = Multiple antibiotic resistance index

Pefloxacin (PEF) 10 µg, Gentamycin (CN) 30 µg, Ampliclox (APX) 30 µg, Zinnacef (Z) 20 µg, Amoxacillin (AM) 30 µg, Rocephin(R)25 µg, Ciprofloxacin (CPX)30 µg, Streptomycin (S) 30 µg, Septrin (SXT) 30 µg and Erythromycin (E)10 µg

Antibiotics susceptibility test of *Vibrio* isolates for dry season before and after plasmid curing is presented in Table 4.6.

Before plasmid curing the *Vibrio* isolates showed resistant to most of the antibiotics disc while some showed sensitivity. After plasmid curing some still remained resistance such as AugumentIn (AU), while others became sensitive, and there was a reduction in the multidrug antibiotics resistance index (MARI) after curing.

Antibiotics susceptibility profile of *Vibrio* isolates for dry season before and after plasmid curing is presented in Table 4.7. All the *Vibrio* isolate showed resistant to septrin (SXT) before curing, but after curing isolates beame sensitive, the isolates also showed resistant to streptomycin (S) before curing and after curing they showed sensitivity. All the isolates showed resistant to chloramphenicol before and after plasmid curing. And also after curing there was a reduction in multidrug antibiotics resistance index (MARI).

Antibiotics susceptibility profile of heterotrophic bacteria isolates for wet and dry seasons is presented in Table 4.8. *Escherichia coli* showed resistant to all the antibiotics except sparifloxacin (SP) for wet season, while for dry season it showed sensitivity to streptomycin (S) but showed resistant to all other antibiotics. augmentin (AU) showed no inhibition to all the isolates, during wet season while the other antibiotics discs showed sensitivity and resistance, for at dry season gentamicin (CN), ampiclox (APX) and zinacef (Z) showed no inhibition to all the isolates, Some of the other antibiotics demonstrated sensitivity, whereas others showed resistance.Some of the other antibiotics demonstrated sensitivity, whereas others showed resistance.

TABLE 4. 5: Antibiotic susceptibility test of *Vibrio* isolates for wet season before and after plasmid curing

Before Curing											
ISOLATES	SXT	CH	SP	CPX	AM	AU	CN	PEF	OFX	S	MARI
Vibrio paraheamolyticus	4(R)	6(R)	17(S)	18(S)	4(R)	3(R)	6(R)	8(R)	18(S)	4(R)	0.7
Vibrio cholerae (1)	6(R)	8(R)	6(R)	8(R)	3(R)	6(R)	4(R)	17(S)	4(R)	4(R)	0.8
Vibrio cholerae (2)	4(R)	6(R)	8(R)	6(R)	4(R)	18(S)	17(S)	4(R)	17(S)	6(R)	0.7
After Curing											
ISOLATES	SXT	CH	SP	CPX	AM	AU	CN	PEF	OFX	S	MARI
Vibrio paraheamolyticus	17(S)	4(R)	17(S)	18(S)	3(R)	4(R)	6(R)	18(S)	17(S)	17S	0.3
Vibrio cholerae (1)	18(S)	6(R)	17(S)	17(S)	6(R)	8(R	8(R)	18(S)	18(S)	18S	0.3
Vibrio cholerae (2)	17(S)	8(R)	6(R)	17(S)	4(R)	17(S)	S(17)	4(R)	17(S)	17S	0.4

Keys: Resistant (R)= 0-10mm, Intermediate (I) = 11-16mm, Sensitive (S) =17mm and above

MARI = Multiple antibiotic resistance index

Septrin (SXT) 30 µg, Chloramphenicol (CH) 30 µg, Sparifloxacin (SP) 10 µg, Ciprofloxacin (CPX) 30 µg, Amoxacillin (AM) 30 µg, Augmentin (AU) 10 µg, Gentamycin (CN) 30 µg and Pefloxacin (PEF) 30 µg

TABLE 4 6: Antibiotic susceptibility test of *Listeria* isolates for dry season before and after plasmid curing

Before curing											
ISOLATES	PEF	CN	APX	Z	AM	R	CPX	S	SXT	E	MARI
Listeria monocytogenes (1)	11(I)	0(R)	0(R)	0(R)	6(R)	3(R)	10(R)	17(S)	5(R)	6(R)	0.8
Listeria monocytogenes (2)	17(S)	18(S)	0(R)	2(R)	6(R)	17(S)	17(S)	5(R)	2(R)	5(R)	0.9
Listeria monocytogenes (3)	18(S)	17(S)	2(R)	8(R)	4(R)	4(R)	18(S)	6(R)	6(R)	4(R)	0.8
After curing											
ISOLATES	PEF	CN	APX	Z	AM	R	CPX	S	SXT	E	MARI
Listeria monocytogenes (1)	12(I)	17(S)	17(S)	9(R)	8(R)	0(R)	0(R)	18(S)	2(R)	0(R)	0.5
Listeria monocytogenes (2)	17(S)	17(S)	18(S)	4(R)	17(S)	2(R)	17(S)	0(R)	0(R)	17(S)	0.4
Listeria monocytogenes (3)	17(S)	18(S)	18(S)	3(R)	5(R)	1(R)	3(S)	0(R)	0(R)	4(R)	0.6

Keys: Resistant (R)= 0-10 mm Intermediate (I) = 11-16 mm Sensitive (S) =17 mm and above

MARI = Multiple antibiotic resistance index

Antibiotics susceptibility profile of heterotrophic bacterial isolates for wet and dry seasons is presented in Table 4.8. For wet season chloraphenicol (CH) and augmentin (AU) showed resistance to all the isolates, while the other antibiotics disc some showed sensitivity and resistance., for dry season gentamicin (CN), ampiclox (APX) and zinacef (Z) showed resitance to all the isolates in dry season, while the others antibiotics some showed resistance while others showed sensitivity Agarose gel electrophoresis of plasmid profile of bacterial isolates for wet season is presented in Plate 4.3

The sample ID of *Vibrio* and *Listeria* isolate and their plasmid size for wet season is presented in Table 4.9. The V9- *Vibrio parahaemolyticus* had one plasmid band with molecular size of 1300 bp, v10- *Vibrio cholerae* had one plasmid with molecular size of 1600 bp, *Vibrio vulnificus* had one plasmid band with 1500 bp. The V13- *Vibrio cholera*e had one plasmid band with 1600 bp,V-15 *Vibrio fluvialis* had one olasmid band with 1000 bp, while for *Listeria* species. Also, V 18- *Listerua monocytogenes* had one plsmid band size with molecular msize of 1300 bp. The V19- *Listeria monocytogenes* 2 had one plasmid band with molecular size of 1400 bp *Listeria monocytogenes* 3 had one plasmid band with molecular size of 800 bp, *Listeria monocytogenes*4, had four (4) plasmid bad with molecular sizes of 1500 bp, 1400 bp,900 bp, and 800 bp and *Listeria ivanovii* had one plasmid band with molecular size of 1400 bp.

TABLE 4.7: Antibiotics susceptibility test of *Vibrio* isolates for dry season before and after plasmid curing

Before curing											
ISOLATES	SXT	CH	SP	CPX	AM	AU	CN	PEF	OFX	S	MARI
Vibrio parahaeamolyticus	6(R)	4(R)	17(S)	17(S)	7(R)	3(R)	0(R)	3(R)	7(R)	3(R)	0.7
*Vibrio cholerae (*1)	3(R)	5(R)	17(S)	18(S)	5(R)	17(S)	2(R)	17(S)	17(S)	2(R)	0.5
Vibrio cholerae (2)	5(R)	5(R)	3(R)	2(R)	5(R)	18(S)	17(S)	3(R)	17(S)	2(R)	0.7
After curing											
ISOLATES	SXT	CH	SP	CPX	AM	AU	CN	PEF	OFX	S	MARI
Vibrio parahaemolyticus	17(S)	3(R)	17(S)	17(S)	2(R)	2(R)	0(R)	17(S)	17(S)	17(S)	0.3
Vibrio cholerae (1)	17(S)	2(R)	18(S)	18(S)	3(R)	17(S)	2(R)	18(S)	18(S)	18(S)	0.2
Vibrio cholerae (2)	17(S)	2(R)	0(R)	18(S)	5(R)	17(S)	18(S)	0(R)	17(S)	17(S)	0.4

Keys: Resistant (R)= 0-10 mm Intermediate (I) = 11-16 mm Sensitive (S) =17 mm and above

MARI = Multiple antibiotic resistance index

Septrin (SXT) 30 µg, Chloramphenicol (CH) 30 µg, Sparifloxacin (SP) 10 µg, Ciprofloxacin (CPX) 30 µg, Amoxacillin (AM) 30µg, Augmentin (AU) 10 µg, Gentamycin (CN) 30 µg, Pefloxacin (PEF) 30 µg, Tarivid (OFX) 10µg and Streptomycin (S) 30 µg.

Table 4.8: Antibiotics susceptibility test of heterotrophic bacteria isolates for wet and dry seasons

Wet season											
ISOLATE\	PEF	CN	AU	AM	CPX	SP	S	CH	SXT	OFX	MARI
Shigella flexneri	2(R)	18(S)	0(R)	18(S)	17(S)	13(I)	0(R)	0(R)	13(I)	0(R)	0.6
Pseudomonas aeruginosa	18(S)	3(R)	3(R)	3(R)	18(S)	17(S)	3(R)	3(R)	14(I)	3(R)	0.5
Esherichia coli	0(R)	2(R)	2(R)	0(R)	12(I)	17(S)	3(R)	3(R)	0(R)	2(R)	0.8
Serratia marcescens	17(S)	12(I)	3(R)	0(R)	17(S)	18(S)	0(R)	3(R)	0(R)	2(S)	0.5
Dry season											
ISOLATE	PEF	CN	APX	Z	AM	R	CPX	S	E	OFX	MARI
Samonella enterica	0(R)	3(R)	3(R)	3(R)	17(S)	11(I)	2(I)	17(S)	3(R)	0(R)	O.6
Pseudomonas aeruginosa	17(S)	0(R)	2(R)	3(R)	2(R)	3(R)	2(I)	17(S)	11(I)	17(S)	0.3
Esherichia coli	3(R)	0(R)	3(R)	3(R)	2(R)	3(R)	3(R)	18(S)	3(R)	2(R)	0.9
ISOLATE	PEF	CN	APX	Z	AM	R	CPX	S	SXT	E	MARI
Bacillus cereus	20(5)	16(I)	2(R)	2(R)	6(R)	14(S)	20(R)	16(I)	14(R)	16(I)	0.3

Keys: Resistant (R)= 0-10 mm Intermediate (I) = 11-16 mm Sensitive (S) =17 mm and above
MARI = Multiple antibiotic resistance index Septrin (SXT) 30 µg, Chloramphenicol (CH) 30 µg, Sparifloxacin (SP) 10 µg, Ciprofloxacin (CPX) 30 µg, Amoxacillin (AM) 30 µg, Augmentin (AU) 10 µg, Gentamycin (CN) 30 µg, Pefloxacin (PEF) 30 µg, Tarivid (OFX) 10 µg, Streptomycin (S) 30 µg and Levofloxacin (LEV) 5 µg

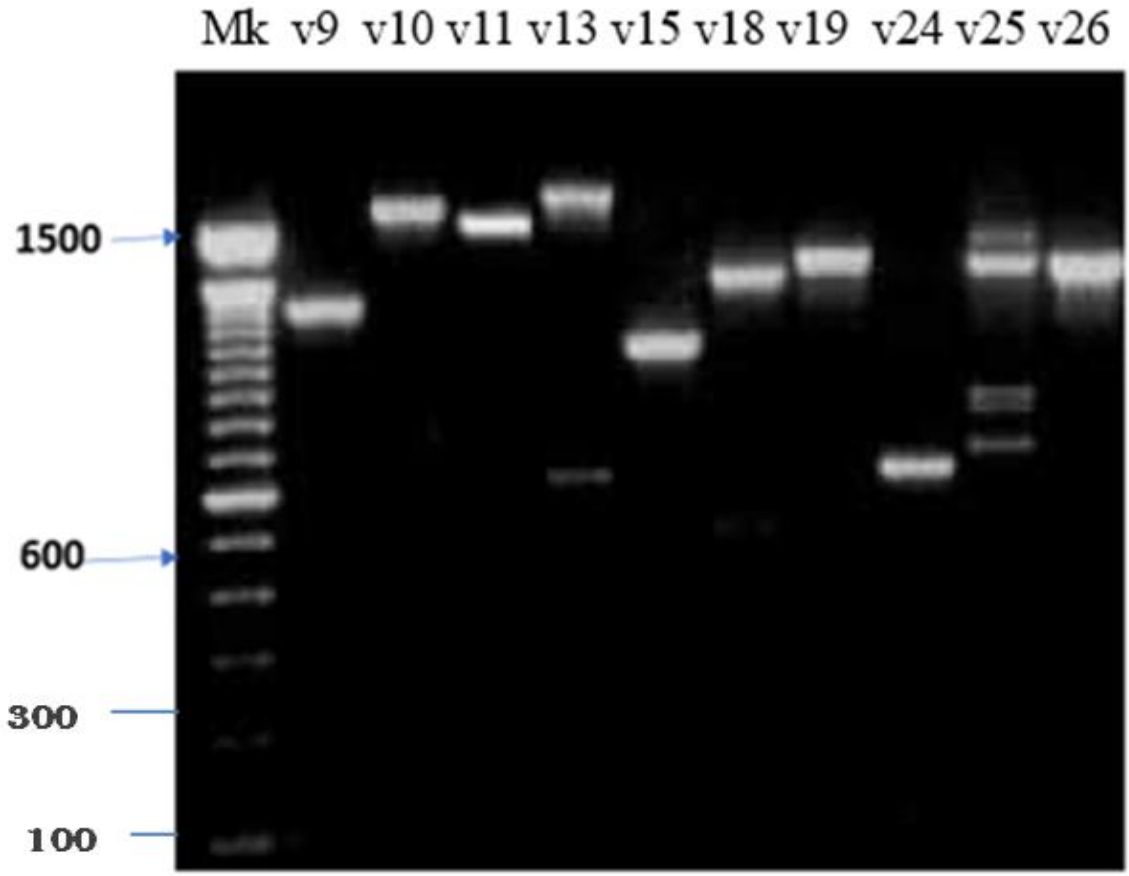

Plate 4.3: Agarose gel electrophoresis of plasmid profile of bacterial isolates, for wet season

Key;

MK: molecular weight marker.

v9- *Vibrio parahaemolyticus*,

v10- *Vibrio cholerae,*

v11- *Vibrio vulnificus*,

v13- *Vibrio parahaemolyticus*,

v15- *Vibrio fluvialis*,

v18- *Listeria monocytogenes,(1*)

v19- *Listeria monocytogenes* (2),

v24- *Listeria monocytogenes* (3)

v25- *Listeria monocytogenes* (4)

v26- *Listeria ivanovii*

Table 4. 9: *Vibrio* and *Listeria* isolate and their plasmid size for wet season

Sample ID	Organisms	No of plasmid	Size (bp)
V9	*Vibrio parahaemolyticus* 1	1	1300
V10	*Vibrio cholerae*	1	1600
V11	*Vibrio vulnificus*	1	1500
V13	*Vibrio cholerae*	2	1600,800
V15	*Vibrio fluvialis*	1	1000
V18	*Listeria monocytogenes* 1	2	1300,600
V19	*Listeria monocytogenes* 2	1	1400
V24	*Listeria monocytogenes* 3	1	800
ssssV25	*Listeria monocytogenes* 4	4	1500, 1400, 900,800
V26	*Listeria ivanovii*	1	1400

Agarose gel electrophoresis of plasmid profile of bacteria isolate for dry season. is presented in Plate 4.4. *Vibrio* and *Listeria* isolates and their plasmid size for dry season is presented in Table 4.10. *Listeria monocytogenes* 1 showed no plasmid band and size, *Listeria monocytogenes*2 had one plasmid band with molecular size of 1200 bp, *Vibrio cholera* 1 had one plasmid band with 1200 bp. Also, *Vibrio cholera* 2 had one plasmid band with 1200 bp. *Vibrio parahaemolyticus* showed no plasmid band and no molelecular size too. *Listeria monocytogenes* 3 had one plasmid band with molecular size 1200 bp.

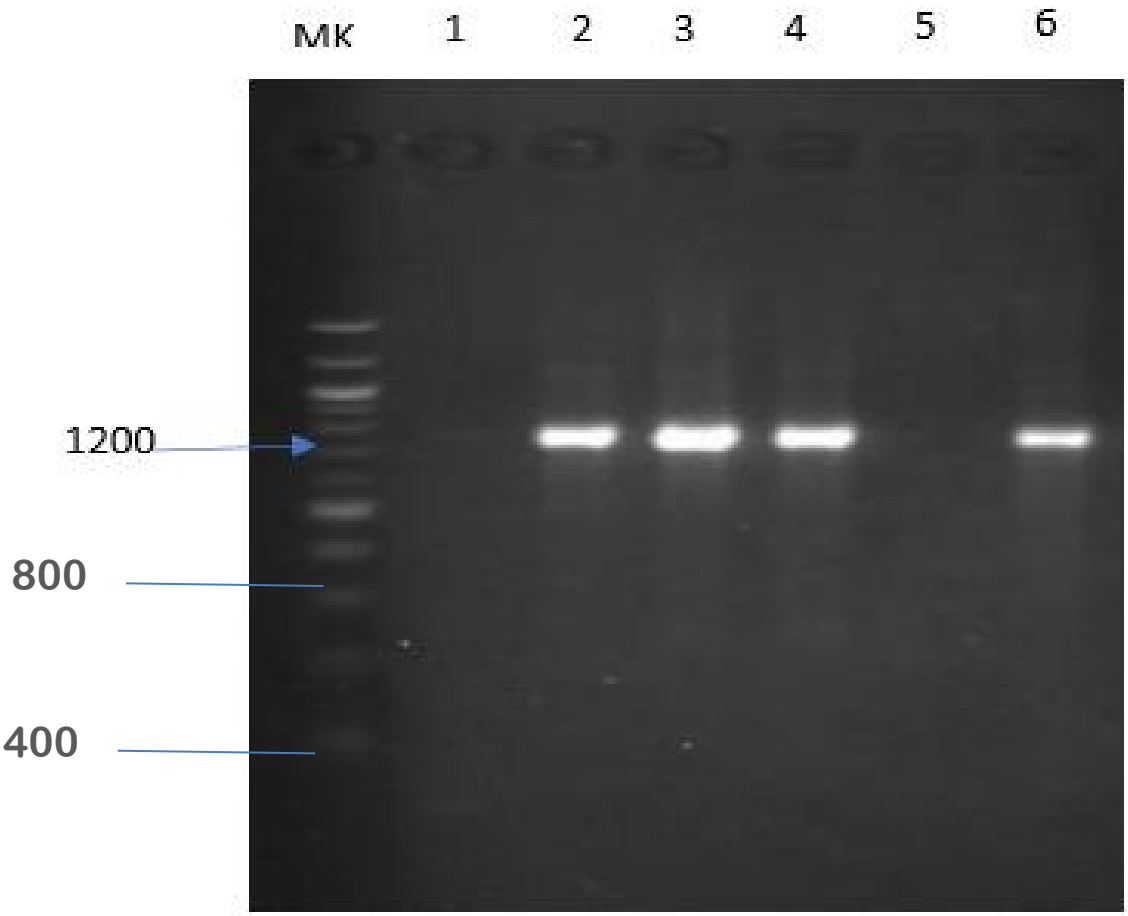

Plate 4.4: Agarose gel electrophoresis of plasmid profile of bacteria isolate for dry season.

Key:

MK: molecular weight marker.

1- *Listeria monocytogenes* (1)
2 – *Listeria monocytogenes*(2)
3 –*Vibrio cholerae (*1)
3 – *Vibrio cholerae (*2)
4 5 – *Vibrio parahaemolyticus* , 6 –*Listeria monocytogenes* (3).

Table 4.10: *Vibrio* and *Listeria* isolates and their plasmid size for dry season

	Organism	No of plasmid	Size (bp)
1	*Listeria monocytogenes (*1)	-	-
2	*Listeria monocytogenes* (2)	1	1200
3	*Vibrio cholerae* (1)	1	1200
4	*Vibrio cholerae* (2)	1	1200
5	*Vibrio parahaemolyticus*	-	-
6	*Listeria monocytogenes* (3)	1	1200

Agarose gel electrophoresis of the PCR products of penicillin binding protein (*pbp)* genes of *Listeria* isolates for wet season is presented in Plate 4.5. The sample ID indicated in the plate represents the following isolates listed alongside the sampel ID. At 1500 bp

Agarose gel electrophoresis of the PCR products of *aph(2")-If* resistant gene of *Vibrio* isolates for wet season. is presented in Plate 4.6. The sample ID indicated in the plate represent the *Vibrio* isolate along side, at molecular size of 600 bp for *Vibrio parahaemolyticus and Vibrio fluvialis,* while others did not have the *aph(2")-If* reistant gene.

Agarose gel electrophoresis of PCR of penicillin binding protein(pb2p) resistant gene of *Listeria* isolates for dry season is presented in Plate 4.7.The sample ID indicated in the plate represent the *Listeria* isolate along side at molecular size of 500 bp. Agarose gel electrophoresis of the PCR products of *aph(2")-If* resistant gene of *Vibrio* isolates for dry season. is presented in Plate 4.8. The sample ID 1-4 indicated in the plate represent the *Vibrio* isolate alongside, Agarose gel electrophoresis of the PCR products of *HlyA* gene amplified from selected *Listeria* isolates (Band size approximately 230 bp). Gel image indicates a positive of *HlyA* in all samples except V19 for wet season as presented in Plate 4.9.

Agarose gel electrophoresis of the PCR products of *ctxA* virulent gene amplified from selected *Vibrio* isolates (Band size approximately 563bp) for wet season is presented in Plate 4.10. Gel electrophoresis of the PCR products of *ctxA* virulence gene amplified from selected *Vibrio* isolates (Band size approx. BV15 from dry season is presented in Plate 4.11. Agarose gel electrophoresis of the PCR products of *ctxA* gene amplified from selected *Vibrio* isolates (Band size approximately 563 bp). Gel image indicates a positive of *ctxA* in all samples except BV15 from dry season is presented in Plate 4.12.

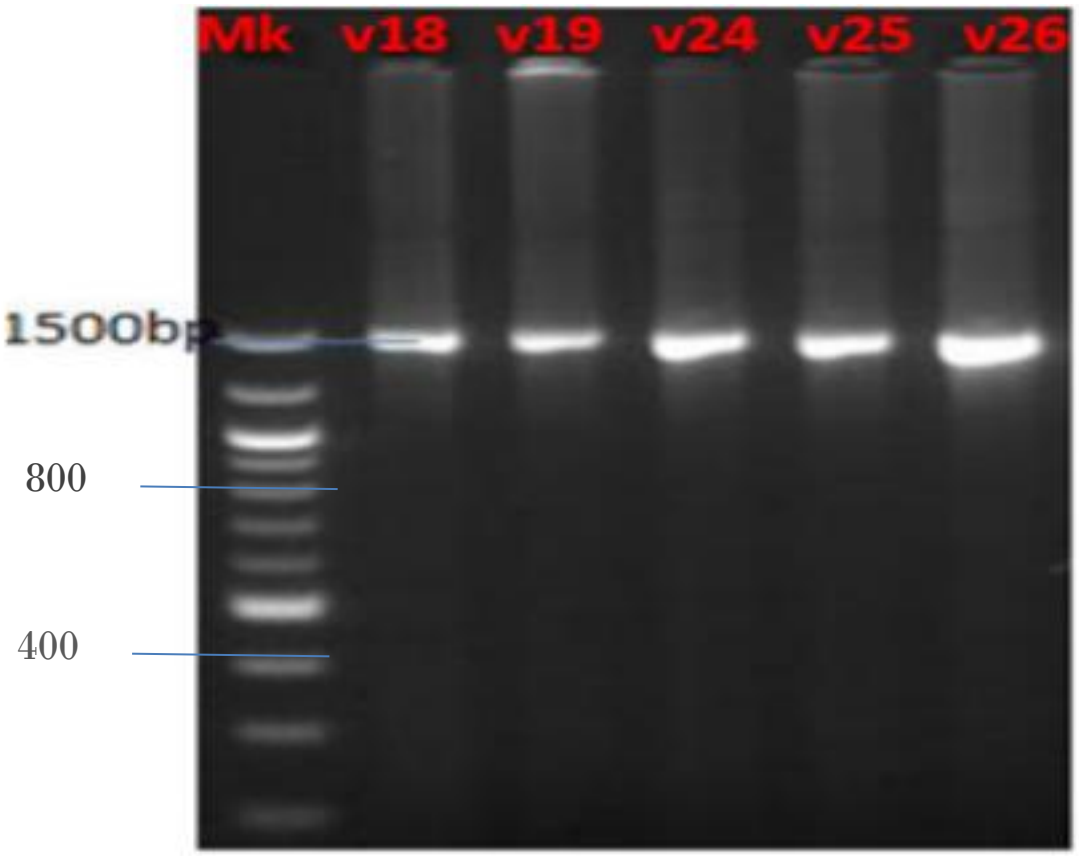

Plate 4.5: Agarose gel electrophoresis of the PCR products of penicillin binding protein (*pbp)* resistant genes of *Listeria* isolates for wet season.

Key:

MK: molecular weight marker.

V18- *Listeria monocytogenes* (1)

V19 - *Listeria monocytogenes* (2)

V24- *Listeria monocytogenes* (3*)*

V25- *Listeria monocytogenes (4)*

V26- *Listeria ivanovii*

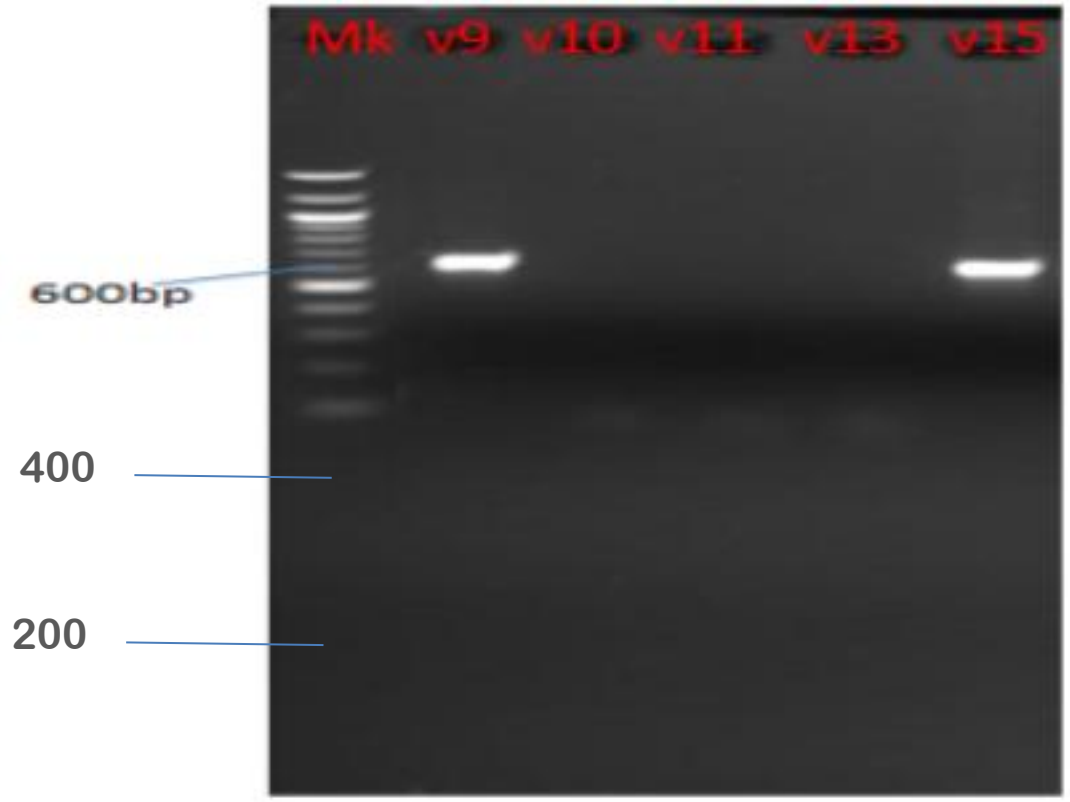

Plate 4.6: Agarose gel electrophoresis of the PCR products of *aph(2")-If* resistant gene of *Vibrio* isolates for wet season.

Key:

MK: molecular weight marker.

V9- *Vibrio parahaemolyticus*

V10 – *Vibrio cholera*

V11- *Vibrio vulnificus*

V13- *Vibrio parahaemolyticus*

V15- *Vibrio fluvialis*

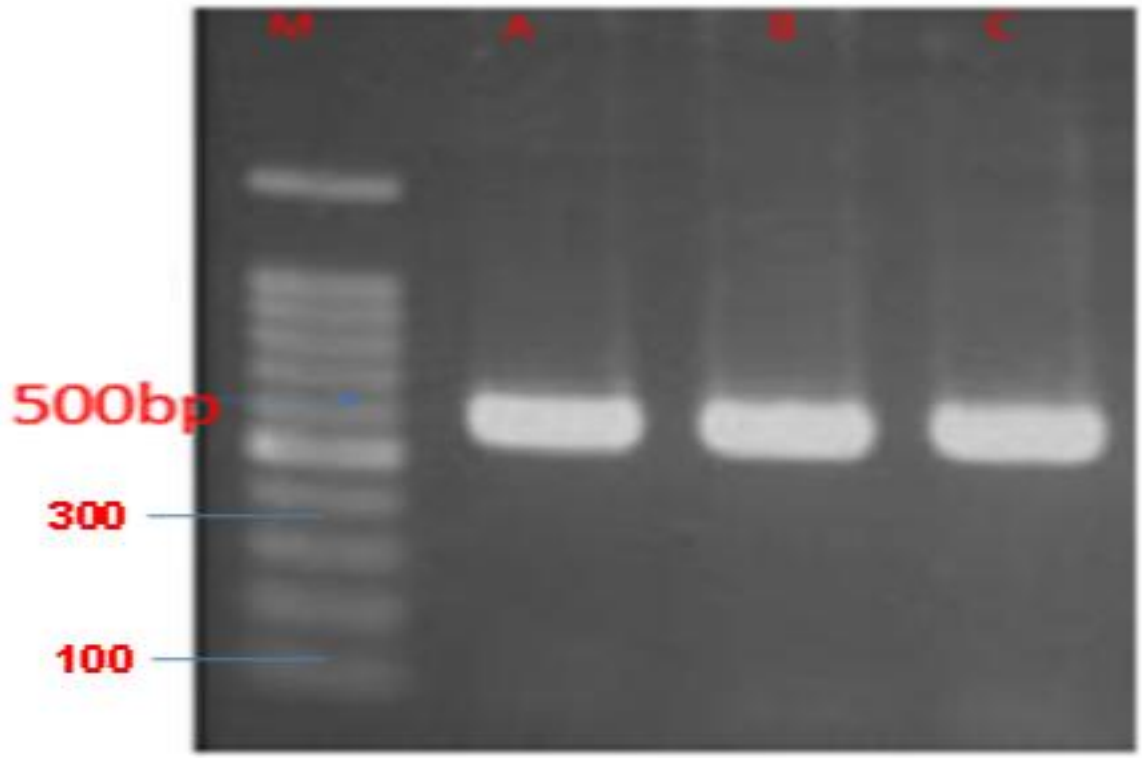

Plate 4.7: Agarose Gel Electrophoresis of PCR of penicillin binding protein (pb2p) resistant gene of *Listeria* isolates for dry season

Key:

M-Molecular weight marker

A –*Listeria monocytogenes* (1)

B- *Listeria monocytogenes* (2)

C -*Listeria monocytogenes* (3)

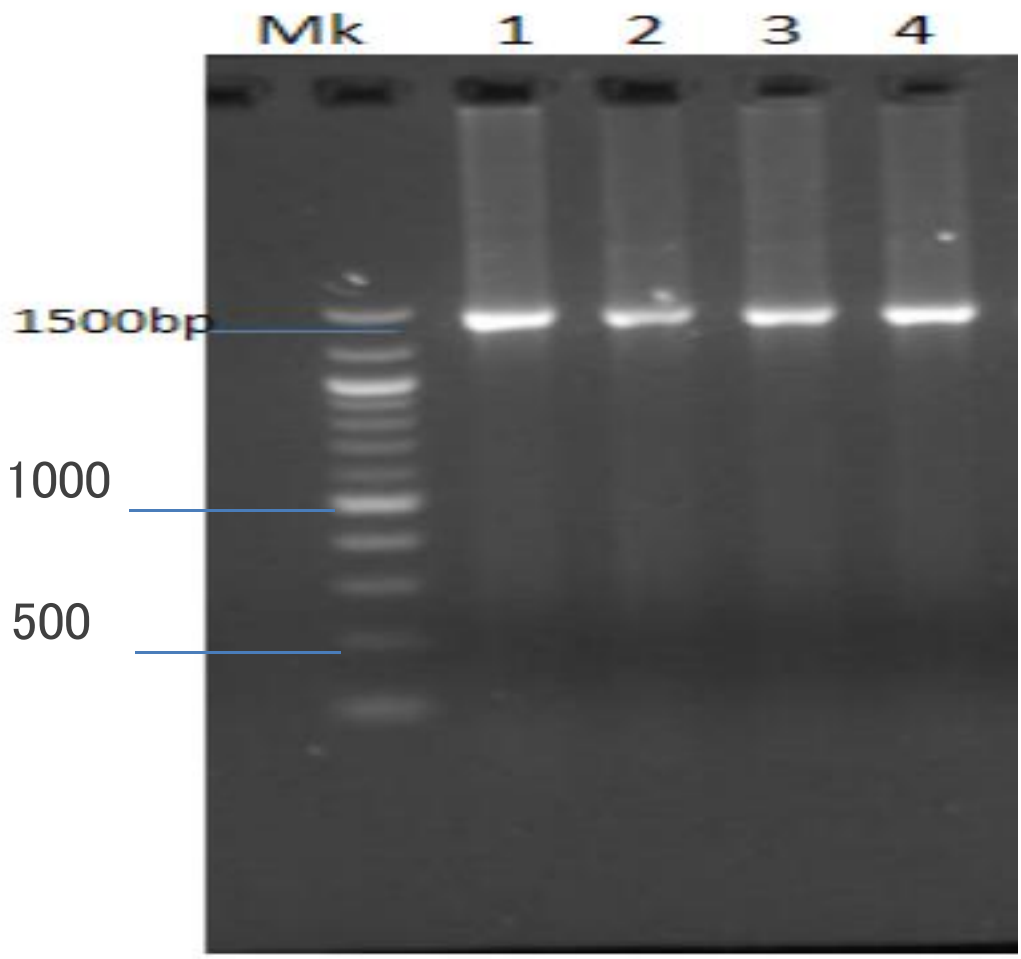

Plate 4.8: Agarose gel electrophoresis of the PCR products of *aph(2")-If* resistant gene of *Vibrio* isolates for dry season.

Key

MK -molecular weight marker

1-*Vibrio parahaemolyticus*

2-*Vibrio cholerae*

3-*Vibrio cholera*

4 - *Vibrio cholera.*

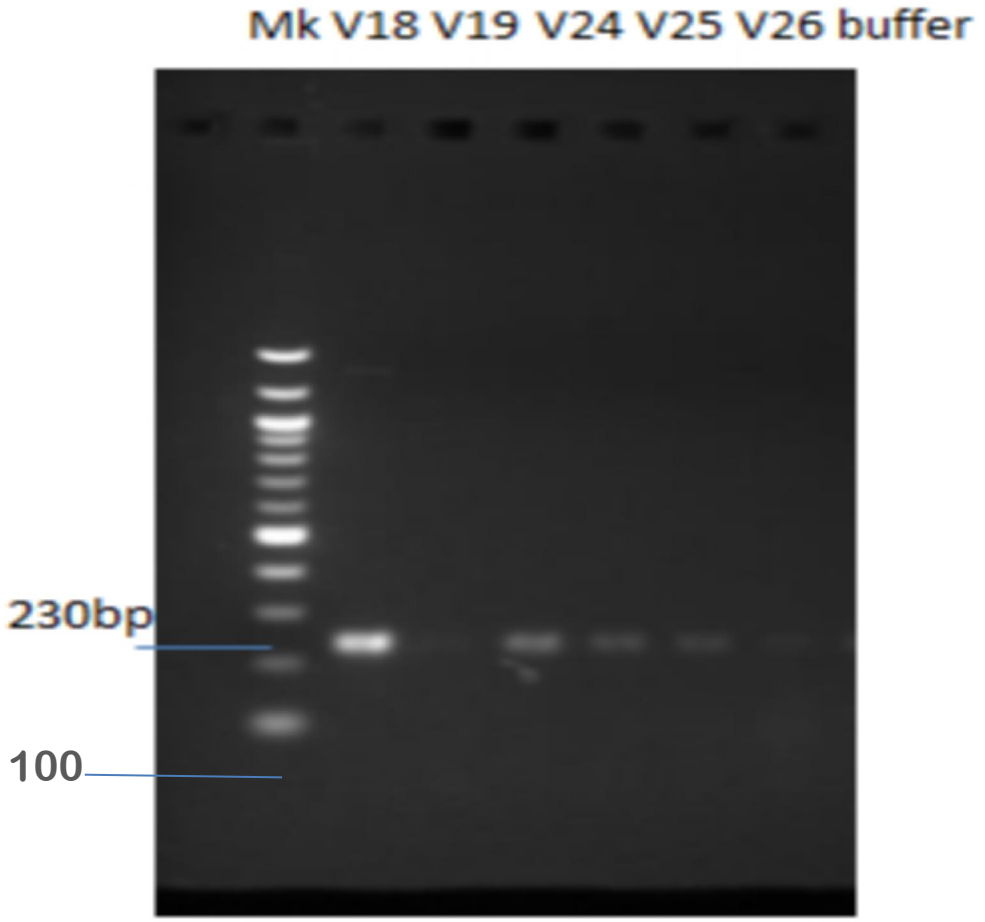

Plate 4.9: Agarose gel electrophoresis of the PCR products of *hlyA* gene amplified from selected *Listeria* isolates. (Band size approximately 230bp). Gel image indicates a positive of *hlyA* in all samples except V19. For wet season

Key:

MK – molecular weight marker,

V18- *Listeria innocua,S* V19- *Listeria monocytogenes* (1) V24- *Listeria monocytogenes* (2) V25 – *Listeria monocytogenes* (3) V26- *Listeria ivanovii.*

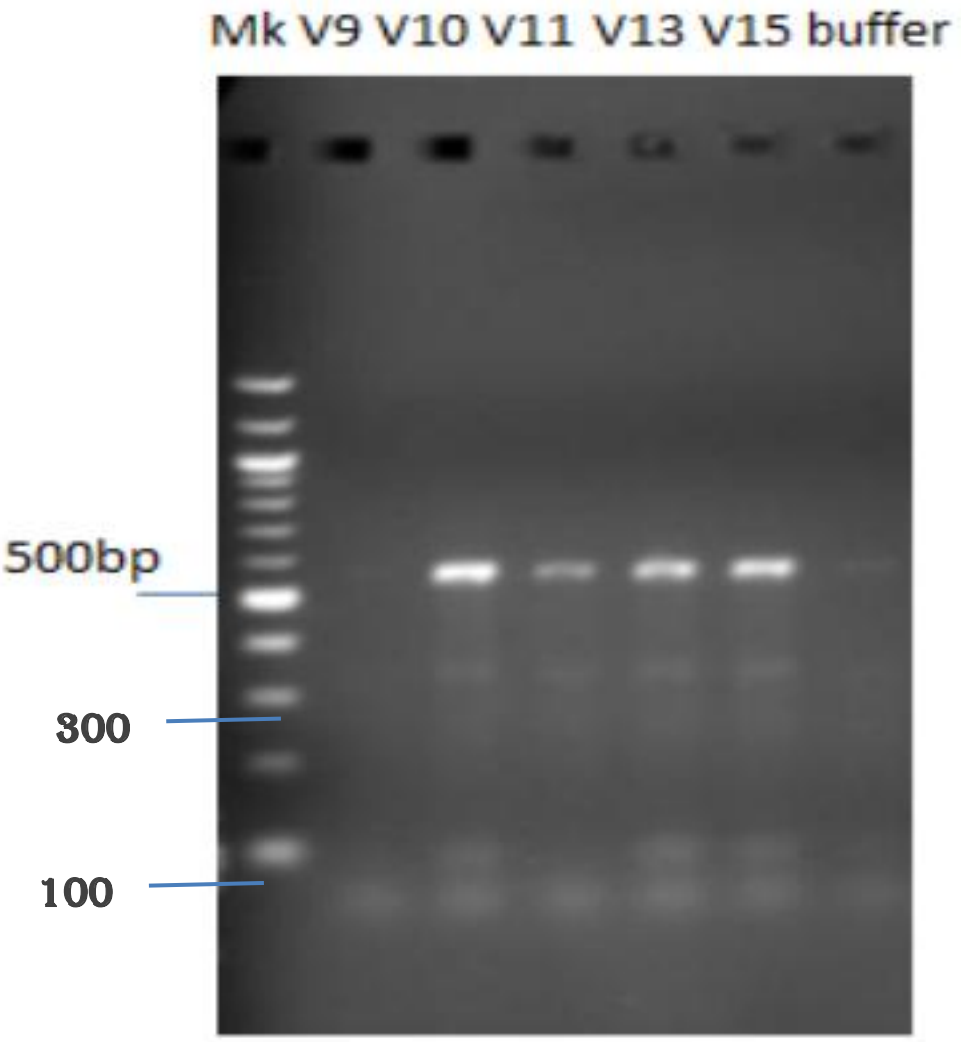

Plate 4.10: Agarose gel electrophoresis of the PCR products of ctxA virulence gene amplified from selected *Vibrio* isolates. (Band size approximately 563bp). Gel image indicates a positive of ctxA in all samples except V9 for wet season.

Key:

Mk- molecular weight

V9- *Vibrio prarahaemolyticus,* V10 – *Vibrio cholerae,*V11- *Vibrio vulnificus,* V13 – *Vibrio parahaemolyticus,* V15 – *Vibrio fluvialis.*

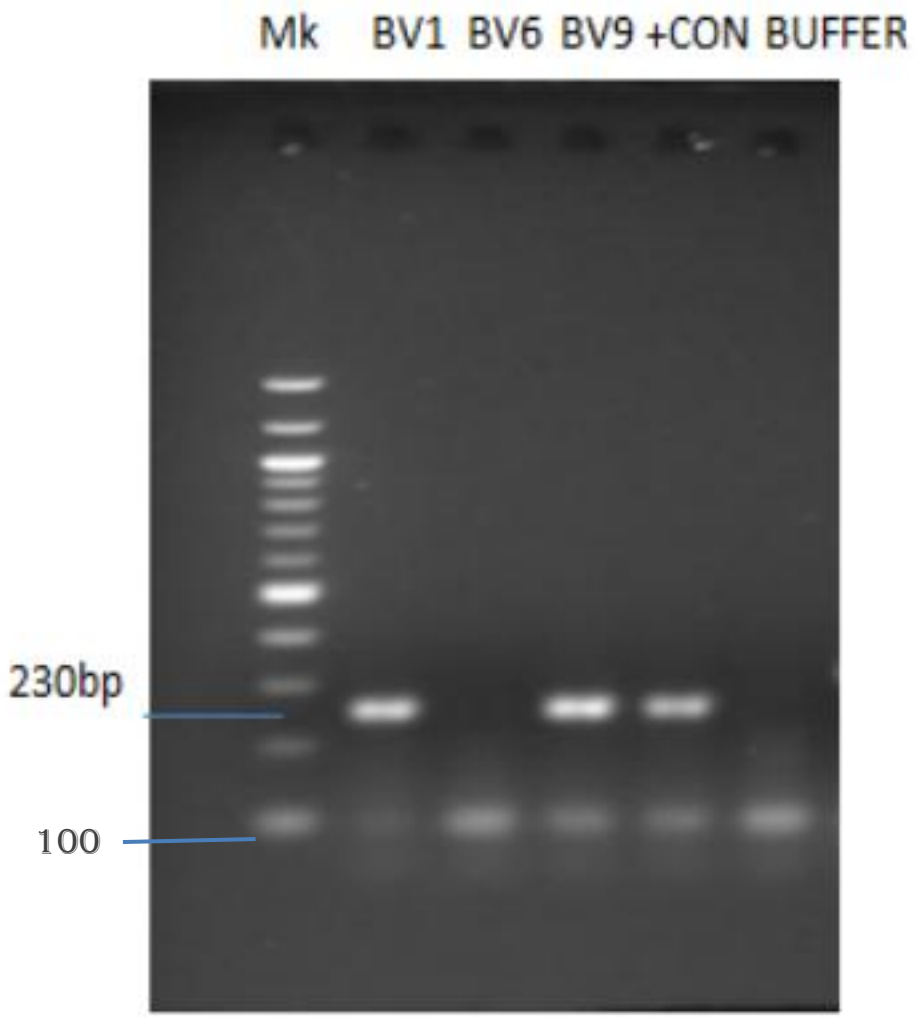

Plate 4.11: Agarose gel electrophoresis of the PCR products of *hlyA* gene amplified from selected *Listeria* isolates (Band size approximately 230bp) for dry season.

Key:

Mk- molecular weight

BV1 - *Listeria monocytogenes* (1)

BV6- *Listeria monocytogenes* (2)

BV9 - *Listeria monocytogenes* (3)

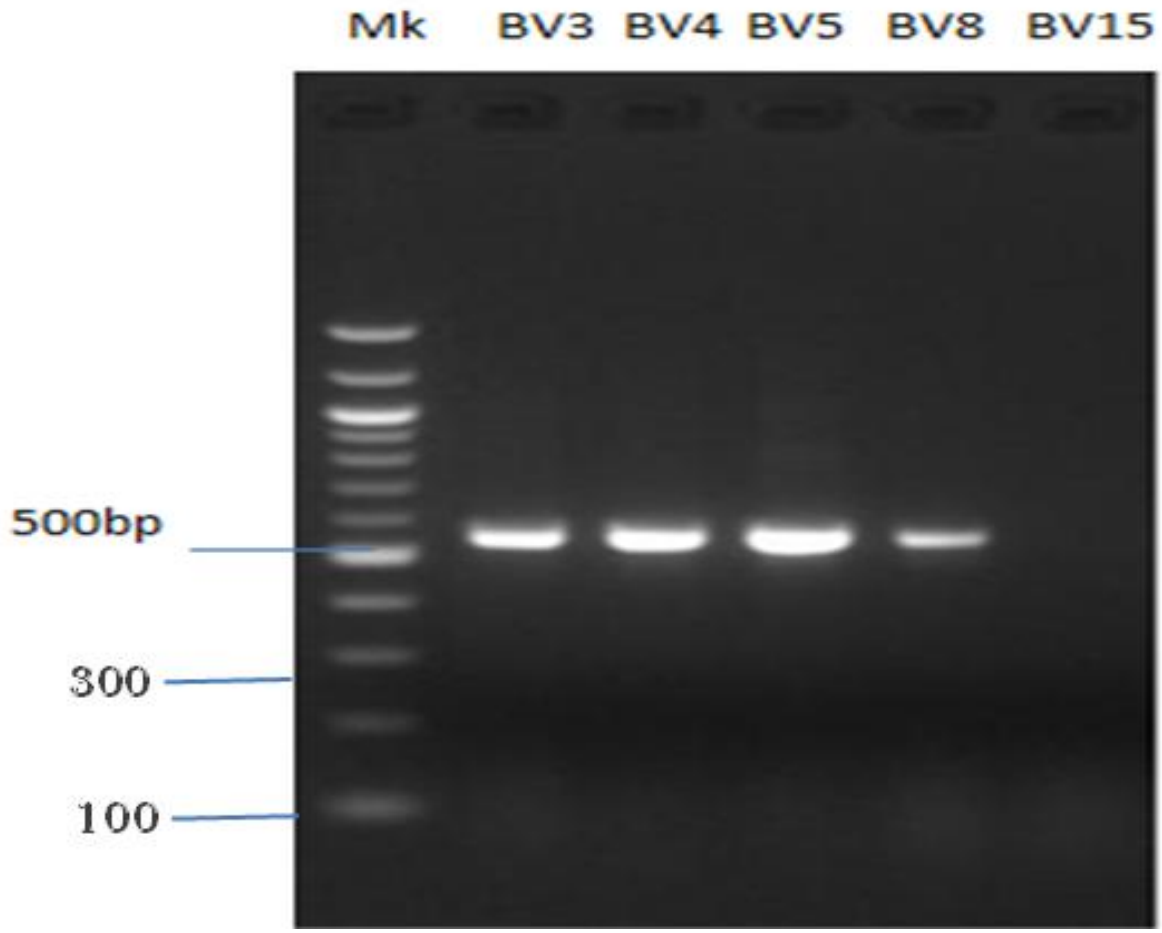

Plate 4.12: Agarose gel electrophoresis of the PCR products of ctxA gene amplified from selected *Vibrio* isolates (Band size approximately 563bp). Gel image indicates a positive of ctxA in all samples except BV15 from dry season

Key:

MK- Molelcular weiht maker

BV3 – *Vibrio parahaemolyticus*

BV4- *Vibrio cholerae*

BV5 – *Vibrio- vulnificus*

BV 8– *Vibrio – parahaemolyticus*

BV15 – *Vibrio cholerae*

The proximate composition of bivalve (*Ergeria radiata*) from different Coastal water, Opolo and Swali market for wet and dry season is presented in Figure 4.4. For wet season; high rmoisture content was obtained from Coastal waters and Opolo market, while thel least moisture content was from Swali market. Again, for dry season,Moisture content was also higher for Coastal water and Opolo market, while the least was obtained for Swali market Higher ash content, during the wet season was obtained from Coastal waters , followed by Opolo and the least content was from Swali market. Where as for dry season ash content was low from the three sources the least was obtained from Coastal waters, followed by Swali then Opolo market. For wet season, fat content was low in all the sources while for dry season, the fat content were also low. Wet season for protein Coastal waters had the highest protein content followed by Swali market then Opolo market whereas for dry season the protein content where all within same range. For wet season; NFE (Cabohydrate) content obtained were within same range from the three sources for dry season NFE (carbohydrate) content obtained was higher than in the wet season from the three sources and where all within same range.. Fibre was low compared to carbohydrate, for wet season fibre content obtained from the three sources were within same range, while for dry season fibre content obtained from the three sources were also within the same range. Dry matter had the highest content in all sample during wet and dry season compared to fiber, ash and fat which was the least.

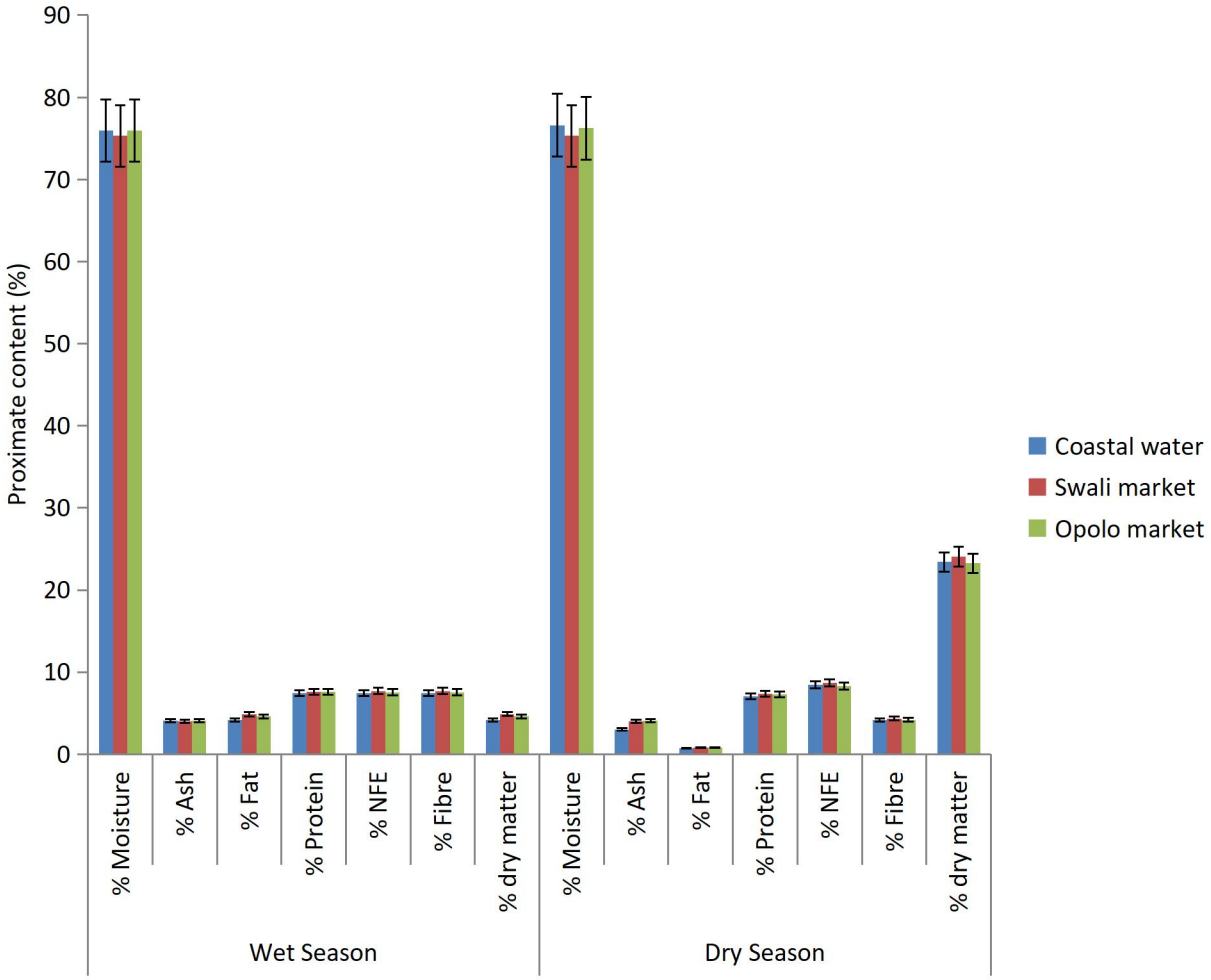

Figure 4.4: Proximate composition of *Ergeria radiata* for wet and dry seasons

Key**:** NFE **-** Nitrogen free extract

The mineral composition (of bivalve (*Ergeria radiata*) from different sources Coastal water Swali and Opolo market for wet and dry season is presented in Figure 4. 5. For wet season the composition of magnesium was higher from the three sources than in dry season. The Na content was high compared to magnesium, while Na content was higher in wet season than in the dry season in all the three different sources. Potassium had the highest content amongst all other mineral content and the K content was high for wet season though they were all within same range whereas for dry season. Potassium content was higher from Coastal than the content obtained from Swali market and Opolo market. The potassium content obtained from the three sources were within same range for both wet and dry season Mn content was low compared to the other mineral content. For both wet season and dry season, Mn content obtained were all within same rangefrom the three sources.

The heavy metal analysis (mg/kg) of bivalve (*Ergeria radiata*) from different sources for wet and dry season is presented in Figure 4.6. The composition of Fe content was highest compared to other heavy metals analyzed such as Pb Cu and Zn. The Fe content obtained from Coastal water was higher than the other two sources, Swali and opolo market. For dry season the Fe content obtained from Coastal waters, was also higher followed by Swali and the least was obtained from Opolo. Also, for Zn content obtained from the three sources were within same range of values for both wet and dry season. The Cupper content obtained was low compared to Zn, Cu content obtained from Coastal waters, Swali and Opolo market durig dry and wet season were within same range of values.

Pb content was lower compared to Cu content, Pb content obtained for both wet and dry season were within the same ranged of values. Hg and As content were not detected from any of the different sources Coastal waters, Swali and Opolo market for both wet and dry season.

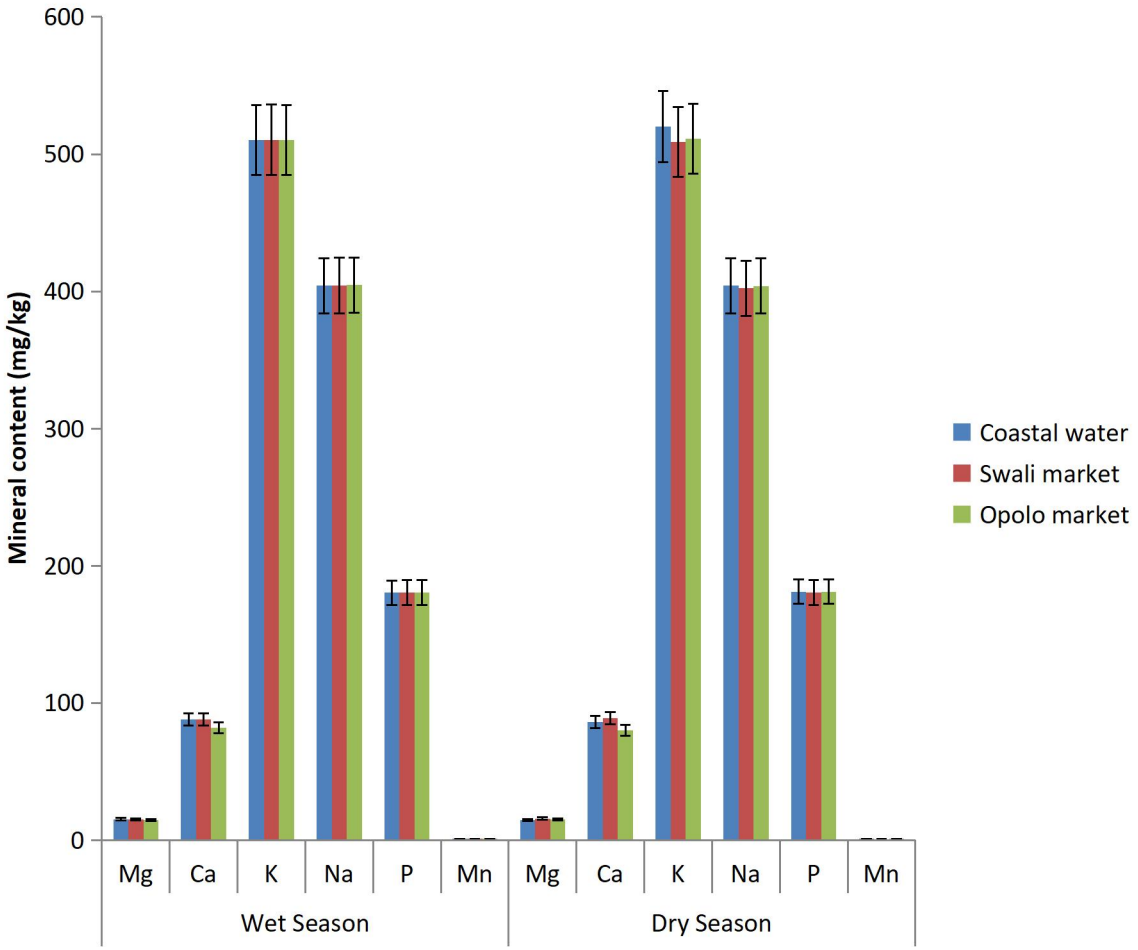

Figure 4.5: Mineral composition (mg/kg) for wet and dry seasons of clam collected from Coastal waters, Swali market and Opolo market

The GCMS chemical constitutents for polycyclic aromatic hydrocarbons (PAHs) in bilvalve (*Ergeria radiata*) collected during wet season is presented in Table 4.11. The ompounds napthalene was present in *Ergeria radiata* from Swali market and there was no detection [ND] in both Coastal waters and Opolo market. Acenapthylene compound was present in *Ergeria radiata* obtained in Opolo market and Coastal water, but not detection of any, from Swali market. acenapthene and anthracene compounds were present in *Ergeria radiata* from Swali and Opolo market but there was no detection of the compound in *Ergeria radiata* from the Coastal waters. Phenanthrene compound was present in *Ergeria radiata* from Swali and Opolo market and there was no detection in *Ergeria radiata* from the Coastal waters. Fleoranthrene compound; concentration in *Ergeria radiata* was present from the three sources ;Coastal waters, Swali and Opolo market. Pyrene compound concentration in *Ergeria radiata* was also present in all the three sources Coastal waters, Opolo and Swali market Benzo [a] anthracene compound was present in *Ergeria radiata* from Swali and Opolo was not detected in any of the sources. Flourene compound was present in *Ergeria radiata* from Swali market Opolo market. On the other hand, neither flourene compound nor Ergeria radiata from Coastal waters were found in the market. .Chrysene compound was present in *Ergeria radiata* from Opolo market whereas there was no detection of any concentration of the compound in Swali market and the Coastal waters. Benzo [b] florathracene compound was present in *Ergeria radiata* from Opolo market while there was no detection of any concentration in Swali market and the Coastal waters. Benzo [k] flourathrane compound was also present in *Ergeria radiata* from Opolo market and none was detected from Swali market and the Coastal waters. Benzo [a] pyrene compound was present in *Ergeria radiata* from Coastal waters, and Opolo market and none was detected from Swali market.In deno (1, 2, 3-cd) pyrene compound was present in *Ergeria radiata* from Opolo market whereas none was detected from both Swali market and the Coastal waters.Dibenz

(a,b) anthracene compound was present in *Ergeria radiata* from the three sources, Coastal waters, Swali and Opolo market. Benzol (ghi) perylene compound was present in *Ergeria radiata* from Swali market and Opolo market and non was detected from Coastal waters. The GCMS spectra for polycyclic aromatic hydrocarbon (PAH) in bivalve (*Ergeria radiata*) collected during dry season from various sources: Coastal waters, Swali market and Opolo market is presented in Table 4.12. Among the targeted PAH compounds. Naphthalene compound was present in *Ergeria radiata* obtain from Opolo market whereas it was not detected in *Ergeria radiata* from Swali market and Coastal waters . Acenapthylene compound was present in *Ergeria radiata* from Coastal waters, and Swali market whereas there was no detection from Opolo market. Acenaphthene compound was not detectable in *Ergeria radiata* from all the sources Coastal waters, Swali and Opolo market. Fluorine compound was present in *Ergeria radiata* from Coastal waters and Opolo market whereas there was no detection in *Ergeria radiata* from Swali market.Anthracene compound was present in *Ergeria radiata* from Swali and Opolo market, but was not detected from Coastal waters.

Phenanthrene compound was present in *Ergeria radiata* from Swali market whereas there was no detection in *Ergeria radiata* from Coastal waters.Flouranthene compound was present in *Ergeria radiata* from Coastal waters, and Swali and Opolo market, but was detected from costal waters. Pyrene compound was present in *Ergeria radiata* from Coastal waters, and Opolo market and was not detectable from Swali market. Benzo (a) anthracene compound as well as chrysene compound, benzo (k) flourathene compound, benzo (a) pyrene compound, dibenz (a,b) anthracene and benzol (ghi) perylene compound waere present in *Ergeria radiata* from the three different sources Coastal waters; Swali and Opolo market. . Whereas In deno (1,2,3-cd) pyrene compound was not detected in *Ergeria radiata* from any of the sources (Coastal waters, Swali and Opolo markets).

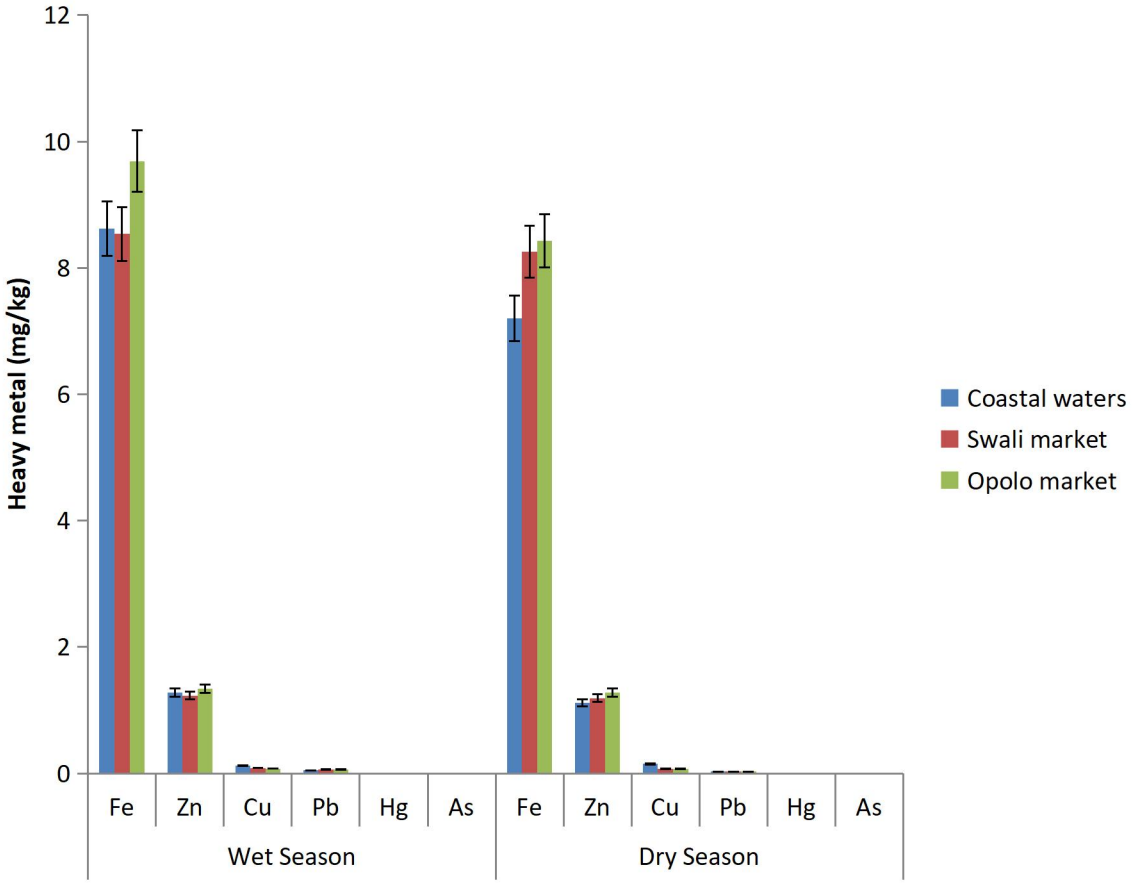

Figure 4. 6: Heavy metals content of *Ergeria radiatafor* wet and dry seasons

Table 4.11: GCMS chemical constitutent of polycyclic aromatic hydrocarbons (PAHs) in *Ergeria radiata* collected during wet season

S/N	Compound Target compound	Mass no	Wet Season (ppm) Coastal waters	Swali Market	Opolo Market
1.	Naphthalene	128.2	ND	0.17+0.01[a]	ND
2.	Acenaphthylene	152	0.06÷0.01[a]	ND	0.08+0.01*
3.	Acenaphthene	152.19	ND	ND	ND
4.	Fluorene	166.22	ND	0.14÷0.01*	0.08+0.01*
5.	Anthracene	178.23	ND	0.23÷0.01	0.10÷0.01*
6.	Phenanthrene	178.22	ND	0.0540.01^a	0.14÷0.01*
7.	Fluoranthene	202.256	0.10+0.01^a	0.42+0.01^a	0.32÷0.11*
8.	Pyrene	202.26	ND	0.16+0.01^a	0.49÷0.13*
9.	Benz/alanthracene	228.29	ND	0.15+0.01	0.41÷0.01*
10.	Chrysene	228.29	ND	ND	0.35÷0.16*
11.	Benzo[b]fluoranthene	238.326	ND	ND	0.31÷0.01*
12.	Benzolk]fluoranthene	252.316	ND	ND	0.25÷0.01*
13.	Benzo[a]pyrene	252.316	0.15÷0.01*	ND	0.34÷0.01*
14.	Indeno[1,2,3-cd]pyrene	276	ND	ND	0.24÷0.01*
15.	Dibenz[a,h]anthracene	278.354	0.061+0.01[a]	0.05÷0.01[a]	0.39÷0.12*
16.	Benzo[ghi]perylene	276.33	ND	0.12÷0.01*	0.27÷0.11*

Key: ND = Not detected

Table 4.12: GCMS chemical constitutent of polycyclic aromatic hydrocarbons (PAHs) in *Ergeria radiata* collected during dry season

S/N	Compound Target compound	Mass no	Dry Season (ppm) Coastal waters	Swali Market	Opolo Market
1.	Naphthalene	128.2	ND	ND	0.07÷0.01*
2.	acenaphthylene	152	0.13+0.01*	0.38÷0.37"	ND
3.	Acenaphthene	152.19	ND	ND	ND
4.	Fluorene	166.22	0.08÷0.01*	ND	0.13÷0.01[a]
5.	Anthracene	178.23	ND	0.11+0.01*	0.05÷0.01[a]
6.	Phenanthrene	178.22	ND	0.28:0.01[a]	0.08+0.01*
7.	Fluoranthene	202.256	0.22+0.10°	0.41+0.17[a]	0.28+0.03*
8.	Pyrene	202.26	0.98+0.13*	ND	0.28÷0.02*
9.	Benz/alanthracene	228.29	0.38÷0.15"	0.19+0.01*	0.05÷0.01*
10.	Chrysene	228.29	0.35÷0.11[a]	0.31÷0.01*	0.43+0.01*
11.	Benzo[b]fluoranthene	238.326	0.12+0.01"	0.38÷0.17*	0.39÷0.19[a]
12.	Benzolk]fluoranthene	252.316	0.24÷0.01[a]	0.23÷0.11[a]	0.37÷0.13*
13.	Benzo[a]pyrene	252.316	0.32÷0.21[a]	0.34÷0.21[a]	0.29÷0.01[a]
14.	Indeno[1,2,3-cd]pyrene	276	ND	ND	ND
15.	Dibenz[a,h]anthracene	278.354	0.11+0.01[a]	0.26+0.07[a]	0.10+0.01*
16.	Benzo[ghi]perylene	276.33	0.07=0.01[a]	0.19+0.01"	0.27÷0.05*

Key: ND - indicate No Detection

The physicochemical quality of Coastal water from Ekowe community for dry and wet season is presented in Table 4.13. The following parammeters where analysed namely; pH, total dissolve solid (TDS), EC (µS/cm), alkalinity mg/l, hardness (g/l), turbidity (FTU), chloride (mg/l), SO_4 (mg/l,), PO_4 (mg/l), and NO_3 (mg/l.). They were analysed from station one (1) to station three (3), pH for wet season ranged for station 2 and station 3 were within the permissible limit but the pH for station 1 was slightly above the permissible limit.set by WHO. The WHO. Where as for dry season. The pH for station 1-3 were all within the permissible limit set by WHO. TDS (mg/l) for wet season for station 1 -3 were above the permissible limit set by WHO, while for dry season for station 1-3 they were slightly above the permissible limit set by WHO limit. The EC (µS/cm) for wet season, the values obtained from station 1-3 were above the permissible unit set by WHO. Alkalinity (mg/l) for wet season only station 1 was within the permissible limit set by WHO, while station 2and 3 were above the permissible limit whereas station 1-3 were within the permissible limit for dry season, station 1-3 were within the permissible limit set by WHO. Hardness (mg/l) for wet season for station 1-3 were above the permissible limit set by WHO, while for dry season station 1 w was above the permissible limit set by WHO. Water temperature ºC for wet and dry season do not have permissile llimit set by WHO. The DO (mg/l) for wet season for station 1-3, were above the permissible limit set by WHO. whereas for dry season and station 1-3 were within the permissible limit set by WHO.

BOD (mg/l) for wet season for station 1-3 were above the permissible limit set by WHO whereas for dry season station 1-3 wthe values obtained were within the permissible limit set by WHO.Turbidity (FTU) for wet season for station 1-3 the values obtained were above the permissible limit set by WHO where as for dry season station 1-3, the values obtained were within the permissible limit set by WHO. Chloride for wet season for station 1 and 2 were within the permissible limit set by WHO while station 3 was slightly above the limit.

While for dry season, staion 1-3 were within the permissible limit set by WHO. The SO_4 (mg/l) for wet and dry season, staion 1-3 were above the permissible limit set by WHO. Also, PO_4 (mg/l,) for wet and dry season for station 1-3 were all above the permissible limit set by WHO as well as NO_3 (mg/) for wet and dry season.

Table 4.13: Physicochemical qualities of Coastal water from Ekowe community.

	Wet season			Dry season			
Parameters	Station 1	Station 2	Station 3	Station 1	Station 2	Station 3	WHO limit
pH	7.9±0.33^{b}	6.1±0.35^{a}	6.0±0.36^{a}	6.9±0.37^{a}	7.4±0.32^{b}	6.8±0.36^{a}	6.9-7.1
TDS (mg/l)	40.0±1.25^{a}	25.0±1.14^{c}	57.0±1.19^{b}	14.0±1.13^{b}	13.0±2.12^{a}	15.0±1.50^{c}	10
EC (µS/cm)	81.0±3.42^{b}	99.0±3.59^{c}	72.0±2.00^{a}	26.0±1.46^{c}	24.0±1.46^{b}	19.0±1.39^{a}	10
Alkalinity (mg/l)	23.0±1.42^{a}	32.0±1.84^{b}	40.0±2.67^{c}	15.0±1.50^{b}	12.0±1.12^{a}	18.0±1.11^{c}	25
Hardness (mg/l)	45.7±2.66^{c}	34.0±2.15^{b}	28.1±0.40^{a}	10.2±1.23^{b}	14.7±1.15^{c}	9.8±0.89^{a}	10
Water temperature (ºC)	22.0±1.84^{b}	20.0±1.45^{a}	20.0±1.45^{a}	29.0±2.36^{b}	31.0±2.09^{a}	30.0±1.45^{c}	-
DO (mg/l)	69.15±0.72^{b}	54.11±1.01^{a}	71.66±1.38^{c}	15.0±1.14^{b}	14.0±1.13^{a}	16.1±1.18^{c}	30
BOD (mg/l)	87.9±0.68^{c}	75.80±0.24^{a}	79.16±0.41^{b}	9.10±0.70^{c}	7.10±0.47^{b}	6.15±0.35^{a}	20
Turbidity (FTU)	49.0±0.69^{c}	30.0±1.25^{b}	22.0±1.12^{a}	10.0±1.25^{b}	7.0±0.45^{a}	12.0±1.12^{c}	0.5
Chloride (mg/l)	39.1±2.43^{a}	38.7±1.21^{b}	54.5±2.31^{c}	26.2±2.50^{b}	28.0±2.22^{a}	32.0±2.17^{c}	50
SO_4 (mg/l)	25.0±0.20^{b}	14.0±0.16^{a}	33.0±0.11^{c}	15.0±0.20^{b}	14.0±0.16^{a}	15.0±0.20^{b}	0.5
PO_4 (mg/l)	31.01±0.13^{b}	23.07±0.10^{a}	42.10±0.02^{c}	11.90±0.42^{a}	11.51±0.25^{a}	11.71±0.30^{a}	0.5
NO_3 (mg/l)	31.2±0.20^{c}	19.4±0.25^{a}	27.0±0.10^{b}	10.9±0.38^{a}	11.7±0.30^{b}	11.2±0.20^{b}	0.5

Values are presented as mean ± SEM; n=3. Mean values with similar superscripts within a row in wet and dry seasons are not significantly different, P>0.05.

Key: EC- electrical conductivity, TDS- total dissolved solid, DO- dissolved oxygen, BOD- biological oxygen demand, SO_4 - sulphate, PO_4 - phosphate, NO_3 – nitrate.

CHAPTER FIVE

5.1 DISCUSSION

Samples of edible bivalve (*Ergeria radiata*) were collected randomly from three locations; Coastal waters, Swali and Opolo markets from Ekoewe community, Bayelsa state, Nigeria during wet and dry season.

Bivalve (*Egeria radiata*) are voracious filter feeders and pump water through their gills to trap food. Unfortunately as a consequence of filter feeding, toxins and other environmental contaminants such as mercury, polycyclic aromatic hydrocarbon and polychlorinated biphenyls are sometimes found in their tissues which could cause implications when consumed by human (Uchidah *et al*., 2017),

In this study higher microbia counts were observed in the wet season relative to the dry season. This could be as a result of many anthropogenic activities and rainfall. Run-offs, from rain might carry raw sewage from the surrounding villages and leachate from waste sites in the catchment area into the Coastal waters. So the bivalves being filter feeders are able to accumulate the isolates in their tissues to a level twice that in the surrounding waters (Ukwo *et al*., 2019). Also, during the wet season there was increase in precipitation which can lead to runoff from land carrying pollutants such as sediments, agricultural runoff and urban pollutant into the Coastal waters where bivalve (*Ergeria radiata*) resides (Udoh *et al.,* 2017). This run off can introduce high microbial counts including bacteria and viruses into the aquatic environment potentially impacting the bivalve (*Ergeria radiata*) lives (Udoh *et al.,* 2017). This agrees with the findings of Antai, (1998) who reported that the high microbial load in the clam is a clear indication that the freshwater clam serves as a medium through which microbes multiplied rapidly. Ekanem and Adegoke (1995), also reported that Cross

River waters were generally of unacceptable quality for growing market shellfish. The authors also posited that clam samples collected from the waters were found to be highly contaminated by bacteria. The findings of high microbial counts during wet season in his study is in accordance with the findings reported by Ejiko and Otene (2019) who reported higher microbial counts during wet season and suggested that the creek receives heavy influx of organic and inorganic materials from surroundings, which are all from anthropogenic activities. Conversely Anagboso (2023) reported higher microbial counts in clam during dry season and which suggested that this could be associated with dilution effect of the water body in clam. It has also been suggested that higher microbial counts which in any season depend on the human activities that is prevailent in the season (Edun *et al*., 2018).

It was observed in this study that bacterial counts were higher from the market sources. This could be attributed to improper handling of bivalve (*Ergeria radiata)* and post harvest practices might have been resulted to higher bacterial load. From both wet and dry season. This was inconcordance with the findings of Ukwo *et al*. (2019).

The total heterothrophic bacteria count in this study from both the wet and dry season ranged from 1.33 ± 0.51 x 10^3 cfu/g to 50±2.00 cfu/g with the highest count obtained from Opolo market. This could be attributed to improper handling and postharvest practices which might have resulted to high microbial load. It could also be attributed to environmental conditions which favours the abundance of many pathogens contamination in the open market. The high total heterothrophic bacterial counts in this study was higher than the findings of Adebayo-Tayo *et al*. (2011) who reported 4.0 x 10^7 to 14.2 x10^8 cfu /g on studies of microbiological of fresh water snails from Niger Delta Creek in Nigeria as well as the findings reported by Udoh *et al*. (2017) who reported high heterotrophic bacterial counts. A huge heterotrophic bacteria counts does not itself present a risk to human health; nevertheless, heterotrophic

counts are good indicator of the overall quality of food consumption (Ekundayo and Fagade, 2005).

The heterotrophic bacteria isolated for wet and dry season included; *Escherichia coli*, *Shigella flexneri* S*almonella enterica, Serretia mercescens, Pseudomonas aeruginosa* and *Bacillus cereus. Escherichia coli* had the highest percentage frequency (18%) of heterotrophic bacterial counts. This was also observed by Adebayo Tayo *et al*. (2011) who reported *E coli* as the most occurring bacteria and stated that this could be indicative of faecal pollution and perhaps part of the normal floral of the bivalve. The findings of this study also agreed with the findings of Udoh *et al.* (2017) and Ukwo *et al*. (2019). *Escherichia coli* gives relevant information regarding the food safety and sanitary conditions of clams and the freshwater respectively (Udoh *et al.*, 2017). The presence of *E. coli* serves as an indicator for pathogenic organisms and faecal contarmination.

The total *Vibrio* counts obtained in this study for both seasons was in the range 9.96±0.95 x 10^5 cfu/g - 19.90±1.27x 10^5 cfu/g. This findings was higher than the *Vibrio* counts of 1.6 to 3.2 x 10^3 cfu/g reported by Adebayo- Tayo *et al*. (2008). According to Lee (2010), the bacteria found in bivalve could be indigenous to marine or estuarine environment, non-indigenous to marine / enteric bacteria that occur maybe due to faecal contamination and bacteria from cross contamination during food preparation and processing. Bioaccumulation of harmful microorganisms in shellfish are compounded by the traditional consumption of certain bivalve shellfish in raw or mildly cooked dishes. The most notable examples include *Vibrio* species which account for 20% of all outbreaks of *Vibrio cholra* (Goel *et al.,* 2010).The *Vibrio* are involved in the transmission and epidemiology of disease leading to endermic and pandermic levels (Goel *et al*., 2010).

In this study, the *Vibrio* species isolated and identified by molecular techniques were *Vibrio paraheamolyticus, V . Cholerae, V. vulificus* and *V. fluvialis,* This findings is in

aconcordance with Ukwo *et al.* (2019) who isolated *Vibrio* species from the Estuary in Niger Delta, and observe them to be far above the stipulated Food and Drug Administration (FDA.2007). Standard for molluscan shellfish . They subsequently concluded that bivalve clam harvested from the study location is not considered safe for human consumption without proper treatment and processing to reduce the level of contaminant which may pose health risk to the consumer.

The total *Listeria* counts in this study for both seasons ranged from $6.12 \pm 0.67 \times 10^3$ - $11.52 \pm 0.76 \times 10^3$cfu/g. This findings is in discordant with 0-1% from fish sample reported by Autio *et al*. (1999). However, higher result of 10 - 55% have been reported by Rocout *et al*. (2000). According to Lee *et al.* (2008), *Listeria monocytogene* is indigenous to marine environment and can be present in bivalve eaten raw or fermented. *L. monocytogenes* have been Implicated as a causative agent is invasive listeriosis, meningitis and febrile gastroenteritis. However, Aygun (2006) stated that the presence of *Listeria* in food might be an indicator of poor hygiene. Ooi and Lober (2005) also reported that, the contamination of food by this bacterium is quite severe since it is one of the few pathogens that can multiply at refrigeration temperature. The most suitable foods for the proliferation of *Listeria monocytogenes* are non-thermally processed products, food kept in refrigeration for a long time, food produced under unsanitary conditions, and cooked and frozen ready-to-eat meals (Wu *et al*., 2022). The *Listeria* species isolated and identified by molecular techniques in this study were *Listeria Monocytogenes*, *L.* Innocoua, *L. Ivanovii.* This findings is similar to that of identified species by Jenney *et al*. (2015) who reported the characterization of *Listeria* spp. from sea food. Antibiotics susceptibility test was carried out on the *Vibrio* and *Listeria* isolates as well as plasmid curing for both wet and dry season isolates. All isolates were at least resistant to one or two antibiotics. After curing, there was a reduction in the resistance to the bacteria isolate to antibiotics. During the wet season *Listeria monocytogene* before curing was resistant to

perfloxacin (PEF), getamycin (CN), zinacef (Z), ampiclox (APX) and streptomycin (S) after curing it was still resistant to perfloxacin (PEF), gentamycin (CN), ampiclox (APX) but after curing it became sensitive to gentamycin (CN) zinacef (Z) and septrin (SXT). During dry season before curing the *listeria monocytogenes* isolated, one showed intermediate to Perfloxacin, the others showed sensitivity. However, the isolates still remained the same after curing, For other antibiotics used some showed sensitivity and others resistant for example the isolates showed resistant to ampiclox before curing, but after curing the isolates became sensitive. After curing there was a reduction in the multidrug antibiotics resistance index (MARI). The *Vibrio* isolated during wet season, showed resistant to septrin (SXT), chloraphenicol CH) ampiclox (AMP) and streptomycin (S). However after curing streptomycin and septrin became sensitive while chloraphenicol and ampiclox remained resistant. Some of the antibiotics showed resistant and others showed sensitivity. While for dry season before curing the *Vibrio* isolate showed resistant to Septrin (SXT), chloraphenicol (CH), and streptomycin (S), while curing septrin (SXT) and streptomycin became sensitive where as chloraphenicol (CH) and ampiclox (AM) remained resistant. High level of bacterial resistantce to antibiotics are indications that such bacterial groups cohabiting common environments may express similar pattern (Malik and Ahmad, 1994).

The *ctxA* virulence gene was detected in all the *Vibrio* species except *V. paraheamolyticus.* Microbiological standard for shell fish as outlined by FDA (2011), indicated that *V. cholera* should be absent in sea food for human consumption, while counts of *V. parahaemolyticus* should not exceed 1000 cfu/g.

The virulence gene *hlyA* (hemolysin gene) were detected using specific primer. The *HlyA* genes were detected in all the *Listeria* species except *L. Monocytogene,* for dry season This is in discordant with the findings reported by Jenney *et al*. (2015) who reported the presence of *hlyA* genes in all the *Listeria monocytogenes is*olated and identified from sea food. *Listeria*

monocytogenes is widely recognized as a principal human pathogen that causes serious diseases, also *Listeria monocytogenes* from sea food suggests that there is risk of acquiring *Listeriosis.*

Antibiotics resistant gene, penicillin binding protein (Pbp) and virulence gene hemolysin gene (*HlyA)* were detected in the *Listeria* species identified and *aph(2")-If* gene of *Vibro* spp. and ctxA gene. All isolates were at least resistant to one or two antibiotics, while some were multi antibiotic resistant. After curing, there was a reduction in the resistance to the bacteria isolate to antibiotics. Ojo and Oso (2009) reported that after curing the number and sizes of the plasmids present in the isolate are critical and that the number of different plasmid carried by an organism varies from species to species.

Antibiotics resistant gene, penicillin binding protein (Pbp) detected in the *Listeria* isolates is a class B transpeptidase involved in cell division and target the beta lactam antibiotics like the penicillin and ampicillin. The Pbp2b is crucial for synthesis of peptidoglycan,the rigid layer of the bacteria cell wall. It functions as a traspeptidase catalsing the crosslinking peptidoglycan chains which is essential for maintaining the the shape of the cell and it interacts with various structure. The pbp gene is a major target for betam lactam antibiotics,it works by inhibiting the pbp in those antibiotics and its inhibition leads to cell lysis and death

The *aph(2")-If* gene of *Vibro* confers the resistance to aminoglycoside antibiotics by encoding the enzyme that modifies and inactivate the drugs(Olaniron *et al.*, 2015)

Aminoglycoside gene encode an aminotransferase an enyme that adds phosphste to the aminoglycoside molecule. The modification prevents the antibiotic from binding to its bacterial target, effectively neutralizing the anti bacterial effect (Okorie-kanu *et al*., 2015). The *aph(2")-If* gene is normally located on the plasmid, which are mobile genetic element. This means it can easily be transferred between different bacteria.

The presence of e*aph(2")-If* gene and other antibiotic resistant gene in *Vibrio* spp can lead to treatment failure.

The economic and health problems caused by this shellfish contamination are further compounded by the development of antibiotic resistant among some of the isolates such as *Listeria monocytogene* and *Vibrio paraheamolyticus.* According to the findings reported by Malik and Ahmad (1994), high levels of bacterial resistance to antibiotics are indications that bacterial groups cohabiting common environments may express a similar antibiotic pattern.

The *hlyA* gene which code for hemolysin gene play a role in lysis of eythrocyte by forming pores of varying diameter in the membrane. The *ctxA* virulence gene is responsible for diarrhea symptoms of cholera (Olaniron *et al*., 2015). *CtxA* gene is not found in all *Vibrio*s spp, *ctxA* gene has toxic strain and non toxic strain. Not detecting the ctxA gene in any of the *Vibrio* spp could be attributed that it is non toxic strain.

In this study, the plasmid profile of the *Listeria* and *Vibrio* isolates had one , two or more plasmid band size, the number of plasmid and band size identified varies among all the isolates. *Listeria monocytogene* had four band sizes of 1500 bp, 1400 bp, 900 bp and 800 bp while other *L monocytogenes* and *L invanovii* had one plasmid band size. *Vibrio paraheamolyticus* had two plasmid band size of 700 bp and 600 bp, while *V Cholerae* had one plasmid band size of 1500 bp, another *V paraheamolyticus* had 1300 bp. This study shows that the number of plasmid and band size identified varies among all the isolates. Ozbey *et al.* (2007) reported that the greater number of plasmids, the easier it is to identify a particular strain among a collection of isolates in an outbreak investigation.

In this study, proximate composition was carried out to determine the nutritional value of the bivalve (*Errgeria radiata*). Thsee included moisture content , protein, fat content, fibre, ash carbohydrate and dry matter. These parameteres were all analyzed during wet and dry

season and showed that, there was no significant difference in the results obtained during the wet and dry season.

The moisture content for both seasons which ranged from 75.52 ±1.0 % to 77.98 ± 25.77 %.There was no seasonal variation in bivalve (*Ergeria radiata*). The moisture content is in concordance with Eneji *et al.* (2008) who reported 78.64 % from land and water snail This is high compared to Opeh *et al.* (2018) who reported 20.47± 0.4 %. The high moisture content is in agreement with 77.11±2.05% from bivalve in Num river, Niger Delta, Nigeria as reported by Chinnadurai *et al.* (2023) who suggested that the high moisture content of bivalve is very high during monsoon period could be due to the decrease in salinity. This might be due to the loss of salt and gain of water to compensate the osmotic pressure. An inverse relationship is usually observed between the salinity and percentage of moisture content in many bivalves (Maqbool, 1993).

The protein content of bivalve (*Ergeria radiata*) for both seasons in this study ranged from 7.70±0.26 % to 8.25±1.60 %. This is in concordance with report of Vineather *et al.* (2024) who suggested the protein level of molluscan are greatly influence by the reproductive cycle. Fagbuaro *et al*. (2006) has also reported that proteins from giant land snails are essential nutrients for human body. This protein sources are one of the building blocks of body tissue and can serve as fuel source. Protein deficiency and malnutrition can lead to a variety of ailment including mental retardation and kwashiorko (Miletic *et al*., 1991). Protein plays a very important role in growth and maintenance of vital bodily function. Protein is vital for most cellular processes and is the major component of lean muscle mass. Molluscan shellfish protein is considered high-quality protein because of its essential amino acid profile and it is classified as a highly digestible protein source (Miletic *et al*., 1991).

The ash content of bivalve (*Ergeria radiata*) in this study for both season ranged from 3.02±0.47 % to 4.11±0.88% and is in concordance with 4.72±0.60% reported by Vineather *et al*. (2020) and 3.22% repored by Engman *et al*. (2013).

The crude fibre content of bivalve (*Ergeria radiata*) obtained for both seasons were in the range of 4.19 ± 0.06 to 4.49 ± 0.34 %. The levels of crude fibre obtained in bivalve (*Ergeria radiata*) in this study is not in concordance with the range values reported by Eneji *et al*. (2008) and Adebayo- Tayo *et al.* (2011) an higher values reported by Soniran *et al*. (2013). However, Eruvbetine (2012) reported total absence of crude fibre in snail meat.

Dry matter content of bivalve (*Ergeria radiata*) obtained in this study, this result is not in concordance with the range repored by Adebayo- Tayo *et al*. (2011).

The fat content obtained in bivalve (*Ergeria radiata*) in this study for both seasons is in agreement the with report of Eneji *et al.* (2008) from land and water snail species. The values obtained for the fat content indicated that clam meat is low in fat. The carbohydrate content in bivalve (*Ergeria radiata*) in this study for both season ranged from 7.42 ± 0.72 %. - 8.69 ± 1.96%. This is low compared to 58.85 ± 9.53 % reported by Opeh *et al*. (2018). Where as itis is in agreement with 6.57% reported by Eneji *et al*. (2008) and Vineather *et al*. (2020) .

The mineral content of bivalve (*Ergeria radiata*) analysed in this study for wet and dry season were Na, K, Ca P and Mn. It was oserved that there was no seasonal variation. of the result in this study.

The magnesium content of bivalve (*Ergeria radiata*) for both season in this study ranged from 14.60 ± 0.51 to 15.72±0.51mg/kg. There was no seasonal variation in the content of magnesium analyzed. This is in concordance with 9.68 to 13.28 mg/kg as reported by Ukwo *et al*. (2020). In a study reported by Eneji *et al*. (2008) magnesium content was in

concordance with 34.73±0.02 mg/kg. Magnesium plays important role in human and animal nutrition as a constituent of bone, necessary for healthy muscles, nerves and metabolism. Magnesium is an essential trace element which plays a major role in the metabolic processes that take place in human system and regulation of blood. It may function as cofactor to some enzymatic activities (Grzebisz, 2011).

The content of manganese for both seasons in this study was in the range 0.081 ± 0.14 mg/kg to 0.85 ± 0.14 mg /kg. Manganese is involved in enzyme function and is even a component of several enzymes, such as arginase, concanavalin A, glutamine-synthase, and many others There appears to be a number of roles that manganese plays in osteoporosis, diabetes, and seizure disorder. Manganese toxicity may result in moderate to severe neurological symptoms to those similar to Parkinsons disease (Horning *et al*., 2015).

Phosphorus content of bivalve (*Ergeria radiata)* in this study for both seasons was in the range 180.45±0.27mg/kg - 181.11± 0.21 mg/kg. This findings is not in concordance with 51.49 – 59.79 mg/100g as reported by Eneji *et al*. (2008). It is also not in concordance with 61.24 mg/100 as reported by Imevbore and Ademosun (1988). This findings is lower than the values 272 mg/ 100g and 269.20 ± 3.90 mg/100g reported by Eruvbetine (2012) and Engmann *et al*. (2013) respectively for snail meat. phosphorus plays a major role in glucose metabolism and also essential element of the DNA molecule. Phosphorus is necessary as an essential mineral that is primarily used for growth and repair of human cells and tissues, as well as healthy energy levels (Kraft, 2015).

In this study, potassium content in bivalve (*Ergeria radiata*) for both seasons was in the ranged between 508.92 ± 0.1 - 520.1 0± 0.3 mg /kg. This was higher compared to 63.30 mg, 60.79 – 69.00 mg 100 g- as reported by Imevbore and Ademosun (1988); Eneji *et al*. (2008) . This findings was not in agreement with low values of 3.8 mg 100 g-1 as reported by

Eruvbetine (2012) though slightly differ from the range of 162.13 – 179.99 mg 100 g^{-1} and 331.8±4.6 mg $100g^{-1}$ as reported by Ubua (2011) and Engmann *et al*. (2013) respectively. Potassium aids in fluid balance, regular heart rhythm, regulation of nerve impulse conduction and cell metabolism. Potassium is also involved in muscle contraction, healthy blood pressure, fluid balance in cells, and bone longevity (Kraft, 2015).

Sodium content of bivalve (*Ergeria radiata*) in this study for both seasons was in the ranged from 402.2 ± 0.1 – 404 ± 0.71 mg/kg which was higher than 36.20 mg/100g as reported by Kalio and Etela (2011) and 70.00 mg/100g as reported by Eruvbetine, (2012). Sodium is a vital electrolyte that plays a crucial role in maintaining fluid balance, nerve and muscle function, and blood pressure regulation in the body. It helps the body keep fluids in a normal balance, including blood volume, and ensures proper nerve and muscle function. Sodium also helps cells absorb nutrients (Wright *et al*., 2018).

The calcium content in bivalve (*Ergeria radiata*) in this study was in the range of 80.13± 0.22 to 88.67 ±0.13 mg/kg. This findings is in agreement with the report of Simpson (1990) and Imevbore and Ademosun (1988), also with 162 mg as reported by Wosu (2003) and 152.12 – 181.50 mg 100 g^{-1} report of Eneji *et al*. (2008). The findings was not in corcondance with 585.50±5.6 mg 100 g-1 as reported by Engmann *et al*. (2013). Calcium is the most abundant metal in the human body and is essential to bone biogenesis, as deficiencies can cause osteoporosis

The iron content in bivalve (*Ergeria radiata*) in this study for both season ranged from 7.22 ± 0.11 - 9.69 ± 0.28 mg/kg. The iron content observed in the study was high compared to the 0.79±0.04 as reported by Opeh *et al.* (2018), which agreed with 9.69 ± 0.29 as reported by Okuzumi and Fujii (2000). Iron content of 6.79±11.0 mg/ for mollusc has also been reported by Obande *et al*. (2013). The content of iron in the present study was high compared to that

reported by Imevbore and Ademosun, (1988) and 0.9 – 1.5 mg as reported by Omole *et al*. (2007) and Okon and Ibom (2012). The primary metabolic function of iron is the transport of oxygen throughout the body as acomplex with hemoglobin and as a cofactor for electron transport (Eguchi and Saltman, 1987). Iron deficiency is called anemia, and symptoms include fatigue, weakness, and difficulty maintaining body temperature (Miller, 2013). The concentration of zinc in bivalve (*Ergeria radiata*) in this study for both seasons ranged from 1.12 ± 0.12 mg/kg to 1.34±0.06 mg/ kg. This value was similar to 1.00 mg/100g^{-1} and 0.70 mg/100g^{-1} as reported by Eruvbetine (2012). Low zinc content in bivalves (clam) implies that clam meat, is non-toxic to health. Zinc toxicity is rare, but, at concentrations in water up to 40 mg/kg, may induce toxicity, characterized by symptoms of irritability, muscular stiffness and pain, loss of appetite, and nausea (WHO, 2007).

No seasonal variations., were observed the mental (Cu, Hg, Pb and As).

The concentration of copper in bivalve (*Ergeria radiata*) in this study for both season ranged from 0.12 ± 0.20 - 0.87±0.0 mg/kg. The Cu in this study. the recorded low concentration compared to 12.6 - 16.3 mg/kg as reported by Adebayo-Tayo *et al*. (2011). Copper is essential part of several enzyme and it is necessary for the synthesis of haemogloblin. The recommended daily allowance (RDA) for copper in growing children is 440 mg/day and 900 mg/day in adults (Ukwo *et al.,* 2019).

The lead content in bivalve (*Ergeria radiata)* for both seasons ranged from 0.03 ± 0.01 -0.06 ± 0.01 mg/kg. This is in agreement with the findings of Adebayo-Tayo *et al.* (2011) who reported 0.03 mg/kg from bivalves. Lead causes renal failure and liver damage in humans. There was no detectable concentration of arsenic and mercury present in the samples for both seasons. Mercury is a highly toxic element, as high concentration of mercury is toxic to the central and peripheral nervous systems in humans with no known safe level of exposure.

Mercury concentration in the whole body is usually lower than 10 m/l. Arsenic is highly toxic to humans as well.

Low concentrations of all the PAHs in the samples for both season (<0.98±1.33ppm) were observed in this study. These PAH sources were from anthrogenic activities surrounding the Coastal waters. This agrees with low concentration of 0.77±0.2 ppm in tissue concentration of PAH in bivalve shellfish from Niger Delta, as reported by Ukwo *et al*. (2022). It was suggested that though the consumers may not consider the possibility of instant negative health consequences resulting from the accumulation of these contaminant in their tissues , cannot be ignored. This is because PAH have effect in human lives and exposure of aquatic life to oil spill need to be monitored closely because the key outcome considered to PAH is the related health risk of carcinogenic effect. Therefore, maintaining clean environment is important for several socio-economic reason. The finding obtained in this study was in disagreement with higher concentration (1.7– 7.33 ppm) as reported by Alani *et al*. (2004). The PAHs could be from combustion sources and PAH like napthalene and phenanthrene are acutely toxic at whole body concentration above 50000 ppm. The PAHs are found throughout the environment in the air, water and soil as complex mixtures (Jacob, 1994). According to Hellou *et al*. (2002), PAHs potentially contributed to modern day diseases including cancer, damage reproductive system, disrupted endocrine and immune system neurobehavioural effect. Pavola *et al*. (2003) stated that their low aqueous solubilty limited volatility and recalcitrance towards degradation, thus allow PAHs to accumulate to level at that can exert toxic effect upon the environment. Manoli (1996) reported that PAHs are probably the most important analytes because many of these compounds are potential or proven carcinogens.

The bivalve (*Ergeria radiata*), habitat (Coastal water) was accessed to determine the pollution from anthropogenic activities for wet and dry season.

The physiochemical paramteres of the Coastal water in the wet season exceeded the permissible limit set by WHO. This could be as a result of anthropogenic activities such as industrial waste, runoff from surroundings which may introduce pollutants into the Coastal water influencing the various parameters exceeding the permissible limit (Okoro and Akande, 2025).

The pH of the Coastal waters of this study showed narrow range of variation near neutrality for wet and dry season (7.11±1.12 and 7.27±1.30). This is in corcondance with the pH value of 7.45 as reported by Leizou *et al*. (2017). Similar findings has also been reported by Passanna and Ranjan (2010). The dry sesason physiochemical parameters of Ekowe Coastal waters were within the permissible limit set by the WHO. This might be due to the dilution effect. of the Coastal water. The findings in this study agreed with that treported by Okoro and Akande (2025) during which it was suggested that seasonal variation can signinificantly affect the water properties of Coastal waterscons idering the amount of precipitation, dilution rate and flow of velocity into the Coastal waters.

5.1 Contribution to knowledge

The study has contributed to knowledge in the following ways;

It is now established that bivalves (*Ergeria radiata*) habour *Listeria* and *Vibrio* species. These included *Listeria innocua, Listeria monocytogenes, Listeria ivanovii, Vibrio parahaemolyticus*, *Vibrio cholerae, Vibrio vulnificus,* and *Vibrio fluvialis*.

The *Listeria* and *Vibrio* species isolated from bivalves (*Ergeria radiata*) revealed the presence of *hylA*, *pbp2b*, and *ctxA*, *aph*(2")-*If* virulence and antimicrobial resistance genes, for *Listeria* and *Vibrio* respectively.

Heavy metals concentration in bivalves (*Ergeria radiata*) was low with the absence of arsenic and mercury. The polycyclic aromatic hydrocarbons (PAHs) in bivalves (*Ergeria radiata*) were within the permissible limit of World Health Organization (WHO).

5.2 Conclusion and Recommendation

Food-borne illnesses due to consumption of bivalves (*Ergeria radiata*) may occur when molluscs that contain pathogenic microorganisms are consumed raw or under cooked. Findings from this study revealed that bivalves (*Ergeria radiata*) harbour bacterial pathogens and low concentration of polycyclic aromatic hydrocarbons (PAHs). The bivalve (*Ergeria radiata*) meats are highly rich in protein, low in fat, and a good source of minerals. However, despite their nutritional value, bivalve harbour some pathogenic bacteria of public health concern, particularly when consumed raw or not properly cooked. Therefore, it is imperative to ensure adequate measures in their preparation to prevent food-borne illnesses on consumption.

It was observed that bacterial counts was higher from the markets sources, therefore there should be a strict adherence to good Agricultural Practices (GAP) among the market sellers. Deligent and strict enforcemement of sanitary conditions of food contact surfaces and handling areas and good personal hygiene practices should be embark upon to reduce the potential contanmination of sea food causes by *Listeria monocytogenes* in the retail level. Prohibition of anthropogenic activities from the environment of the Coastal waters. To achieve this, monitoring team should be set up to monitor the anthropogenic activities going on around the Coastal waters. It is important to highlight the fact that bivalve (*Ergeria radiata*) from Ekowe community Coastal water harbour pathogens that could lead to disease outbreak. Therefore, in order to promote desirable goods and prevent the growth of infections and spoiling organisms in the interest of public health, suitable handling and post-

harvest best practices are used. It is essential to comprehend the physiochemical characteristics of coastal water in order to assess the aquatic ecosystem's health.

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APPENDICES

APPENDIX I

MORPHOLOGICAL, BIOCHEMICAL AND SUGAR TEST OF BACTERIA ISOLATES

Gram stain	-	+	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+
Cell type	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Cell arrangement	C	P	P	C	C	C	C	C	S	S	S	C	S	S	S	S	S	S	S	S	S	S	S
Urease	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Indole	+	-	-	-	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
Citrate	-	-	+	+	-	+	+	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
H_2S	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Oxidase	-		-	+	-	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
Lactose	+	-	-	-	-	-	-	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+
Sucrose	+	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+
Glucose	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fructose	+	+	+	-	+	+	-	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Starch	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sorbitol	+	-	+	-	-	+	+	+	+	+	+	+	+	-	+	-	+	-	-	-	+	+	-
Organism	*Escherichia coli*	*Salmonella enterica*	*Serratia marcescens*	*Pseudomonas aeruginosa*	*Shigella enterica*	*Vibrio* spp [1]	*Vibrio* spp [2]	*Vibrio* spp [3]	*Vibrio* spp [4]	*Vibrio* spp [5]	*Vibrio* spp [6]	*Vibrio* spp [7]	*Vibrio* spp [8]	*Listeria* spp [1]	*Listeria* spp [2]	*Listeria* spp [3]	*Listeria* spp [4]	*Listeria* spp [5]	*Listeria* spp [6]	*Listeria* spp [7]	*Listeria* spp [8]	*Listeria* spp [9]	*Listeria* spp [10]

Keys: + = Positive - = Negative R = Rod C = Clusters P = Pairs S = Single

APPENDIX II

Proximate analysis (%) for wet and dry seasons of *Ergeria radiata* for wet and dry season

	WET SEASON			DRY SEASON		
	Coastal water	**Swali market**	**Opolo market**	**Coastal water**	**Swali market**	**Opolo market**
Moisture	75.96±0.74^{a}	75.29±2.63^{a}	75.96±0.74^{a}	76.60±1.52^{ab}	75.29±2.63^{a}	76.25±0.63^{ab}
Ash	4.077±028^{ab}	4.00±0.31^{ab}	4.10±0.27^{ab}	3.023±0.28^{a}	4.00±0.31^{ab}	4.11±0.88^{ab}
Fat	0.743±0.05^{a}	0.77±0.09^{a}	0.74±0.49^{a}	0.74±0.04^{a}	0.82±0.06^{a}	0.78±0.07^{a}
Protein	7.42±0.72^{a}	7.59±0.70^{a}	7.59±0.67^{a}	7.03±0.25^{a}	7.34±0.20^{a}	7.29±0.24^{a}
NFE	7.42±0.72^{a}	7.69±0.35^{a}	7.53±066^{a}	8.44±1.70^{ab}	8.69±1.96^{ab}	8.31±2.02^{ab}
Fibre	4.17±0.29 ^{a}	4.34±0.50 ^{a}	4.49±0.34 ^{a}	4.18±0.07 ^{a}	4.34±015 ^{a}	4.19±0.06 ^{a}
dry matter	24.04±0.74^{ab}	22.74±1.48^{a}	24.14±1.23^{ab}	23.40±1.53^{a}	24.07±1.17^{ab}	23.26±1.24^{a}

TABLE 2:Mineral analysis (mg/kg) f *Ergeria radiata* for wet and dry season

	WET SEASON			DRY SEASON		
	Coastal water	**Swali market**	**Opolo market**	**Coastal water**	**Swali market**	**Opolo market**
Mg	15.20±0.14^{ab}	15.10±0.55^{ab}	14.60±0.51^{a}	14.70±0.11^{a}	15.72±0.51^{ab}	15.11±0.55^{ab}
Ca	88.10±0.11^{ab}	88.00±0.15 ^{a}	81.80±0.16 ^{a}	86.11±0.14^{ab}	88.67±0.13^{ab}	80.13±0.22 ^{a}
K	510.10±0.12^{a}	510.50±0.32^{a}	510.20±0.52^{a}	520.10±0.13^{ab}	508.92±0.10^{a}	511.10±0.25^{a}
Na	404.10±0.51^{ab}	404.2±0.51^{ab}	404.55±0.71^{ab}	404.10±0.22^{ab}	402.20±0.19^{a}	403.99±0.51^{a}
P	180.20±0.12^{a}	180.45±0.2^{a}	180.52±0.90^{a}	181.10±0.17^{ab}	180.45±0.27^{a}	181.11±0.21^{a}
Mn	0.85±0.11^{a}	0.78±0.78^{a}	0.81±0.50^{a}	0.85±0.14^{a}	0.73±0.55^{a}	0.84±0.15^{a}

APPENDIX III

Heavy metals (mg/kg) for wet and dry seasons of *Ergeria radiata* for wet and dry reason

	DRY SEASON						DRY SEASON					
	Fe	**Zn**	**Cu**	**Pb**	**Hg**	**As**	**Fe**	**Zn**	**Cu**	**Pb**	**Hg**	**As**
Coastal waters	8.62±0.41[a]	1.28±0.21[a]	0.12±0.20[ab]	0.047±016[a]	ND	ND	7.22±0.11[a]	1.12±0.12[a]	0.15±0.03[a]	0.03±0.01[a]	ND	ND
Swali market	8.54±0.37[a]	1.23±1.22[a]	0.087±0.01[a]	0.060±0.01[a]	ND	ND	8.26±0.14[ab]	1.19±0.67[a]	0.07±0.02[a]	0.03±0.01[a]	ND	ND
Opolo market	9.69±0.29[ab]	1.34±0.13[a]	0.077±0.06[a]	0.06±0.01 [a]	ND	ND	8.43±0.28[ab]	1.28±0.02[a]	0.07±0.01[a]	0.03±0.01[a]	ND	ND

Mean values with similar superscripts within a column are not significantly different, P<0.05.

Key: Fe = Iron, Zn = zinc, Cu = Copper, Pb = Lead, HG = Mercury, As = Arsenic

APPENDIX IV

Gene sequency of *Vibrio* and *Listeria* isolte for wet season

WET SEASON

>V9Vibrio parahaemolyticus

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>V13 Vibrio parahaemolyticus
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>V10 Vibrio cholerae

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>V11 Vibrio vulnificus

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>V15 Vibrio fluvialis

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>V18 Listeria innocua

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>V19 Listeria monocytogenes

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>V24 Listeria monocytogenes

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>V25 Listeria monocytogenes

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>26 Listeria ivaovii

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APPENDIX V

Gene sequency of *Listeria* and *Vibrio* isolate for dry season

DRY SEASON

>BV1 Listeria monocytogenes

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>BV3 Vibrio parahaemolyticus

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>BV4 Vibrio cholerae

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>BV5 Vibrio vulnificus

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>BV6 Listeria monocytogenes

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> BV8 Vibrio parahaemolyticus

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>BV9 Listeria monocytogenes

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>BV15 Vibrio cholerae

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APPENDIX VI

Selective Supplement

A **selective supplement** used for the detection and isolation of Listeria species when incorporated into PALCAM Agar. Active Ingredients per 5-mL Vial (Each vial prepares 500-mL of media) Polymixin B 5-mg Acriflavine 2.5-mg Ceftazidime 10-mg

Reconstitution Procedure

The lyophilized supplement was reconstituted prior to use:

1. Aseptically 2.5-mL of ethanol was added; vial was swirl briefly and 2.5-mL of sterile, purified water was added to the vial.

2. vial was mixed gently until supplement was completely dissolved.

Procedure for Media Preparation

PALCAM Agar

one 5.0-mL vial of PALCAM Supplement was allowed to adjusted to room temperature prior to use.

2. 500-mL of PALCAM Agar was prepared and sterilized according to the manufacturers' recommendations. (it was boiled and then autoclave for 15 minutes at 121°C)

3. medium was cooled to 45 to 50ºC in a water bath.

4. supplement was mixed very well before adding.

5. 5.0-mL vial of PALCAM Supplement was aseptically added to the medium; it was mixed very well to incorporate the supplement thoroughly into the medium.

6. The medium was dispense into sterile petri dishes. medium was allowed to set on a cool, level surface.

PALCAM Broth

1. one 5.0-mL vial of PALCAM Supplement was allowed to be adjusted to room temperature prior to use

2. 500-mL of PALCAM Broth was prepared and sterilize according to the manufacturers' recommendations. (it wasboiled and then autoclave for 15 minutes at 121°C).

3. medium was cool to room temperature.

4. supplement was mixed thoroughlyl before adding.

5. the 5.0-mL vial of PALCAM Supplement was aseptically added to the medium;it was mixed verywell to incorporate the supplement thoroughly into the medium.

6. the medium was aseptically dispense into sterile tubes or containers.

APPENDIX VII

COMPOSITION OF MEDIA

NUTRIENT AGAR

Peptone	-	5.0g
Beef extract	-	1.50ml
Sodium chloride	-	5.0g
Agar	-	15.0g
pH	-	7.3 ±0.2

Procedure

28g of the powder was dispersed in 1 liter of deionized water. The medium was allowed to soak for 10 minutes, swirled to mix and sterilized by autoclaving at 121 ^{0}C for 15 minutes at 1.5psi. The medium was allowed to cool at 47 ^{o}C, mixed well then poured aseptically into sterile petri-dish and was allowed to solidify.

Tryptone Soy Agar with 0.6%yeast extract agar

Formula * in g/L

Trypton..... 17.00

Yeast Extract..... 6.00

Soybean Peptone.....3.00

Sodium chloride..... 5.00

Dextrose 2.50

Di-Potassium Phosphate.....2.50

Agar.....15.00

Final pH at 25ºC 7.3 ± 0.2 F

procedure

51 g of powder was suspended in 1 L of distilled water and brought to boil. Then it was istributed into containers and sterilize in the autoclave at 121ºC for 15 minutes.

Thiosulphate citrate bile salt sucrose agar (total *Vibrio* count)

Ingredients	gm/L
Yeast extract	5.0 g
Proteose Peptone	10.0 g
Sodium thiosulfate	10.0 g
Sodium citrate	10.0 g
Ox gall	5.0 g
Sodium cholate	3.0 g
Bromothymol blue	0.04 g
Thymol blue	0.04 g
Saccharose	20.0 g
Sodium chloride	10.0 g
Ferric citrate	1.0 g

Agar	15.0 g

Procedure

88g of the medium was suspended in 1 litre of purified water and it was heated with frequent agitation and boiled for 1 min to completely dissolve the medium. It was allwed to cool at room temperature before it was poured into sterile petri dishes.

Mueller Hinton Agar (MHA)

Ingredients	**In Gram/Litre**
Beef Extract	2.00 gm
Acid Hydrolysate of Casein	17.50 gm
Starch	1.50 gm
Agar	17.00 gm
Distilled Water	1000 ml

Final pH 7.3 ± 0.1 at 25ºC

Procedures

A 38mg of the medium was suspended in a liter of water (distilled water). it was boiled for one minute; just enough for the medium to be dissolved completely. Was autoclave for 15 minutes at 121 ^{o}C and allowed to cool down at room temperature. Once the agar has cool down, pour into the sterile petri dish, the agar was in uniform depth and was allowed to cool at room temperature. The final pH level was 7.3 ± 0.1 at 25ºC. The plate was stored at a temperature.

GRAM STAINING AND BIOCHEMICAL REAGENTS

STAIN

Formulation		**Weight**
Crystal violet	-	56 g
Ethanol (95%)	-	400ml
Lugol iodine	-	60ml
Safranin	-	2.5g
Distilled water	-	220ml

REAGENTS

Composition	**Amount**
CATALASE REAGENTS	
Hydrogen peroxide	3%
OXIDASE TEST REAGENTS	
Tetramethly-p-phenylenediamine-dihydrochloride -	0.1g
Distilled water -	1000ml
INDOLE MEDIUM	
Peptone -	20.0 g
Sodium chloride -	5.0 g
Distilled water -	1000 ml
pH -	7.4
SIMMON CITRATE AGAR	
Sodium ammonium phosphate -	1.50 g
Potassium dihydrogen phosphate -	1.0 g
Magnesium sulphate -	0.2 g
Sodium citrate -	2.5 g
Bromothymol blue -	0.016 g

Distilled water	-	1000 ml
pH	-	7.0

UREA AGAR BASE

Peptone	-	1g
Sodium Chloride	-	5g
Potassium dihydrogen- Sulphate K H_2PO_4	-	2g
Glucose	-	5g
Agar powder	-	20g
Distilled water	-	1000ml

PEPTONE WATER

This medium was used to enrich and develop the inoculums that were used to inoculate the agar plates. It was also used to maintain the culture for some biochemical tests.

Composition

Peptone	-	10g
Sodium Chloride	-	5g
Distilled Water	-	1000ml
pH	-	7.6

Preparation

The powdered medium was used and it was prepared as directed by the manufacturer. Fifteen grams of the powdered medium (Oxoid) was dissolved in 1000 ml of distilled water. The medium was sterilized by autoclaving at 121°C for 15 minutes

Sugar Utilization Medium

Peptone water	2.0 g
Sodium chloride	5.0 g
Water	1000 ml
Phenol red	7.0

Weighed 10% v/v of each of the following sugar was dissolved in 1000 ml of distilled water and autoclaved at 121 ^{0}C for 15 minutes. This was dispensed aseptically into sterile test tube containing Durham's tubes

APPENDIX VIII

CHROMATOGRAM GRAPHS

```
File       :C:\gcms\1\data\Dr Agoro\D.D
Operator   : EISL LAB
Acquired   :  5 Apr 2024  18:05     using AcqMethod PAH_24.M
Instrument :    GCMSD
Sample Name: D
Misc Info  :
Vial Number: 5
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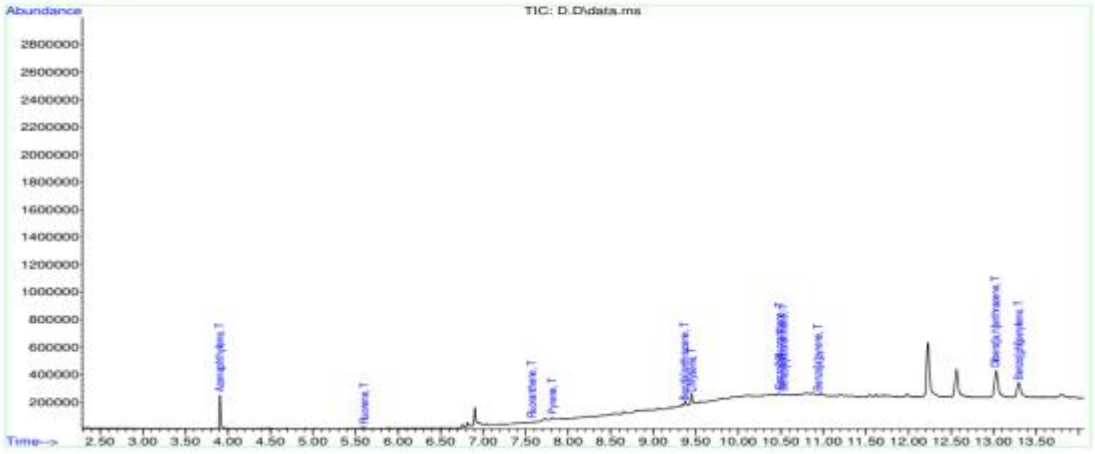
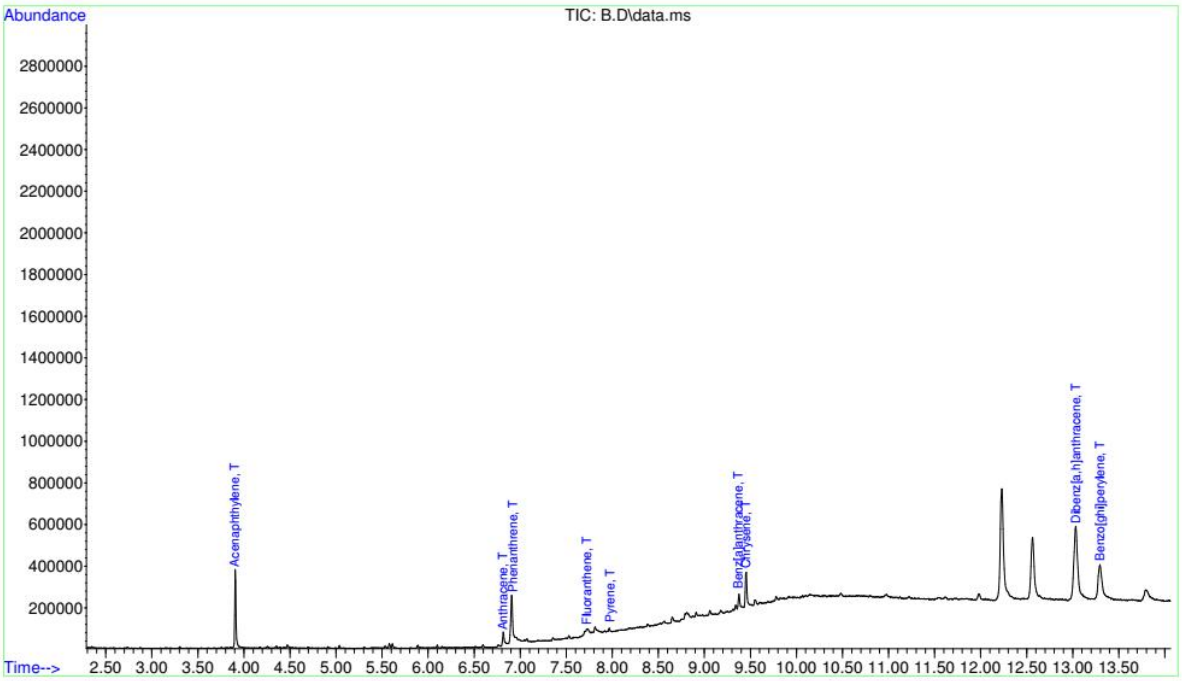
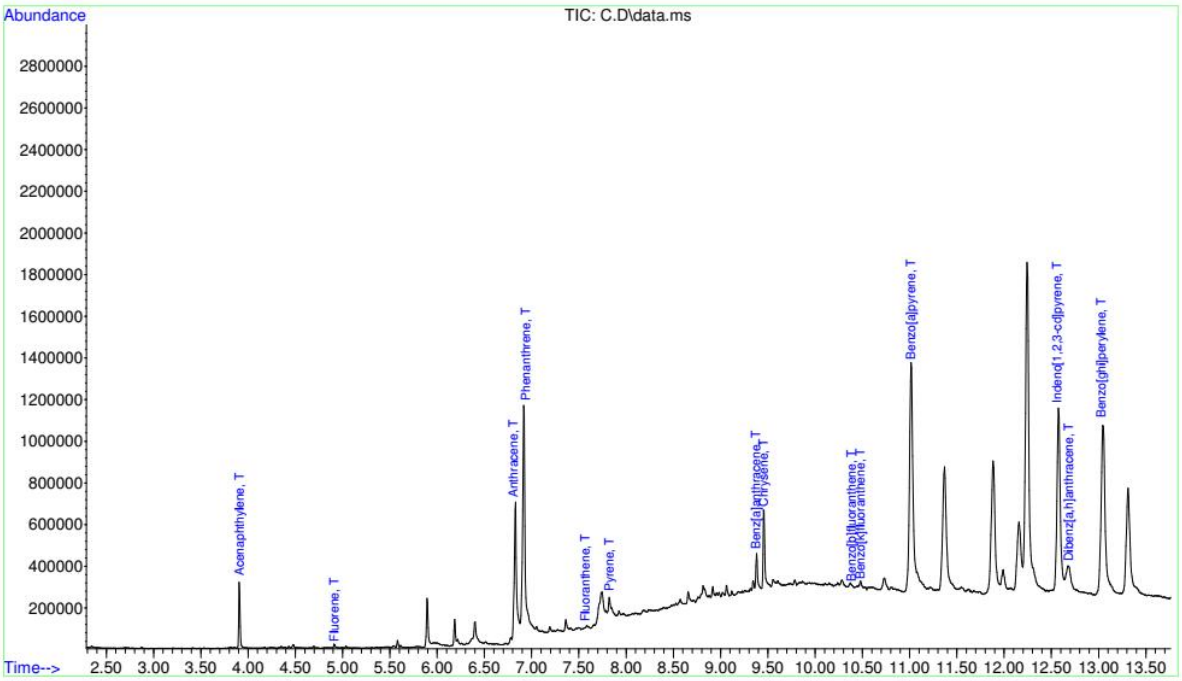



```
File       :C:\gcms\1\data\Dr Agoro\B.D
Operator   : EISL LAB
Acquired   :  5 Apr 2024  17:46     using AcqMethod PAH_24.M
Instrument :    GCMSD
Sample Name: B
Misc Info  :
Vial Number: 3
```



```
File       :C:\gcms\1\data\Dr Agoro\C.D
Operator   : EISL LAB
Acquired   :  5 Apr 2024  17:09     using AcqMethod PAH_24.M
Instrument :    GCMSD
Sample Name: C
Misc Info  :
Vial Number: 4
```

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File       :C:\gcms\1\data\Dr Agoro\E.D
Operator   : EISL LAB
Acquired   :  5 Apr 2024  18:25     using AcqMethod PAH_24.M
Instrument :    GCMSD
Sample Name: E
Misc Info  :
Vial Number: 6
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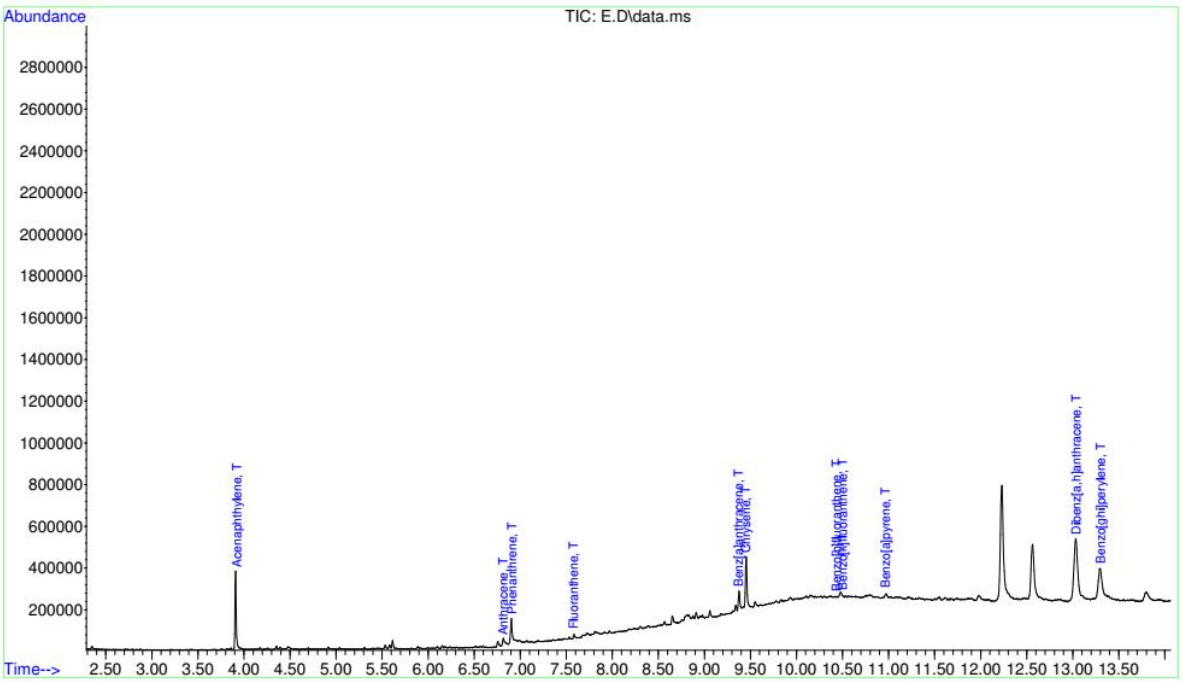

```
File       :C:\gcms\1\data\Dr Agoro\F.D
Operator   : EISL LAB
Acquired   :  5 Apr 2024  18:48     using AcqMethod PAH_24.M
Instrument :    GCMSD
Sample Name: F
Misc Info  :
Vial Number: 7
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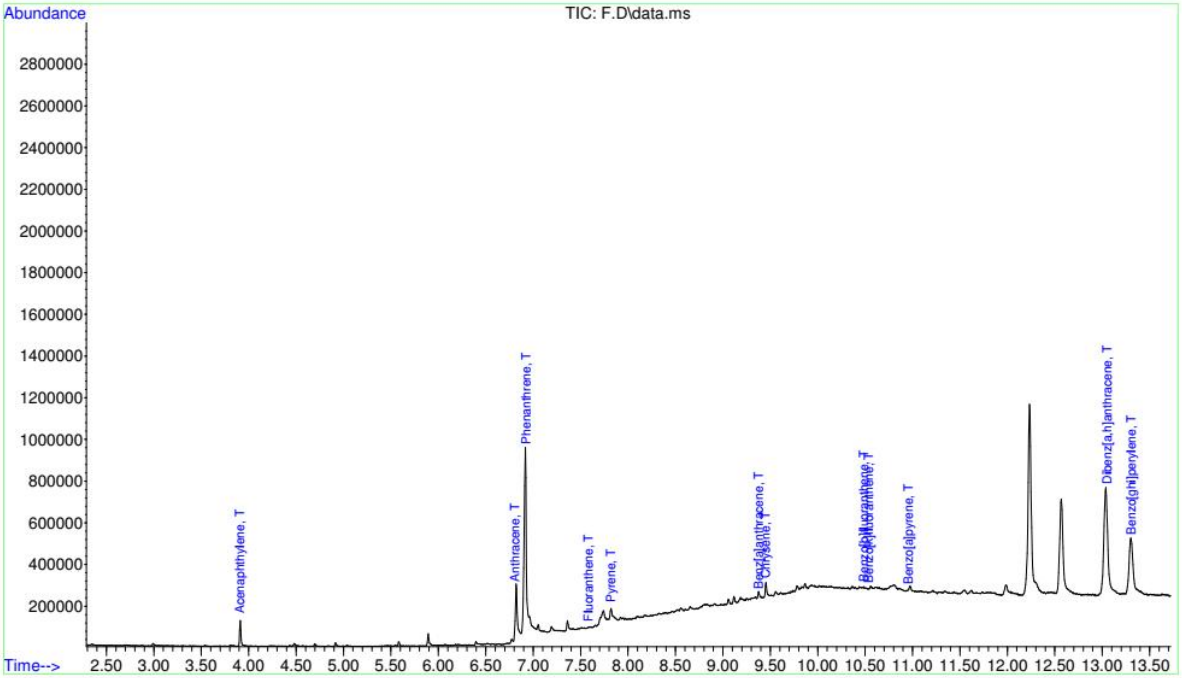

APPENDIX VIX

PROXIMATE ANALYSIS FOR WET AND DRY SEASONS

MOISTURE CONTENT (WET SEASON)

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
Coastalwater	3	75.11	76.42	75.9600	.73695
Swalimarket	3	72.35	77.42	75.2933	2.63181
Opolomarket	3	75.11	76.42	75.9600	.73695
Valid N (listwise)	3				

ASH MATTER CONTENT (WET SEASON)

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
Coastalwater	3	3.78	4.34	4.0767	.28148
Swalimarket	3	3.70	4.31	4.0033	.30501
Opolomarket	3	3.80	4.31	4.1000	.26665
Valid N (listwise)	3				

FAT CONTENT (WET SEASON)

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
coasterwater	3	.71	.80	.7433	.04933
swaliwater	3	.67	.85	.7700	.09165
Opolowater	3	.71	.80	.7433	.04933
Valid N (listwise)	3				

PROTEIN CONTENT (WET SEASON)

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
coasterwater	3	7.00	8.25	7.4167	.72169
swaliwater	3	7.07	8.39	7.5967	.69924
Opolowater	3	6.89	8.22	7.5900	.66776
Valid N (listwise)	3				

NFE CONTENT (WET SEASON)

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
Coastalwater	3	7.00	8.25	7.4167	.72169
Swalimarket	3	7.31	8.00	7.6933	.35133
Opolomarket	3	7.08	8.29	7.5267	.66425
Valid N (listwise)	3				

FIBRE CONTENT (WET SEASON)

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
coasterwater	3	4.00	4.50	4.1667	.28868
swaliwater	3	3.78	4.70	4.3467	.49571
Opolowater	3	4.24	4.88	4.4900	.34220
Valid N (listwise)	3				

DRY MATTER CONTENT (WET SEASON)

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
coasterwater	3	23.58	24.89	24.0400	.73695
swaliwater	3	21.11	24.00	22.7433	1.48136
Opolowater	3	22.79	25.19	24.1433	1.22904
Valid N (listwise)	3				

PROXIMATE ANALYSIS FOR DRY SEASONS

MOISTURE CONTENT (DRY SEASON)

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
Coastalwater	3	75.21	78.24	76.6033	1.52959
Swalimarket	3	72.35	77.42	75.2933	2.63181
Opolomarket	3	75.56	76.81	76.2467	.63406
Valid N (listwise)	3				

ASH CONTENT (DRY SEASON)

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
Coastalwater	3	2.71	3.25	3.0233	.28024
Swalimarket	3	3.70	4.31	4.0033	.30501
Opolomarket	3	3.11	4.78	4.1133	.88444
Valid N (listwise)	3				

FAT CONTENT (DRY SEASON)

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
Coastalwater	3	.71	.80	.7433	.04933
Swalimarket	3	.77	.89	.8167	.06429
Opolomarket	3	.71	.84	.7767	.06506
Valid N (listwise)	3				

PROTEIN CONTENT (DRY SEASON)

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
Coastalwater	3	7.00	7.05	7.0267	.02517
Swalimarket	3	7.15	7.55	7.3367	.20133
Opolomarket	3	7.11	7.56	7.2867	.24007
Valid N (listwise)	3				

NFE CONTENT (DRY SEASON)

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
Coastalwater	3	6.69	10.09	8.4367	1.70192
Swalimarket	3	6.78	10.69	8.6867	1.95679
Opolomarket	3	6.16	10.19	8.3067	2.02786
Valid N (listwise)	3				

FIBRE CONTENT (DRY SEASON)

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
Coastalwater	3	4.10	4.25	4.1833	.07638
Swalimarket	3	4.17	4.46	4.3400	.15133
Opolomarket	3	4.13	4.23	4.1933	.05508
Valid N (listwise)	3				

DRY MATTER CONTENT (DRY SEASON)

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
Coastalwater	3	21.76	24.79	23.3967	1.52959
Swalimarket	3	22.72	24.78	24.0700	1.16966
Opolomarket	3	21.88	24.29	23.2600	1.24254
Valid N (listwise)	3				

APPENDIX X

Ergeria radiata

Bivalve (***Ergeria radiata***)