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WEATHER PATTERN AND DIARRHOEAL DISEASE VECTORS IN SOME COASTAL AREAS IN SOUTHERN GHANA

BY

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DECLARATION

I, Naomi Elikplim Amekugee, hereby certify that this thesis is the result of research conducted by me at the Noguchi Memorial Institute for Medical Research and University of Ghana, Department of Animal Biology and Conservation Science and the in pursuit of a Master of Philosophy degree in Entomology at the African Regional Postgraduate Programme in Insect Science (ARPPIS), University of Ghana. This thesis has not been submitted for any other degree, in part or in full, and all citations to other people's work have been properly acknowledged.

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DEDICATION

I dedicate this work to my parents, Mr and Mrs Cornelius Maku Amekugee, as well as all of my siblings, professors, friends, and my future self. With dedication and hard work, things will improve.

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ABBREVIATIONS

%	Percentage
°C	Degree Celsius
e.g.	Example
mg/l	Milligrams per litre
ml	millilitre
µl	microlitre
µmax	maximum specific growth rate
Topt	Optimum temperature
A_1	Anal wing vein
API	Analytical Profile Index
C	Costal vein
CT	Cholera toxin
CDC	Centers for Disease Control and Prevention
CO_2	Carbon dioxide
CuA	Anterior cubitus vein
CuP	Posterior cubitus vein
CTX-M	Cefotaxime - Munich
EIEC	Enteroinvasive *Escherichia coli*
ECBAS	Ethics Committee for Basic and Applied Sciences
ESBL	Extended spectrum beta-lactamase
ETEC	Enterotoxigenic *Escherichia coli*
FIB	Faecal indicator bacteria
GDP	Gross Domestic Agricultural Product

GHS-ERC	Ghana Health Services Ethics Review Committee
HEA	Hektoen Enteric Agar
HUS	Haemolytic uraemic syndrome
IND	Indole
KIA	Kligler iron agar
LDT	lower development threshold
MLA	MacConkey Lactose Agar.
MR	Methyl Red
NaCL	Sodium chloride
N_2	Nitrogen
PCR	Polymerase chain reaction
RH	Relative Humidity
Sc	Subcostal vein
Spp	Species
S.S A	Salmonella Shigella Agar
TCBS	Thiosulfate citrate bile salts sucrose Agar
TCP	Toxin-coregulated pilus
TDA	Tryptophan Deaminase Reaction
TTGA	Tellurite- taurocholate-gelatin agar
TSIA	Triple sugar iron agar
VBD	Vector-borne disease
VP	Voges-Proskauer
XLD	Xylose Lysine Deoxycholate Agar
WHO	World Health Organization

ABSTRACT

Dipterans belonging to Muscidae, Sarcophagidae and Calliphoridae families may mechanically transmit many microorganisms to humans like diarrhoea causing pathogens. It was imperative to determine seasonal variations in the diversity and density of dipteran vectors of diarrhoeal diseases in some coastal communities in the southern part of Ghana namely Anyako, Anyanui, Opetekwei and Mumford. This study investigated the seasonal variations in the diversity and density of dipteran vectors of diarrhoeal diseases relative to climatic factors in these coastal communities. The flies were collected by the use of plastic water bottle fly traps baited with mango, fish and meat. The collection was done in two seasons, dry (January-March) and wet (April-June). The flies were grouped into pools based on insect family, the month of collection and study sites then morphological identification of the flies was done using keys from Kirk-Spriggs & Sinclair (2017). These flies were swabbed for bacteria culture on T.C.B.S agar, S.S agar and Chromogenic UTI clarity agar. Identification of bacteria was performed using biochemical tests.

The seasonality of flies was assessed throughout 6 months with baited traps in these 4 communities. A total of 8817 insects were collected and identified, consisting of 3 families and 3 genera – Muscidae (87.10%), Sarcophagidae (2%), Calliphoridae (10.66%). The greatest number of Muscidae, Sarcophagidae and Calliphoridae peaked from April to May. The abundance levels of Muscidae and Sarcophagidae were positively correlated with relative humidity with p value of $p<0.01$ and $p<0.05$ respectively whiles Calliphoridae had a positive correlation with precipitation with a p value of $p<0.01$ in Anyako. Muscidae abundance level had a positive correlation with a p value of $p<0.05$ with temperature only in Anyanui and Mumford while no correlation was seen in Opetekwei.

Escherichia coli, a diarrhoea-causing bacteria was isolated from all insect genera from all the communities in both the dry and wet seasons. The study has found that there was a seasonal variation in the density of flies across the different sites and these vary with climatic factors such as temperature and precipitation and most flies carried *E. coli*, a diarrhoea-causing bacteria.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

In mechanical transmission, disease-causing pathogens are transferred from an infected substrate or host to another. This type of transmission does not require a biological relation. Mechanical vectors are not limited to arthropods only but may also include other animals such as mice, rats, bats, birds and sometimes even humans (Foil & Gorham, 2000). With this knowledge, vector ecologists should be conversant with their roles in disease transmission (Foil & Gorham, 2000). When disease-causing agents are picked from infected substances that are contaminated with the excretory or secretory products of infected hosts, transmission can be done through two main paths, first is through direct transmission, where the transfer is from one host to another. The second path is indirect or contaminative mechanical transmission, where transmission is through infected secretion or excretion (Foil & Gorham, 2000). Lacrimal or open sored secretion of sick humans or animals attract haematophagous (blood-feeding) flies that act as mechanical vectors. Indirect transmission has been linked to arthropods like ants and cockroaches. House flies and other Diptera, as well as other insects, may also be involved.

Malaria and yellow fever are amongst some of the diseases that are transmitted by biological vectors. Because of the relevance of these biologically transmitted diseases, mechanical transmission of diseases by other arthropods have the tendency to be overlooked (Mullen & Durden, 2009). Despite this, there is a lot of evidence that arthropods act as mechanical vectors for pathogens that cause serious diseases in humans and domesticated animals. Because mechanical transmission does not allow an agent to mature or multiply within a vector, mechanically transmitted pathogens frequently have many routes of infection, unlike biologically transmitted diseases. In animals, the mechanical transmission may be through ear tagging, rectal palpating or shearing (Mullen & Durden, 2009). To function as a point of entry

for agents in bodily secretions, a compromised recipient barrier that is a break in the skin may be employed in transmission. Direct contamination of water or food through secretion or excretion is done by many arthropods. Arthropods transfer chemicals physiologically in the majority of cases, but mechanically in others. Pathogens that are mechanically transported can multiply in several settings, including water, soil, and manure, in addition to the source host (Foil & Gorham, 2000). The morphological structures of several arthropods enable them to be involved in the faecal oral route of contamination. Under laboratory conditions, quite many arthropods are capable of pathogen transfer between the substrate. Domestic ants and flies have a lot of fine setae on their integument, and bacteria and parasite eggs can easily stick to them from polluted substrates. Cockroaches can also pick up and spread pathogenic organisms. Mechanical transmission of pathogens is more likely to be done by haematophagous insects (telmophagous) that have bladelike mouth parts (tabanids) than insects with needle-like mouthparts that are specialised vessel feeders (solenophagous) like the mosquito. Bladelike mouthparts have a bigger surface area that comes into contact with bloodmeal, allowing for a greater amount of blood to be retained (Foil & Gorham, 2000).

Flies, such as the housefly are among the most common insects on the planet and are unquestionably one of the insects most closely associated with humans. They are members of the Diptera order, which includes mosquitoes. These two species, *Musca domestica* (Housefly) and *Chrysomya* spp. (Blowfly), are regarded as medically important because they passively transfer diseases by carrying bacteria from faeces to food, resulting in diarrhoeal disorders.; they are also nonbiting flies (Iqbal *et al.,* 2014). These flies serve as mechanical vectors of causatives agents of diarrhoea and other pathogens which are taken up from substrates contaminated by infected hosts' secretory and/or excretory products. Cyclorrhaphans such as *Chrysomya* spp., *Musca* spp., *Sarcophaga* spp. and *Calliphora* spp. prefer to breed in rotting plant and animal tissues, waste, carrion, and the likes instead of the marine environments

preferred by nematocerans (e.g., mosquitoes, midges and crane flies) and brachycerans (e.g., Horse flies).

The house fly, *Musca domestica*, Blow fly *Chrysomya* spp. and Flesh flies *Sarcophaga* spp. are part of the species that are commonly found in the world and widespread (Khamesipour *et al.,* 2018). They are frequently seen in large numbers in slaughter houses, animal farms and food centres, they serve as mechanical vectors as well as nuisance to animals and humans (Khamesipour *et al.,*2018). Molecular analysis brought to light a diverse group of pathogens that were found on *M. domestica* (Armah *et al.,* 1994; Kababian *et al.,* 2020). Evidently, there is a positive correlation between the increase in fly population and the increase in the frequency of diarrhoea cases (Rudan *et al.,* 2005; Schmidt *et al.,* 2011). *M. domestica* carry pathogens that have different properties depending on where they are collected; yet, there is a link between house flies captured from the environment and the infections prevalent in that environment. This established the link between diseases carried by house flies in hospitals and animal farms where antibiotics are often used as growth boosters (Fewtrell *et al.,*2005; Macro, 2009; Emina & Kandala, 2012).

Bacteria and fungi which were resistant to antibacterial were commonly found on *M. domestica*. Aside from the housefly being a passive transmitter of the diarrhoea pathogens, other flies are medically important, because they also contribute to the transmission of diarrhoea pathogens. Many infectious pathogens can cause diarrhoea, these ranges from bacteria (*Salmonella* spp., *V. cholerae*) viruses like sapovirus, norovirus, rotavirus and astrovirus, *Aspergillus* spp. (fungi) is also capable of causing diarrhoea (Das *et al.,* 2018). Shiga toxin-producing *E. coli* and bacteria that causes bacillary dysentery are some of the less common causative agents. In these times of climate change, the frequency of extreme weather occurrences is projected to increase leading to global warming (Stocker *et al.,* 2013) and negative health effects have been linked to climate change (Patz *et al.,* 2014).

1.3 Justification

The potential impact of extreme weather conditions on diarrhoea vector numbers can in turn have an impact on the diarrhoea disease transmission dynamics, increased reports of diarrhoeal sickness have been linked to an increase in harsh (extreme dry or wet) weather occurrences, according to epidemiological studies (Wu *et al.,* 2014; Xu *et al.,* 2014; Phung *et al.,* 2017). A clear understanding of the interactions between diarrhoeal pathogens-vectors-hosts and environment factors is needed to help develop a holistic approach in the control of these infections.

Understanding vector diversity and density dynamics in response to climate change/ seasonal variations in environmental factors along the Ghanaian coast could help mitigate against the impact of diarrheal diseases that may arise. This research seeks to shed light on the interactions between environmental factors, the specific species of non-biting flies that transmit these diarrhea diseases and the causative agents.

1.4 Purpose and Objectives of the study.

The primary aim of this study is to determine seasonal variations in the diversity and density of dipteran vectors of diarrhoeal diseases in some coastal communities in the southern part of Ghana. The specific objectives are:

1. To explore seasonal variations in species composition and abundance of dipteran vectors of diarrhoeal diseases.
2. To determine the role of biotic (human activities) and abiotic factors (temperature, relative humidity and precipitation) on vector population dynamics.
3. To determine pathogen diversity on diarrhoeal vectors across different seasons.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Vectors of diarrhoea

Collinet-Adler et al. (2015) captured a total of 2,101 flies with 2 (1 per 1,000) belonging to the Sarcophagidae family, 4 (2 per 1,000) belonging to the Calliphoridae family and 2,095 (99.7%) belonging to the Muscidae family. In January 2011, 4212 flies were collected. 1 Sarcophagidae (0.2 per 1,000) flies, 2 Calliphoridae (5 per 1,000) and 4,209 Muscidae (99.9%). Pathogens such as *Salmonella* spp., rotavirus, *E. coli*, *Giardia* spp., and *Cryptosporidium* spp. were found in 43 of 60 (72 %) fly samples (Collinet-Adler *et al.,* 2015). With definite knowledge of houseflies being active transporters of these pathogens, there may be a widow of opportunity for other non-biting dipterans to transport pathogens as well. This could be due to the difference in environmental conditions and influence from other organisms in the environment. These factors may go a long way to influence disease transmission along the coast of Ghana. Taking a critical look at insect families that may be potential carriers of pathogens causing diarrhoea, the biology of these insects has to be considered. The apex of dipteran evolution is represented by the Cyclorrhapha group, and a typical example is the common housefly, *Musca domestica.* These insects are have evolved to be highly adapted to living in close proximity with humans and animals. These flies feed on food and waste products of both humans. This feeding cycle enables them to carry pathogens causing serious diseases.

The Muscidae family includes significant blood-feeding parasites, disease vectors, and parasitic species that harm humans and domesticated animals. These and other flies are frequently referred to as synanthropic flies, which are flies which are adapted to live in close association with human habitations and are capable of transmitting human pathogens either mechanically or biologically through this close relationship. Muscid flies and related species

are divided into groups based on their preferred habitat. House flies, for instance, can be found in a variety of filthy organic substrates such as latrines, trash, manure, and animal waste. Dung flies, such as the horn fly, are dirt insects that only live in cattle droppings as immatures. Sweat flies are another example, as their adults feed on sweat all day. Except for Antarctica, *Musca domestica* is a nonbiting dirt fly that may be found on all continents. Immatures can be discovered in a variety of degraded organic substrates. Major breeding habitats include human garbage dumps, open privies, animal dung, unclean bedding, poultry litter, and waste around fruit and vegetable processing plants. Transfer of pathogens can occur as adults feed on these rotten materials then settle on food there by contaminating it for humans' consumption Breeding occurs all year in tropical and subtropical areas but is stopped in temperate areas by the winter. The house fly is perhaps the most significant nuisance and potential vector of intestinal infections in terms of public health. The Sarcophagidae family includes flesh flies, which are similar to house flies but have blackish stripes on the grey thorax (area behind the head) and a light and dark grey checkered pattern on the abdomen. While the family is common, most flesh flies are tropical. Many are scavengers, feeding on open wounds or carrion whiles females deposit eggs that grow into larvae. The fly feeds on carrion, this can result in the transfer of pathogens as it flies from one carrion to the other. *Sarcophaga nodosa* and *Sarcophaga* (Bercaea) *africa*, for example, feed on living and dead tissue, including snails, rotting debris, and faeces. The size of an adult calliphorids (family Calliphoridae) are usually between that of Muscidae and Sarcophagidae sizes. They are generally intermediate in size and typically show a dazzling metallic blue, green, copper, or black hue. The common names "bluebottle", "greenbottle" and "blowflies" refer to the colouration of these flies. These flies are drawn to decomposing meat, fish, and human and dog faeces, and they are often the first creatures to come into touch with dead animals. Blowfly larvae can infest the dog's live tissues,

particularly sores, wounds, and decomposing flesh, examples of blowflies are *Chrysomya putoria* and *Chrysomya megacephala.*

Sarcophagidae (flesh flies), Calliphoridae (blowflies and bottleflies) and Muscidae (house flies and latrine flies) play significant roles as the mechanical vectors of disease-causing pathogens such as viruses, bacteria, fungi and pathogens (Banjo *et al.,* 2005). The common housefly, *Musca domestica,* is widespread in world including Ghana (Curtis & Hawkins, 1982) as well as other species of calyptrate flies (e.g. Calliphoridae and Sarcophagidae). These species can respond quickly to environmental change, and under the right conditions, it can double in a matter of days (Eesa & El-Sibae, 1993). During low humidity, low rainfall and high temperature, calyptrate species are at their most active (Goulson *et al.,* 2005). According to a recent study, rising temperatures linked to predicted climate change is likely to drastically boost fly population density. This is projected to have an impact on *M. domestica* by increasing its population about 2.5 times by 2080 under the worst-case scenario and by about 1.5 times under the relatively hopeful medium-low emissions scenario (Goulson *et al.,* 2005). The probable relationship between flies, climate variability and diarrhoea occurrence, as well as projected climate change implications in Africa, need immediate attention. Extreme weather conditions changes can interfere with intervention measures.

The life cycle of diarrhoeal vectors such as *Musca domestica* (house fly), *Chrysomya* spp. (Blow flies) and *Sarcophagidae* spp. (flesh flies), are known to be associated with human dwelling. These flies have a taste for human excrement and become polluted with it, as evidenced by a bacteriologic culture of caught flies with many pathogens such as *Shigella* species following contact with infected human faeces (Levine & Levine, 1991). The transmission of enteric pathogens by *Musca domestica* is well documented. A study conducted by Lindsay *et al.* (2012) showed that *Chrysomya putoria* also known as the common African latrine fly was likely to be an important vector of diarrhoea causing pathogens (Lindsay *et al.,*

2012). Fly behavioural studies demonstrated that *C. putoria* are drawn to human faeces, fish and raw meat, indicating that faecal diseases can be transported from faeces to food (Lindsay *et al.,* 2012).

2.2 Muscidae (House flies, *Musca domestica*).

Muscid flies, such as *Musca domestica*, a common *Muscidae* species, would visit a body shortly after it has died, attracted by any exudates rather than the corpse itself. Four short black lines run along its thorax, and its abdomen is greyish or yellowish. Vein 4 has a sharply inclined wing vein. *Musca domestica*, the house fly, is well known and is distributed extensively in the world. Flies, as possible mechanical vectors, are recognized as vectors of enteric illnesses. A classic example of a synanthropic fly is *M. domestica*, it dwells amongst people and their domesticated animals. House flies can be found and thrive everywhere there are humans, but they are extremely rare in natural or wild regions around the world. *Musca domestica* although has gained the greatest attention concerning gastrointestinal disease transmission, other fly species have also been involved in mechanical transmission in epidemic circumstances. Expanding geographic ranges of some fly species could be a role in changes in disease epidemiology, example *Chrysomya megacephala* was introduced to Guam by the Japanese in World War II, these species on Guam swiftly established themselves as a significant mechanical vector of intestinal parasites (Foil & Gorham, 2000). House flies and other muscoids that are drawn to eyes, open wounds, and other sources of infection on afflicted vertebrates may directly transmit these disease agents to noninfected hosts, in addition to acting as indirect vectors of intestinal diseases. Flies belonging to the Chloropidae and Muscidae families typically mechanically transmit infectious illnesses from a host's eyes or wounds. Because infectious agents from lesions or eyes cannot penetrate healthy skin or corneas, infection requires a point of entry compromise (trauma or arthropod bite). Members of the

Chloropidae family, which include eye gnats, ulcer flies, and yaws flies, are recognized for their continual feeding on body secretions from a variety of sources. It has been demonstrated that eye gnats are involved in the transmission of substances that cause yaws, mastitis, and anaplasmosis in humans and animals (Foil & Gorham, 2000).

2.2.1 Description of *Musca domestica.*

The adult house fly (*Musca domestica*) is a grey insect about 6 to 7 mm in length, with the female being somewhat larger than the male. Ruby eyes and spongy mouthparts are evident on its head. The thorax is striped with four slender black stripes, and the fourth longitudinal wing vein has a substantial upward bend. With a black midline and uneven dark side patterns, the abdomen is grey or yellowish. The underside of the male is yellowish. The sexes may be clearly distinguished by the spacing between their eyes, which is nearly twice as wide in females as it is in males. The stable fly (*Stomoxys calcitrans*) and the false stable fly (*Muscina stabulans*), which are all members of the same family, are frequently confused with the house fly.

Figure 1. An adult Housefly (*Musca domestica*) source: (Kababian *et al.,* 2020)

2.2.2 Habitat and Biology

The housefly has four phases in its life cycle: egg, larva or maggot, pupa, and adult. The egg might take anywhere from 6 to 42 days to mature into an adult fly, depending on the temperature. The lifespan is normally 2–3 weeks, but in cooler climes, it can last up to 3 months. Typically, eggs are strewn across organic waste such as dung and garbage in large quantities. In only a few hours, the eggs will hatch. Immature larvae burrow into the breeding material; they require oxygen from the atmosphere to survive, thus they can only survive in areas with plenty of fresh air. They can only exist on the surface of the breeding medium when it is extremely moist, but in drier materials, they can penetrate to a depth of several centimetres. The larvae of most species are tiny, white, legless maggots that grow swiftly and have three instars. Depending on the species, temperature, and the type and quantity of food available, it might take anywhere from three days to several weeks for a species to develop (Resh & Cardé, 2009).

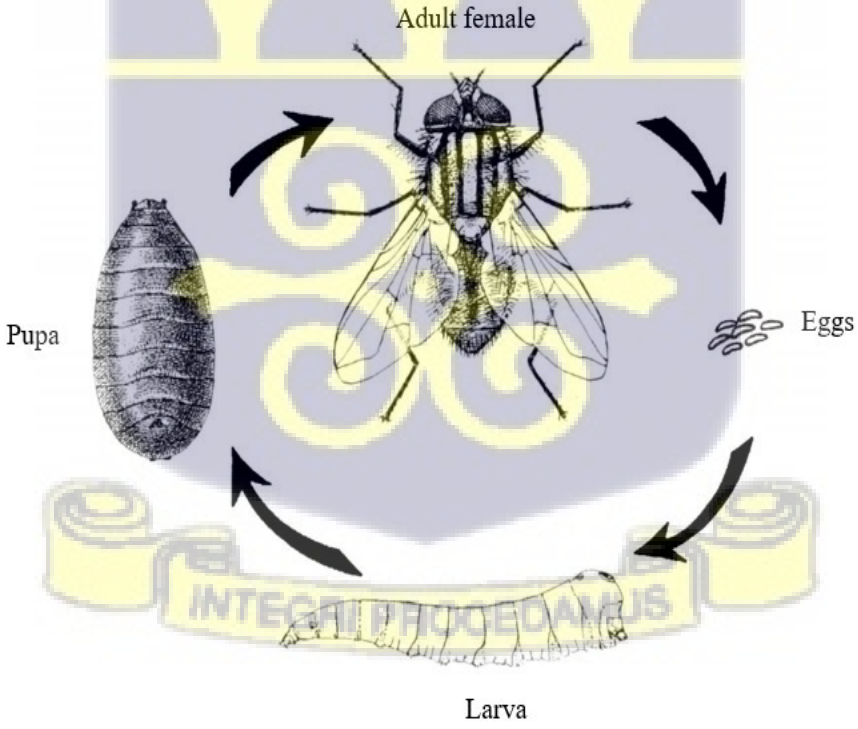

Figure 2. An illustrative diagram of the lifecycle of *Musca domestica.* Source: https://naturalhistorymuseum.blog/2015/12/16/whats-in-a-fly-musca-domestica-the-greatest-traveller-of-them-all-cursator-of-diptera/, Accessed :27/09/2021.

When the feeding stage is over, they move to a drier region and the larvae dig into the soil or hide under items that provide protection. They create a puparium, a capsule-like container in which the larvae mature into adults. The fly then forces its way out of the top of the case and up to the surface, which takes around 2–10 days. Shortly after emerging, the fly spreads its wings and the body dries and hardens. The adult is capable of reproducing after a few days. In natural conditions, a mature female rarely lays more than five eggs at a time, and she rarely lays more than 120–130 eggs each time (Resh & Cardé, 2009).

2.3 Calliphoridae (*Chrysomya* spp., *Lucilia* spp.)

Blow flies (family Calliphoridae) belong to the Diptera order of insects. Metallic blue, green, or black are the colours available. They're a little bigger than houseflies, but they have comparable habits. Screwworms, Bluebottle flies, Greenbottle flies, and Cluster flies are all key members of this group (Resh & Cardé, 2009). Most of these worldwide families (>1500) species are drawn to rotting flesh and can detect the chemical fragrance of decay within minutes after death. Adult blowflies are the first to arrive at human and animal corpses in terrestrial environments. Unless the conditions are normal, such as those seen in dry areas or with buried carrion, they are rarely replaced by other insects. Blowflies feed on exposed body fluids (saliva, blood, mucus) as soon as they arrive, and females with mature eggs begin oviposition right away (Szpila, 2010). Most blow flies (e.g., *Calliphora* spp., *Cochliomyia* spp., *Lucilia* spp., *Phaenicia* spp.) are specialist scavengers in nature, and the adults speed up the decomposition of all types of vertebrate carcasses (Resh & Cardé, 2009).

Lucilia spp. (*Lucilia sericata*) (Meigen) is one of the most widespread and extensively dispersed species in this genus, not just in North America but worldwide (Hall, 1948). It is metallic green, but not as vivid as *L. coeruleiviridis* and *L. mexicana,* and it has a coppery gleam to it. This is a forensically important species, and much has been published about it,

including thorough research on larval growth and development (Rivers & Dahlem, 2014). *Calliphora* spp. adults are typically larger than *Lucilia* spp. adults, and their abdomens are frequently vivid, metallic blue. *Calliphora vicina* and *Calliphora vomitoria* are two species that are prevalent in the United States and many other regions of the world and are considered important vectors of diseases and also important in a forensic investigation (Rivers & Dahlem, 2014).

These are gleaming and metallic in appearance, with blue, green, or black bodies. Members of this family are distinguished by the qualities and arrangement of their hairs. Bristles are seen on the meron of all blowflies. Look for two pleural bristles and a hindmost post humeral bristle that is positioned lateral to the pre-sutural bristle when identifying this family. There is a well-defined posterior Calli and a continuous dorsal suture through the centre of the thorax. Either the post-scutellum is absent or underdeveloped. The costa is intact on the insect, and the sub-costa is evident (Kirk-Spriggs & Sinclair, 2017).

Figure 3. Adult Female Blow fly (*Chrysomya megacephala*).
Source: https://alchetron.com/Chrysomya-megacephala, Accessed: 27/09/2021

2.3.1 Habitat and Biology of Calliphoridae.

Blow-flies like conditions that are moderate to tropical, with a layer of loose, damp soil and litter in which the larvae can develop and pupate. Maggots have hook-like mouthparts that tease the tissues they dwell in apart. Adults have spongey mouthparts that resemble those of house flies. Larvae (maggots) eat mostly dead animals and animal shelters. Any form of fresh meat or road kill left in the field attracts these flies. The majority of the blow fly species analysed are anautogenous, which means that a female needs a lot of protein to create mature eggs in her ovaries (Kirk-Spriggs & Sinclair, 2017).

Females are thought to use carrion for both protein and egg laying, although this has yet to be verified (Kirk-Spriggs & Sinclair, 2017). Blow fly eggs are yellowish or white in colour when deposited, measuring 1.5 mm 0.4 mm and resembling rice grains in size. A female blowfly lays 150–200 eggs every batch on average; however, she is generally iteroparous, depositing around 2,000 eggs in her lifetime. Females of two Chrysomya species (*C. rufifacies* and *C. albiceps*) that are either arrhenogenic (laying exclusively male progeny) or thelygenic (laying both male and female offspring) have a 50:50 sex ratio in their blow fly eggs. It takes roughly 24-48 hours for an egg to hatch into the first larval stage. Larvae develop in three stages (instars), each of which is separated by a moulting process. Blow flies are poikilothermic, meaning their growth and development rates are significantly influenced by temperature and species. It will burrow into the earth to pupate when the third larval stage is completed, emerging as an adult seven to fourteen days later (Kirk-Spriggs & Sinclair, 2017).

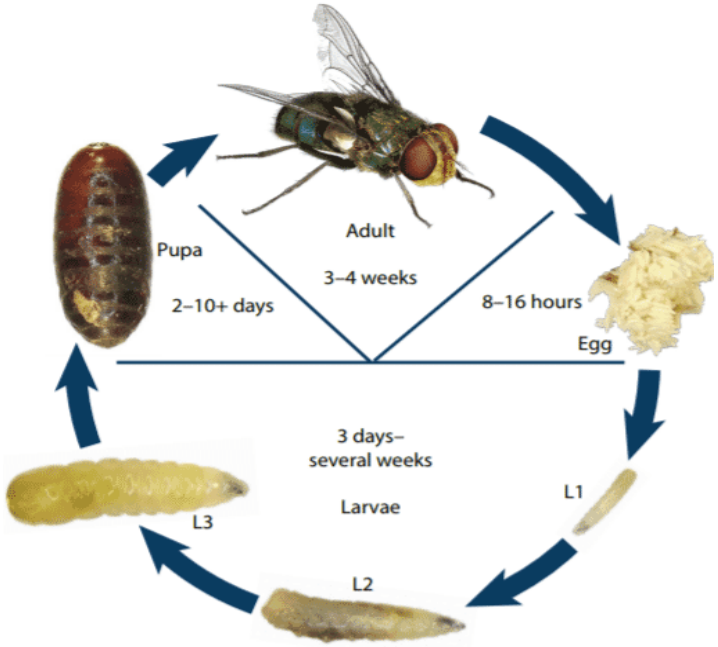

Figure 4. An illustrative diagram of the lifecycle of *Chrysomya megacephala* (Calliphoridae) Source: https://alchetron.com/Chrysomya-megacephala, Accessed 27/09/2021.

2.4 Sarcophagidae

The flesh flies, family Sarcophagidae, is a large family divided into 400 genera with around 3100 species. These species are extremely widespread in suburban and urban regions where the environment has been heavily impacted by humans, but they are rarely seen in more rural or untouched places. Despite having a global distribution, the Sarcophagidae family is remarkably concentrated in tropical and mild temperate climates (Moura & Mello-Patiu, 2015). Despite their widespread label "flesh flies," most Sarcophagidae species are not generally necrophagous, at least when it comes to vertebrate flesh. Grey flies with red eyes, dark stripes (or vittae) on the dorsum of their thorax, and grey tessellated (checkerboard patterned) abdomens are necrophagous sarcophagids. Many insects and arthropods, terrestrial molluscs (snails and slugs), reptiles and amphibians have parasitoid, parasitic, or predatory interactions with them. Many species are scavengers of dead insects and other invertebrates, while others are coprophagous (dung breeding).

Only a few species specialise in colonizing bigger, vertebrate carrion, and these species are invaluable in forensic investigations. While big carrion attracts a small number of species, ground meat can attract these insects even if they do not actively mate on ground meat. A wide range of sarcophagids is attracted to carrion for a protein diet or as a mating place. The fact that all Sarcophagidae flies do not deposit eggs is an interesting scientific fact to note. The flies lay their eggs in a bi-pouched uterus and virtually invariably deposit active first-instar larvae. After deposition, feeding starts practically immediately.

Most Sarcophagidae adults have quite similar outward features. The mesonotum is a bristled meron, developing subscutellum, and abdomen checkered or spotted are all black stripes, and the species are medium to big, ranging from 8 to 14 mm (Carvalho & Mello-Patiu, 2008). Flesh flies are a type of insect that thrives in warm environments, the adults are common insects that feed on sugary substances such nectar, sap, fruit juices, and honeydew (Triplehorn *et al.,* 2005). These flies are drawn to organic items (especially decomposing organic materials) and must consume live or dead tissue to complete their life cycle.

Figure 5. Adult Flesh fly *Sarcophaga africa*. (Sarcophagidae).
Source: Diptera.info - Discussion Forum: *Sarcophaga* (Bercaea) *africa*, Accessed: 27/09/2021.

2.4.1 Habitat and Biology.

Some flesh flies are ovoviviparous, which means they do not lay eggs after they have fully developed. Instead, the larvae hatch inside their mother's "uterus" and are kept there until they find a suitable host. The discharge of the larvae onto the host is referred to as “larviposition”. The larvae start feeding right after being deposited on the substrate. These larvae consume and grow quickly.

Live larvae of flesh flies are born and placed into carrion, faeces, or open wounds to complete their development. This may provide flesh flies an edge over prospective food competitors because predation or parasitism do not kill eggs, and larvae can feed right away rather than waiting for days for them to hatch (Rivers & Dahlem, 2014).

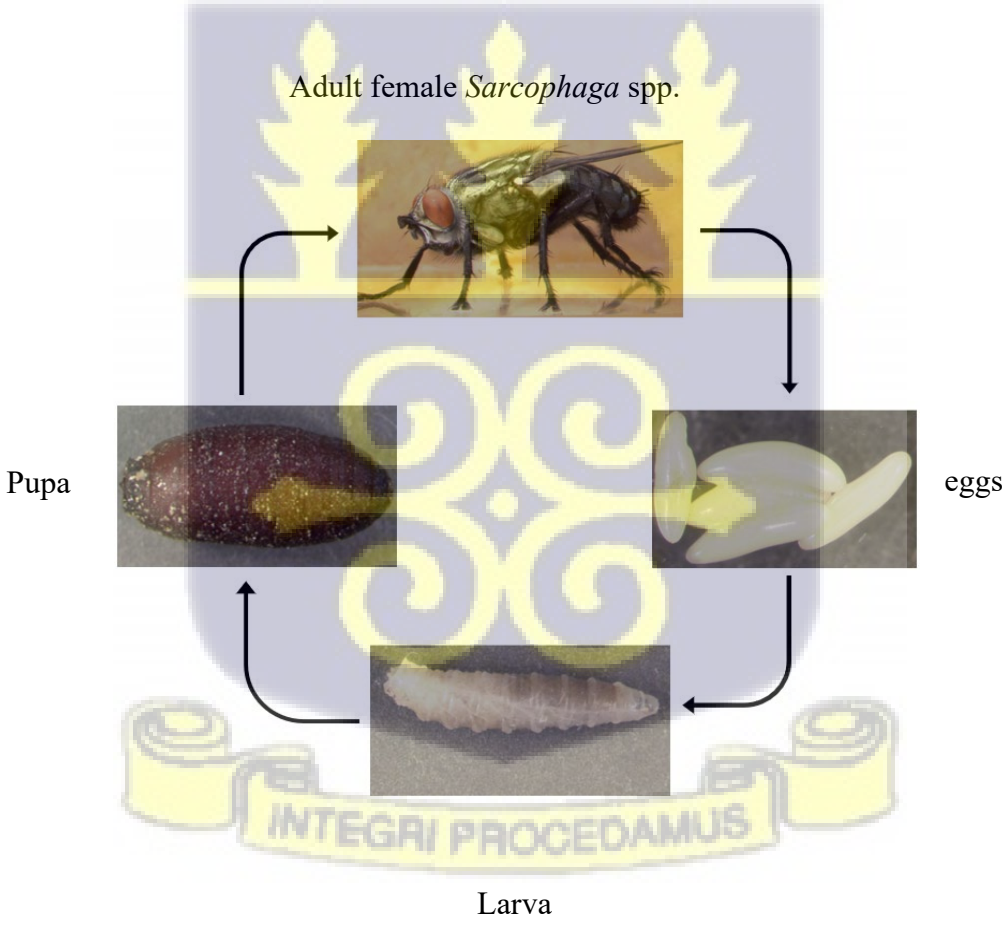

Figure 6. An illustrative diagram of the lifecycle of *Sarcophaga africa* (Sarcophagidae).

Source: Source: Diptera.info - Discussion Forum: *Sarcophaga* (Bercaea) *africa*, Accessed: 27/09/2021.

2.5 Possible vectors of diarrhoea (non-target)

Cockroaches, like flies, have been linked to a wide range of diseases. In two publications, Bell *et al*. (2007) and Foil & Gorham. (2000), the literature on the medical relevance of cockroaches was compiled. Bell *et al.* (2007) studied the interactions of 18 cockroach species with diseases. Bell *et al.* (2007) studied the biotic interactions of cockroaches with pathogenic and non-pathogenic organisms, gathering pathogens that comprised of one virus, 2 protozoan species, 2 fungi, 7 helminths species and 33 bacteria. Cockroaches are less mobile and less visible than house flies. Despite this, they frequently move around within and across structures and can come into contact with human food (Foil & Gorham, 2000). Cockroaches carry a diverse range of bacteria as part of their natural gut flora. However, the isolation of vertebrate infections that aren't found in the regular cockroach gut flora suggests that cockroaches can acquire and spread these pathogens in their environment. There is far fewer research on ants as mechanical vectors than there are on flies or cockroaches. Ants and cockroaches are good sources of *V. cholerae*, according to Do Nascimento *et al.,* (2020). *Serratia* spp., *Citrobacter* spp., *Klebsiella* spp., *Enterobacter* spp., *Proteus* spp., *Staphylococcus* spp., and *Yersinia* spp., including *Yersinia pestis*, were cultured using blood agar plates from collected ants in a veterinary clinic laboratory (Do Nascimento *et al.,* 2020).

2.6 Pathogens (Enteric bacteria)

When compared to faecal-oral and contaminated water transmission, arthropods play a limited role in the spread of faecal-borne viral infections (Do Nascimento *et al*., 2020). Since the discovery of enteric bacteria, arthropods, particularly flies, have been connected to bacteria that cause enteric infections, including *E. coli*, *Salmonella* spp., and *Shigella* spp. In susceptible individuals, an inoculum of only 10-100 Shigella germs can develop shigellosis. The majority of enteric infections are found throughout the world, but they only occur in an epidemic form

periodically. The most common scenario is that carriers of hidden infections team up with a small number of acute patients to function as infection sources (Mullen & Durden, 2009). The most common scenario is that carriers of hidden infections team up with a small number of acute patients to function as infection sources. In these endemic settings, etiologic agents are essentially uniformly present; all that is needed for an epidemic is a reliable method of transmitting the disease from infected to non-infected hosts. Untreated or insufficiently treated drinking water, as well as infected food handlers with low personal hygiene, can be a source of transmission (Foil & Gorham, 2000).

The volume of exposed faeces serving as infectious sources, as well as the larval habitats of possible vectors, have an impact. There are numerous reports available creating a link between the occurrence of enteric bacterial infection and environmental factors or the residents' habits (Foil & Gorham, 2000). Increased occurrences of diarrhoea have been linked to certain communities' vast populations of filth flies, due to the presence of open human excrement and meals that has been frequently exposed to flies (Foil & Gorham, 2000). In Thailand, a similar correlation between fly abundance and the rate of human diarrheal illness was noted (Foil & Gorham, 2000).

According to Squire and Ryan (2017), the protozoans causing diarrhoea in the intestines are *Cryptosporidium* sp. and *Giardia* sp. with *Cryptosporidium* sp. being the most common protozoa of diarrhoea worldwide (Squire & Ryan, 2017). A recent study by Global Enteric Multicenter Study (GEMS) has identified the *Cryptosporidium* spp., which is second only to rotavirus as a cause to moderate to severe diarrhoea in children for the first five years of their life, is the aetiology and population-based burden of paediatric diarrhoea disease in Sub-Saharan Africa (Squire & Ryan, 2017). It was estimated that *Cryptosporidium* spp. has contributed to 2.9 million cases annually in children under the age of fewer than 24 months in

Sub-Saharan Africa. Infection with *Cryptosporidium* spp. is linked to more than two-fold increase in mortality in children aged 12 to 23 months (Squire & Ryan, 2017).

Giardia duodenalis is a diarrhoea-causing parasite that affects mammals, including humans, and is responsible for an estimated 2.8×10^8 cases of the enteric disease worldwide each year (Squire & Ryan, 2017). In Africa's underdeveloped countries, the prevalence is higher. A long-term infestation can cause weight loss, malabsorption, and dehydration. In children in underdeveloped nations, this can lead to stunted growth and wasting (low weight for height) as well as cognitive impairment (Squire & Ryan, 2017). Infection with the above-mentioned protozoan can be spread via direct contact with infected humans, animals, or consuming contaminated foods. Aside from the fore mentioned causatives of diarrhoea others also contributes to diarrhoeal disease and these are enteropathogenic *Escherichia coli*, *Shigella* spp., *Salmonella* spp., *Rotaviruses*, *Campylobacter* spp. and *Entamoeba histolytica* (Khan *et al.,*1990).

2.6.1 *Escherichia coli.*

The genus *Escherichia* belongs to the family Enterobacteriaceae and the tribe Escherichieae, and it contains mostly motile gram-negative bacilli. The type species of the genus *Escherichia* is *E. coli* (Edwards & Ewing, 1972). The non-pathogenic facultative flora of the human intestine is dominated by *Escherichia coli.* On the other hand, even the most resistant human hosts are susceptible to some *E. coli* strains, which can cause gastrointestinal, urinary, and central nervous system illness. There are at least six different types of *E. coli* bacteria that cause diarrhoea, each with its pathogenic scheme. When considered together, these organisms are most likely the most common cause of diarrhoea in children all across the world. Traveller's diarrhoea (enterotoxigenic *E. coli*), watery diarrhoea in new-borns (enteropathogenic *E. coli*), chronic diarrhoea (enteroaggregative *E. coli*), and haemolytic-uremic syndrome haemorrhagic

colitis are all signs of diarrheagenic *E. coli* infection (enterohemorrhagic *E. coli*) (Squire & Ryan, 2017).

Even "non-pathogenic" *E. coli* bacteria can cause sickness in weakened or immunocompromised hosts, or when gastrointestinal barriers are breached. Furthermore, even the most resistant members of humans are susceptible to infection by one of the innumerable highly evolved *E. coli* strains capable of producing a wide range of human diseases (Lupindu, 2017). Pathogenic *E. coli* infections might be localized to the mucosal surfaces or can spread throughout the body. Infection with *E. coli* strains that are essentially hazardous results in three different clinical symptoms: (i) sepsis/meningitis, (ii) enteric/diarrheal disease, and (iii) urinary tract infection (Lupindu, 2017).

Diseased surfaces on both animate and inanimate things can be used to isolate *E. coli*. Human or animal body surfaces are examples of animated surfaces. *Escherichia coli* can be present on food surfaces as well as in working environments such as tables, knives, and clothing. Food surfaces such as meat, eggs, or fish can be used to isolate *E. coli* depending on the study's purpose (Lupindu, 2017). Despite the availability of assays to detect various forms of diarrheagenic *E. coli*, it is not always necessary to link a patient to a specific *E. coli* infection. Patients with enterotoxigenic *E. coli* (ETEC) traveller's diarrhoea, for example, consult a doctor for a stool culture long before the diarrhoea goes away. Despite the fact that diarrhoea normally clears up and patients respond to empirical antibiotics like fluoroquinolones, which are routinely used to treat other bacterial diarrhoeas, the majority of enteroinvasive *E. coli* (EIEC) isolates will go unreported in the clinical laboratory. In cases of persistent diarrhoea, especially in travellers, children, and the immunocompromised, as well as in outbreak situations, stools should be cultured for most types of diarrheagenic *E. coli*. *Escherichia coli* can be isolated from a stool sample and forwarded to a competent reference laboratory for confirmation. The

indications for culturing EHEC are different from those for the other *E. coli* types that cause diarrhoea (Nataro & Kaper, 1998).

At 37°C and aerobic conditions, *E. coli* can be easily recovered from clinical specimens on general or selective media. The most common method for recovering *E. coli* in faeces is MacConkey or Eosin methylene-blue agar, which selectively grows members of the Enterobacteriaceae and allows identifying enteric infections based on appearance. Biochemical reactions are routinely used to identify *Enterobacteriaceae* (Balows & Hausler, 1991). These tests can be carried out in individual culture tubes or with commercially available test "strips." Both methods produce satisfactory outcomes. *Escherichia coli* strains are routinely chosen from agar plates for epidemiologic or clinical objectives after presumptive visual identification. This technique should be used with caution, as only around 90% of *E. coli* strains are lactose positive; many diarrheagenic *E. coli* strains, including many EIEC strains, are lactose negative. The single best test for identifying *E. coli* from other Enterobacteriaceae members is the indole test, which is positive in 99 % of *E. coli* strains (Nataro & Kaper, 1998). Aside from biochemical identification, the other identification methods are serotyping, molecular detection methods.

2.6.2 *Vibrio cholerae*

In poorer countries, *Vibrio cholerae* is a severe public health problem, with epidemics following a predictable seasonal pattern and being connected to poverty and poor sanitation. Debilitating watery diarrhoea causes rapid dehydration, and 50 to 70 % of untreated patients die (Faruque et al., 1998). Cholera is a waterborne infection, and *V. cholerae*'s close interaction with surface water and the people who contract it highlights the importance of water ecology. (Faruque *et al.,* 1998*)*. Cholera toxin (CT) is the toxin that causes diarrhoea. Cholera is characterized by severe watery *diarrhoea* produced by *Vibrio cholerae*, a toxigenic bacterium

that colonizes the small intestine and produces cholera toxin (CT), an enterotoxin (Lebenthal, 1990). Seasonal outbreaks of cholera are widespread in parts of southern Asia, Africa, and Latin America, and are associated with poverty and inadequate sanitation (Faruque *et al.,* 1998). Although *V. cholerae* is a well-defined species, its pathogenic potential is not uniform. For establishing the public health significance of *V. cholerae*, two main characteristics are evaluated. The production of CT, which causes severe diarrhoea, and the presence of the O1 or O139 antigen, which acts as a signal of pandemic potential because the source is unknown (Herrington *et al.,* 1988). Molecular study has revealed that, in addition to genes for CT, all cholera-causing bacteria have genes for a colonization factor known as toxin-coregulated pilus (TCP) and a regulatory protein known as ToxR, which coregulates the production of CT and TCP (Herrington *et al.,* 1988). As a result, cholerae pathogenesis is dependent on the interaction of several infectious substances that are produced by the toxigenic *V. cholerae.*

Cholera outbreaks are responsible each year, for an estimated 120,000 deaths and many more cases, the vast majority of which occur in kids (Faruque *et al.,* 1998). The epidemiology of cholera is characterized by (i) observing endemic locations, children aged 1 to 5 years have the highest rates of infection. (ii) a high degree of case clustering by place and season, (iii) antibiotic resistance patterns that regularly shift year to year, and (iv) disease protection through enhanced sanitation and hygiene., and (iv) clonal diversity of epidemic strains.

Culturing can be used to isolate *V. cholerae*. Traditional culturing methods developed decades ago, are continually being modified, resulting in more accurate identification of *V. cholerae* in environmental samples (water, plankton, sediment, shellfish). The study material is directly plated onto TCBS, TTGA, or CHROMagar Vibrio plates (Huq *et al.,* 2012) or pre-enrichment of the sample in alkaline peptone water, then streaking for isolation onto one or more of these three agars. To increase detection or isolation of environmental samples, a pre-enrichment procedure is strongly suggested. After an incubation period, presumptive *V. cholerae* can be

isolated from these media and confirmed either biochemically or directly by PCR. Antisera for the O1 and O139 antigens in a slide agglutination assay with PCR primers intended to target the O1 and O139 coding sections of the genomic DNA can be used to serogroup isolates as O1, O139, or non-O1/non-O139. These *V. cholerae* isolates can then be stored as a glycerol stock in nutrient agar with 0.5 %t NaCl overlaid with sterile mineral oil at 70°C or as replicates in nutrient agar with 0.5 % NaCl overlaid with sterile mineral oil. To ensure the viability and purity of *V. cholerae* stock cultures, they should be re-streaked for isolation regularly (Huq *et al.,* 2012).

2.6.3 *Salmonella* spp.

Salmonella spp. can be transmitted from chickens to humans through consumables like eggs and meat. *Salmonella spp*. causes Salmonellosis, an intestinal infection in humans (Yaashikaa *et al.,* 2016). Broilers and laying breeding fowls were surveyed for the presence of *Salmonella serovars* in Eastern and during hatching, one *Salmonella*-infected egg could contaminate chicks and eggs (Chowdhury *et al.,* 2011). *Salmonella* infection of food goods can lower customer demand and negatively impact producer revenues (Okoli *et al.,* 2006). *Salmonella* species are the most commonly documented cause of human and animal foodborne disease. With approximately 93.8 million cases yearly, non-typhoidal *Salmonella* gastroenteritis adds to the worldwide public health burden (Ammar *et al.,* 2010). *Salmonella* infections can be divided into four kinds based on their clinical manifestations (Chowdhury *et al.,* 2011). The first is Bacteraemia, reactive arthritis and osteomyelitis these are caused by *Salmonella typhimurium* and *S. enteritidis*. The second is gastroenteric which is caused by *S. enterica* serovar Typhimurium. Enteric fever is caused by *S. typhi* and *S. paratyphi.* The last to mention is the carrier state, this includes the people who have been infected previously with *S. typhi* and *S. paratyphi.*

Salmonella spp. can be isolated by swabbing from a suspected media contaminated with Salmonella. 9 ml of buffered peptone water was inoculated with 1 ml of the swab sample. The cells were cultured for 18 hours at 37°C for pre-enrichment. In addition, for selective enrichment, 10ml of Rappaport-Vassiliadis broth (Hi-Media) was inoculated with 0.1ml of the pre-enriched inoculum for selective enrichment and incubated at the temperature of 42°C for 24 hours. After enrichment, Xylose lysine deoxycholate (XLD) is streaked with (10µl) of inoculated broth then incubated at 37°C for 24 hours. On XLD agar, putative *Salmonella* colonies with a red halo, a black centre, and a pink-red zone were tested for biochemical characterisation (4-5 colonies per plate) (Nair *et al.,* 2015).

2.6.4 *Shigella* spp.

Shigella spp. is a Gram-negative bacterium with four species: *Shigella dysenteriae* (serogroup A), *Shigella flexneri* (serogroup B), *Shigella sonnei* (serogroup C), and *Shigella boydii* (serogroup D). In recent years, *Shigella sonnei* has become the most frequent serotype causing shigellosis in Asian countries (Qu *et al.,* 2012). *Shigella dysenteriae* causes fatalities and has been linked to outbreaks (Raja *et al.,* 2011). Water supply, sanitation, and the residential environment, including fly aggregation, are all risk factors for shigellosis (Gaurav *et al.,* 2013). Shigellosis can potentially cause complications such as deadly toxic encephalopathy (Ekiri syndrome) (Pourakbari *et al.,* 2012), epithelium, and haemolytic uraemic syndrome (HUS) (Butler, 2012). Because of the emergence of different antibiotic resistance forms, *Shigella* spp. has become a public health concern, underlining the significance of ongoing surveillance of the organism. The development of CTX-M-type ESBLs is primarily responsible for resistance and reduced susceptibility to â-lactam antibiotics (Zhang *et al.,* 2011).

Shigella spp. main pathogenic trait is its capacity to infect a wide range of host intestinal cells, including enterocytes, macrophages, and dendritic cells, resulting in severe inflammatory

reactions in intestinal tissue. *Shigella* spp. causes acute bloody diarrhoea by infecting the intestinal epithelium and causing patchy damage. Micro-ulcers and inflammatory exudates occur as a result, and inflammatory cells (polymorphonuclear leucocytes) and blood show in the stool. 106-108 shigellae per gram are seen in the diarrhoeal stool. The bacteria are extremely susceptible to external conditions once discharged, and it dies quickly, especially when dried or exposed to direct sunlight.

In Gaurav *et al.* (2013), researched *Shigella* spp. Samples from suspected sources were acquired. In total, 311 human stool samples, 100 cow faeces samples, and 100 poultry poo samples were collected for three months. The samples were collected cold and forwarded to the laboratory the same day to be processed for Shigella organism isolation.

Shigella spp. was isolated using various bacteriological media. The reagents and chemical used in the study were obtained from Hi-media, Difco Published protocol and Sisco Reference Laboratory and was used to process faecal samples (CDC and WHO, 2003). A loop was used to collect faecal samples and streaked on Xylose Lysine Deoxycholate Agar (XLD) and MacConkey Lactose Agar (MLA) and incubated at 37°C for 24 hours. The presence of convex and colourless colonies on MLA plates was examined for further identification. Similarly, colonies that were translucent or red on XLD plates were taken for identification. Suspected *Shigella* spp. colonies were streaked on XLD media to obtain pure culture before being streaked on selective media such as Deoxycholate Citrate Agar (DCA) and Salmonella-Shigella Agar (SSA), Hektoen Enteric Agar (HEA) (Gaurav *et al.,* 2013).

Kligler iron agar (KIA) and Triple sugar iron agar (TSIA) are used to subculture colonies that have a distinct appearance on selective medium (TSIA). A pure culture was employed to inoculate the relevant media to achieve reactions in KIA and TSIA. One colony was selected and inoculated into each of the test media using straight platinum wire. The KIA and TSIA

slants were infected for 24 hours at 37 degrees Celsius. Other biochemical assays that can be used in identification include Methyl Red (MR), Voges-Proskauer (VP), Indole, Citrate, Urease, and Mannitol Motility (Gaurav *et al.,* 2013).

2.6.5. *Campylobacter* spp.

Campylobacter spp. are Gram-negative, microaerophilic and/or anaerobic bacteria that are mostly spiral-shaped and are known or suspected of being human gastrointestinal pathogens (Skirrow, 1994). Human campylobacteriosis is usually related to *Campylobacter jejuni* and *Campylobacter coli* (Skirrow, 1990). Campylobacteriosis is more common in many Western countries than Salmonella-related illnesses (Rohner *et al.,* 1997). Human campylobacteriosis is spread by poultry and poultry products, which play an essential role in disease transmission (Deming *et al.,* 1987; Evans, 1992). However, due to improper detection in developing countries, some cases could have gone undiscovered (Trachoo, 2003). Ingestion of animal-derived food is the principal mode of infection transmission (Butzler & Oosterom, 1991). *Campylobacter* spp. identification is complicated by their stringent growth requirements, complex taxonomy, and unreliable biochemical assays (On, 2001).

Campylobacter jejuni is a prominent source of foodborne illness globally (Singh *et al.,* 2008) producing human acute bacterial enteritis. Overall, this organism's high incidence of clinical disease, low infective dosage in humans (Robinson, 1981), and potentially serious consequences confirm it as a significant public health issue (Tauxe, 2002). Although the number of infections has decreased marginally in some regions of the world in recent years, the overall illness burden remains high, necessitating a greater understanding of how this disease is spread into and through the human food chain. The fact that an increasing proportion of *Campylobacter* isolates from people and the human food chain are antibiotic resistant, and that antimicrobial resistant *Campylobacter* strains cause more persistent or severe disease than

antimicrobial-susceptible strains, adds to the concerns (Singh *et al.,* 2008; Tauxe, 2002). The most common cause of intestinal infections globally is thermotolerant campylobacters (*Campylobacter jejuni*/*Campylobacter coli*). The most common symptom is diarrhoea, which can range from mild to severe, with watery or bloody stools. Abdominal pain is another typical digestive tract symptom, but vomiting is infrequent. Fever, headache, asthenia, and anorexia can all occur before diarrhoea (Mégraud & Gavinet, 1989). *Campylobacter* spp. are enteroinvasive bacteria that cause colitis and can resemble inflammatory bowel disease in some cases. When pain is the most prominent symptom of the illness, distinguishing it from appendicitis might be challenging.

Due to phylogenetic and genetic similarities between *C. coli* and *C. jejuni* (Dedieu *et al.,* 2004), identifying *Campylobacter* species is problematic (Morris *et al.,* 1985). Differentiation of *C. jejuni* and *C. coli* is required for the treatment of human campylobacteriosis (Cloak & Fratamico, 2002). As a result, simple approaches for detecting and reliably distinguishing thermophilic *Campylobacter* species are required.

Jamshidi *et al.* (2008) researched isolation identification of *Campylobacter* spp. In the lab, filtered chicken rinse water was centrifuged for 10 minutes at 16000 g at 4°C. After the supernatant was discarded, the particle was suspended in 5 mL of enriched Preston broth. After resuspension, the samples were incubated for 24 hours at 37°C in a microaerobic atmosphere (5 % O2, 10% CO2, and 85 % N_2), then for another 24 hours at 42°C. In the enrichment broth, trimethoprim 10 mg/L, rifampicin 5 mg/L, polymyxin B 2500 IU/L, cefoperazone 15 mg/L, and amphotericin 2 mg/L were added to the nutrient soup.

The improved cultures were then plated onto a selective media consisting of a blood agar base supplemented with 7% lysed horse blood and antibiotics such as vancomycin (10 mg/L), polymyxin B (2500 IU/L), and trimethoprim (5 mg/L). In a microaerobic atmosphere for 48

hours, the plates were incubated at 42°C. A phase-contrast microscope and Gram staining were used to assess the morphology and motility of putative colonies on selective media. The suspicious colonies were then isolated on blood agar plates containing 5% sheep blood and incubated at 42°C for 72 hours under microaerophilic conditions, followed by catalase, oxidase, and hippurate hydrolysis biochemical tests. *Campylobacter* spp. colonies having a negative hippurate hydrolysis result were tested for sensitivity to 30 g discs of nalidixic acid on blood agar plates. A loopful of the likely colonies obtained on sheep blood agar was combined with 0.5 ml of a 1 % sodium hippurate solution and incubated for 2 hours at 37°C in a water bath. Then, on top of the hippurate solution, each tube received 0.2 ml of 3.5 % ninhydrin solution in a 1:1 mixture of acetone and butanol. The colour was further developed by incubation at 37°C for 10 minutes. The presence of glycine, which is formed by the hydrolysis of the Hippurate, was shown by rich purple colour, crystal violet-like, indicating a favourable result. (Jamshidi *et al.,* 2008).

2.7 Public health importance.

Because flies dine freely on both human food and unclean stuff, flies can spread diseases. While crawling and feeding, the fly gathers up disease-causing microorganisms. Those that attach to the fly's exterior surfaces may only live for a few hours, but those that are eaten with the meal may live for several days in the fly's crop or gut. When a fly comes into contact with people or their food, it transmits the disease (Foil & Gorham, 2000). The typical link between house flies and pathogenic organisms is that pathogens are picked up on the flies' bodies at one feeding place (e.g., a garbage can or manure pile) and then transferred to human and/or animal food on which they land and eat. Most infections can also be caught more directly by tainted food, drink, air, hands, or direct contact between people. Poliomyelitis and some skin infections (cutaneous diphtheria yaws and mycoses) are among the diseases that flies may spread. Enteric

illnesses (dysentery, diarrhoea, typhoid, cholera, and different helminth infections) and eye infections are only a few of the diseases that flies can spread (such as trachoma and epidemic conjunctivitis) (Foil & Gorham, 2000).

2.8 Weather variation and role of human activities effects on vector population dynamics.

Climate change has been documented to be one of the major causes of anthropogenic and environmental variance around the planet (Stocker *et al.,* 2013). These climatic and weather fluctuations have an impact on insect population dynamics, dispersion, abundance, and feeding behaviour, among other things. Shatz *et al.* (2013) hypothesized that numerous variables, including anthropogenic, biotic, and abiotic, were responsible for the Asian longhorn's dispersal (*Anoplophora glabripennis*) (Shatz *et al.,* 2013). The reproduction of insects is affected by upper and lower thermal abiotic variables. It also has an impact on the emergence, flight, and dispersal rate of insects (Yamamura *et al.,* 2006). Insect functioning is also affected by biotic factors such as low and high temperatures.

Global warming is a result of climate change, as is an increase in the frequency of extreme weather occurrences (Stocker *et al.,* 2013). Climate change has been related to negative health effects (Patz *et al.,* 2014). Climate change has an impact on vector-borne illness transmission and distribution, and the effects are expected to worsen in the future (Rocklöv & Dubrow, 2020). Worldwide projections show the yearly number of diarrhoeal disease episodes among children at 1.73 billion, with 700,000 deaths (Walker *et al.,* 2013). The worldwide burden of diarrhoeal disease is enormous, yet it is disproportionately distributed, with the WHO regions of Africa and Southeast Asia having the greatest burdens (Walker *et al.,* 2013). Weather variability has been related to bad health outcomes (Patz *et al.,* 2014) and increased reports of

diarrhoeal sickness have been linked to an increase in harsh weather occurrences, according to epidemiological studies (Phung *et al.,* 2017; Wu *et al.,* 2014; Xu *et al.,* 2014).

The worldwide burden of diarrheal disease, which accounts for the bulk of global juvenile fatalities worldwide, is predicted to rise as a result of climate change (Rosenthal, 2009). Previous research has linked diarrheal illness to a variety of environmental variables (including temperature, rainfall, and relative humidity) (Alexander *et al.,* 2013). Following a flood, rates of diarrhoeal disease (including cholera) may rise, particularly in communities with inadequate sanitation (Jongman *et al.,* 2012). Even when there's no flooding, excessive rainfall might lead to an increase in diarrhoeal illness rates due to overflowing latrines or sewage systems (Hirabayashi *et al.,* 2013). Just like other insects who take advantage of their environment to succeed in their survival, many vectors of diarrhoeal also flourish when the environmental conditions are favourable. Changes in insect density in reaction to abnormally warm weather offer us important indicators of global warming's possible implications, this can lead to the lengthening of their reproductive season and an increase in the number of generations per year (Kiritani, 2013). An increasing collection of evidence demonstrates that insects are reacting to contemporary climate change both directly and indirectly. Many studies have investigated and discussed the effects of global warming on arthropods and such studies include (Bale *et al.,* 2002; Cammell & Knight, 1992; Cannon, 1998; Kiritani, 2007).

The nature of studies of disease ecology pays special attention to the roles of anthropology. The transmission of many faecal-oral diseases is influenced by the same mechanisms that affect the spread of diseases due to the structure of most sanitation systems. For instance, the proximity of latrines or defecation to water sources may create convenient possibilities for faecal-oral transmission. Faecal-oral transmission could occur through physical contact with faeces near water and food. In tropical areas, several factors impacting faecal-oral transmission

are more essential than in temperate areas. People may, for example, defecate near agricultural fields due to a lack of proper indoor plumbing. Aside from direct touch with the excrement, contaminated soil might adhere to agricultural products. Regular outdoor vegetable markets are held in most regions of the world, where this product is hauled in from the (contaminated) fields and laid out on cloths or directly on the ground. The stage is set for disease spread via faecal-oral transmission unless all produce is thoroughly cleansed before use, which may be challenging in locations without running water. Open sewers, which are still ubiquitous in many developing-world towns, contribute to the spread of such diseases. Deforestation and other environmental changes may have an impact on the spread of faecal-oral illnesses and other related diseases. Human activities can influence the composition and abundance of vectors of diarrhoea. When there is open sewage filled with rotten food, human and animal faeces this serve as feeding and breeding grounds of this several insects that can become potential disease vectors.

2.8.1 Temperature on insect development.

Increasing the temperature range increases the rate of growth, but it also increases the formation of abnormalities and larval mortality (Khaliq *et al.,* 2014). Both land use and climate change have played a role in the decline of 260 macro moth species and the increase of 160 species in the United Kingdom (of a total of 673 species) (Fox *et al.,* 2014). Temperature, among other environmental conditions, can lengthen or shorten the life cycle of insects (Régnière *et al.,* 2012). Temperature changes in China, for example, will have opposing effects: increase in the maximum temperature will reduce malaria transmission in the country's southern regions, while increase in the minimum temperature will increase malaria transmission in the country's northern region, making a previously too cold region suitable for malaria transmission (Fouque & Reeder, 2019).

Khaliq *et al.* (2014) conducted research, investigating insect responses to high temperatures. In this research, it was noted that high thermal temperatures can affect the stage of an insect's life cycle, as well as its growth and several internal metabolic operations. For example, the egg period of *Helicoverpa armigera* was 7.9 days at 28 °C but prolonged to 10.4 days at 25 °C, this indicated the negative impact of decreasing temperature on the lifecycle of insects (Khaliq *et al.,* 2014).

Lyons *et al.* (2013) also conducted research monitoring *Anopheles arabiensis* and *Anopheles funestus*, Malaria vectors, developed at different rates and survived at different temperatures. 25 replicates of 20–30 eggs were implanted at nine constant and two variable temperatures for development rate investigations and survival predictions. Using established methods, many developmental parameters were estimated from the data. For both species, the lower development threshold (LDT) was estimated to be 13-14°C. *Anopheles arabiensis* has continuously developed quicker than *Anopheles funestus*. For overall growth and larval development, the optimum temperature (Topt) and development rate at this temperature (μmax) differed significantly between species. Optimum temperature and max for pupal development, on the other hand, were not significantly different between species. Temperature fluctuations had a deleterious impact on the rate of development and survival of *A. funestus*.

2.8.2 Relative Humidity influence on Insect Fauna.

Abiotic elements such as humidity, thermal effect, light, and food have distinct effects on different insects (Karl *et al.,* 2011). The rate of change of abiotic factors in the physical environment (temperature, light, humidity, etc.) can be evaluated. A change in this factor can go a long way to affect the fecundity, mortality, multiplication rate and generation time. Insect growth and behaviour can be influenced by relative humidity (RH), which affects the insect's capacity to regulate water loss. Insect development is sometimes hampered by low humidity,

yet most insects found in desert crops have evolved physiological and behavioural methods to avoid dehydration (Chik *et al.,* 2018). Many insects' physiology, and consequently their development, longevity, and oviposition, are affected by relative humidity. At low relative humidity, insects or their eggs may drown or become more easily infected by pathogens, as numerous pests of stored items have demonstrated (Gullan & Cranston, 2014).

2.8.3 Precipitation influence on insect Fauna

Because of their substantial disease burden, broad frequency, and great susceptibility to climatic influences, vector-borne diseases are among the most well-studied of the diseases linked to climate change. In contrast to some other climate-related health concerns, such as heat stress or exposure to storms and floods, meteorological conditions have a less direct and more varied impact on particular diseases (Smith *et al.,* 2014). Precipitation influences the biting, survival, and reproduction rates of vectors, as well as the survival and development rates of the infections they carry. Precipitation has a very significant influence on illnesses spread by vectors with aquatic development. Vector-borne disease (VBD) transmission is also influenced by rainfall and environmental humidity. In most cases, greater rainfall and significant flooding are linked to an increased risk of getting VBDs. This phenomenon has been observed in the transmission of malaria in Uganda, Zambia, and Papua New Guinea, as well as dengue fever in Vietnam and the Philippines (Fouque & Reeder, 2019). As environmental factors are affecting the transmission of some vector-borne diseases there is a likelihood of diarrhoea transmission being affected.

2.9 Potential Impact of Diarrheal Diseases on the Value Chain of Fisheries Along the Ghanaian Coast.

Ghana is one of the African countries with natural marine resources, especially the Atlantic Ocean, which has the greatest potential for prosperous fishing (Nyemah *et al.,* 2017). Ghana

has about 550 kilometres of coastline and a total of more than 24,300 square kilometres of the continental shelf to sustain a vibrant fisheries industry. The country also has a river, lake, and lagoon system that forms the base of inland fisheries. Accordingly, Ghana's fishing industry has been a major source of livelihood and jobs for millions of people in the country, accounting for about 5% of Ghana's Gross Domestic Agricultural Product (GDP) as well as 10% of the national animal protein. Ghanaians eat about 850,000 tons of fish per year, and about 440,000 tons are produced locally (Nyemah *et al.,* 2017). Most of the time the beaches along the coast of Ghana are littered with waste materials. The population growth in recent years has been remarkable, accompanied by rapid urbanization and a relative increase in industrial activity, particularly in many developing countries in Africa. Each day, as many as 8 million litter products are allowed to reach the seas worldwide (Nunoo & Quayson, 2003). In general, this debris, which comes from various sources, can be divided into land- and aquatic sources. Land based causes include industrial effluents, wash-out waste from run-off and poorly maintained garbage dumps, litter from beach vacationers, and neighbourhood garbage dumping grounds (Tsagbey *et al.,* 2009). Fishing communities are plagued with opened defaecation and dumping of waste at these areas. These activities may serve as breeding places for non-biting flies and various causatives of diarrhoea. This can impact the fishing industry when fishermen, fish mongers and boat makers fall ill and lessen the fishing force which in turn leads to less fish production. Contamination may also occur when fishes and other products are passed along the production line to the consumer thus leading to the spread of causatives of diarrhoea. Causative agents may be transmitted when flies feed on the processed fish products or when harvested fish come into contact with contaminated sea water.

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study sites

The study was carried out in four (4) towns, namely Anyako and Anyanui in the Volta region, Opetekwei in Greater Accra and Mumford in Central Region.

3.1.1 Anyako.

Anyako is a small island in the Keta Municipal Assembly and its geographical coordinates are 5° 59′ 38.26″ N, 0° 55′ 3.81″E. The town is surrounded by the Keta lagoon with its inhabitants involved in fishing. Some parts of the lagoon are designated for the dumping of waste materials. There is open defecation by children and adults along the banks of the lagoon. The island is plagued with a flood during the raining reason. Anyako land is clayey and doesn't support agriculture so the inhabitants are mainly into fishing.

Figure 7. Map of Anyako. Source: Amekugee N.E

3.1.2 Anyanui

Anyanui is situated in Anlo, Volta, Ghana, its geographical coordinates are 5° 47' 0" North, 0° 44' 0" East, 104 km east of Accra. Anyanui is made up of three sub areas namely Torkor, Wedadanu and Xorsekordzi. Anyanui is a coastal community, the inhabitants are into fishing and farming

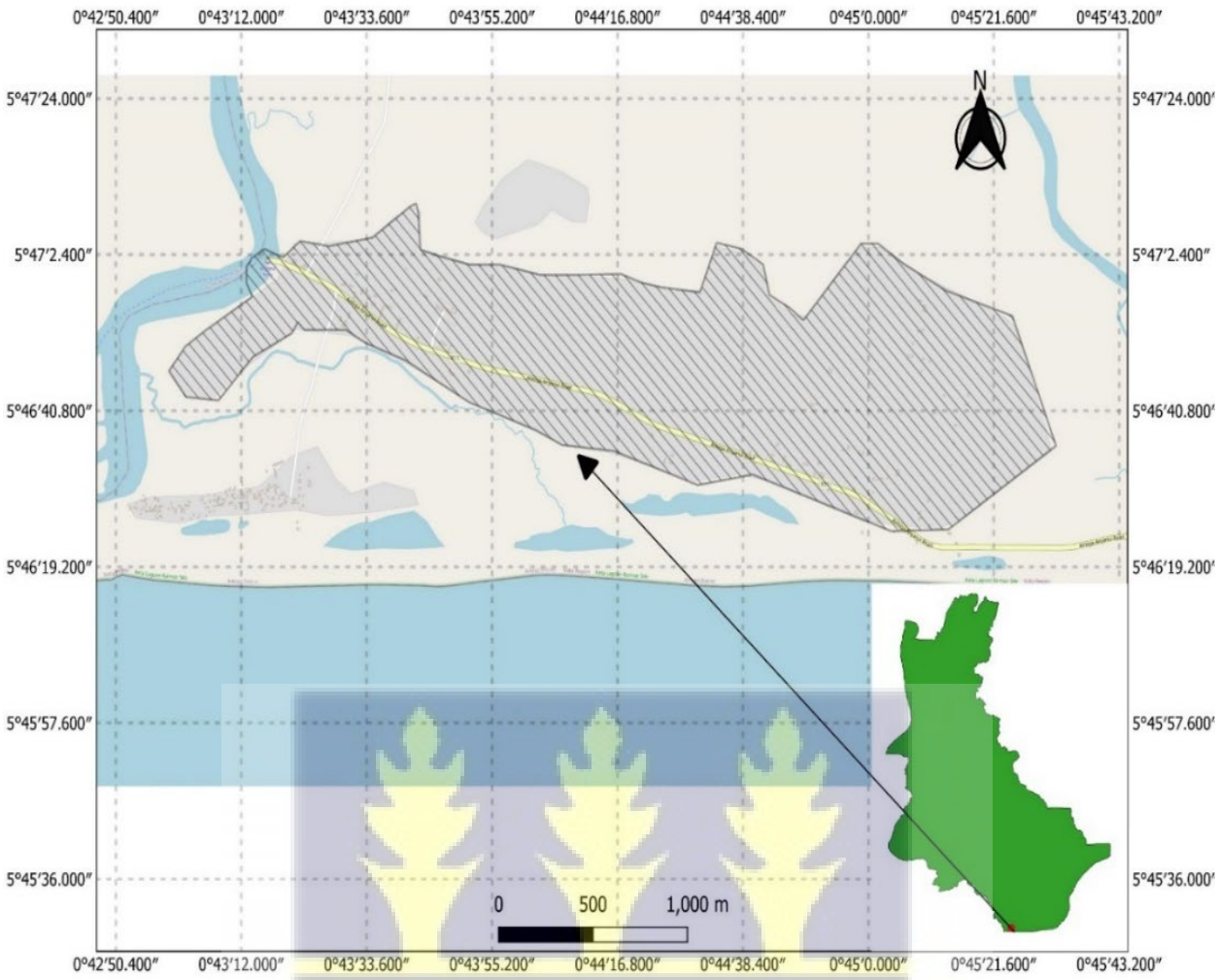

Figure 8. Map of Anyanui. Source: Amekugee N.E

3.1.3 Opetekwei

Opetekwei is in the Ablekuma West Municipal Assembly in Greater Accra and its geographical coordinates are 5° 31' 51" North, 0° 16' 20" West. Opetekwei is part of a fishing community that is densely populated. It is an urban area with an Estate and certain areas with little Infrastructure planning. Some areas are littered with human and animal waste.

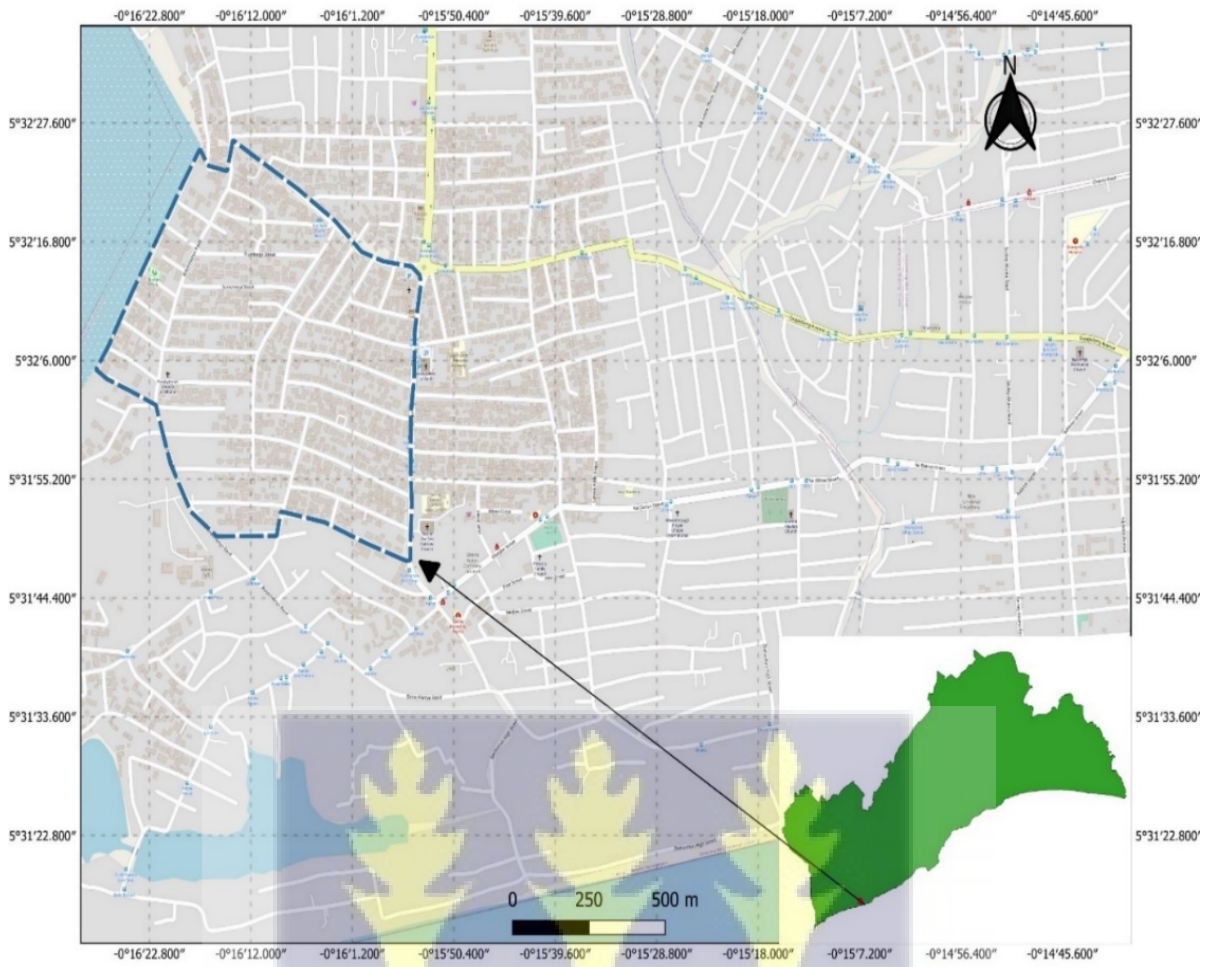

Figure 9. Map of Opetekwei. Source: Amekugee N.E

3.1.4 Mumford

Mumford is a town in Ghana's Central Region, near Apam, in the Gomoa West District. Mumford has a population of 18,368 people according to the 2013 census (Kiggins, 2010). It is a fishing community that also holds an annual celebration called Akwambo in the first week of November, it's geographical coordinates are 5° 15' 55" North, 0° 45' 56" West.

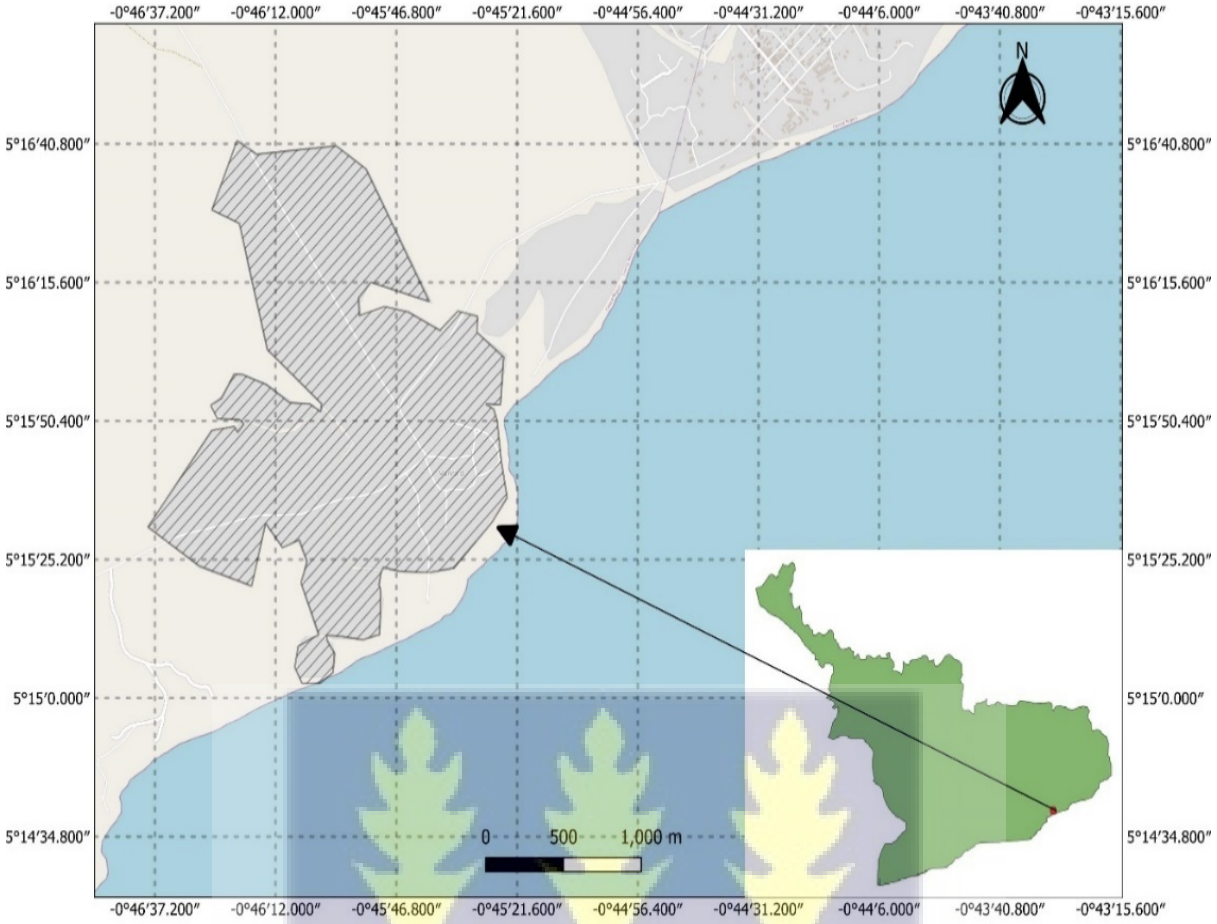

Figure 10. Map of Mumford. Source: Amekugee N.E

3.2 Study design.

A quantitative study design was used to acquire information on the presence of vectors of diarrhoea. The method includes setting traps in the communities, observation of the environmental sanitation in the communities and quantitative data collection and statistical test analysis. Pool test method which requires the combination of samples from test subjects and test them as a unit all at once, was used in the identification of insects with potential pathogens of diarrhoea. Traps used were made from 750ml water bottle. An opening about half an inch was made on the bottle cap. A cut was made at 2 inches below the bottle neck. The upper separated part was inverted into the lower part and taped. Strings were attached for hanging. Holes were made at the bottom for drainage in case of rainfall.

3.3 Ethical Clearance and study communities' entry.

Permission to conduct the study was given after ethical clearance was requested and the proposal was approved to conduct the study in Anyako, Anyanui, Mumford and Opetekwei. The Ghana Health Services Ethics Review Committee (GHS-ERC) reviewed and approved the implementation of the study. Ethics Committee for Basic and Applied Science (ECBAS) ECBAS 044/19-20 approved the commencement of the study. All protocols were adhered to the national guidelines on the prevention of COVID-19.

3.4 Sampling Techniques

A simple random sampling with blocking technique was used in the collection of the insects. Each sample site was divided into the 3 sub-areas A, B and C. The traps used were made and 3 baits were used in the insect collection. Each sub-area had 3 traps each being baited with meat, fish and mango. Based on research by Boonchu *et al.* (2003); Getachew *et al.* (2007); Figueiró *et al.* (2014), mango, fish and meat were among several baits used in their research. The decision to use these baits was because they were easily available and cost effective. The traps were placed randomly in the communities and the environmental factors (Temperature, Precipitation and Relative humidity) were recorded each sampling day. Sampling was carried out for 6 months, from January to June 2021.

Sampling was carried out three days a week, the traps were systematically set on Mondays, Wednesdays and Saturdays from 8 am to 5 pm. After collection, each insect is placed individually into Eppendorf tubes with respective trap names and dates then stored in Fridges are 4-5°C (Sivaramakrishnan & Razia, 2021). The sanitation conditions of the communities were noted and recorded in data books. The samples from each sample site were grouped into 24 pools making a total of 96.

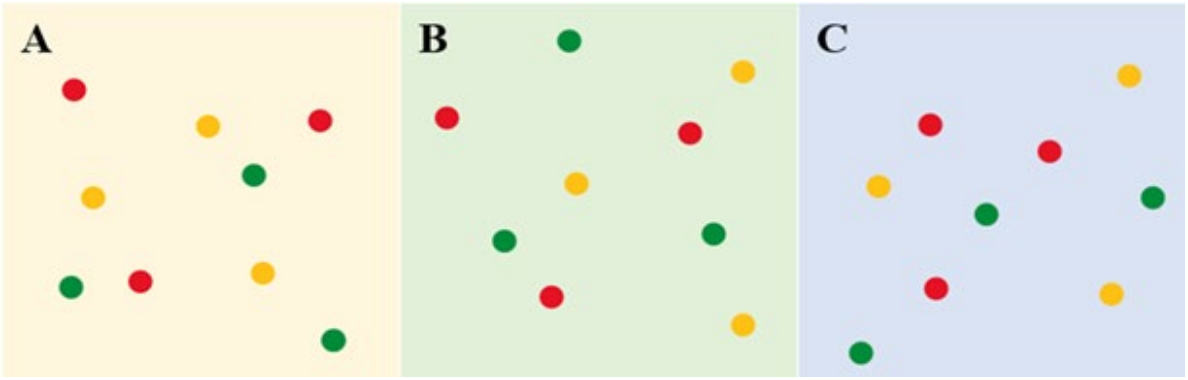

Figure 11. Simple random sampling with blocking. Each sampling site has 3 sub-areas (A, B and C). Red signifies meat, yellow signifies mango and green signifies fish. The baited traps are set randomly with a recorded GPS coordinate.

Figure 12. Water bottle traps. (A) Setting fish baited trap at Anyako. (B) Meat baited trap at Anyako. (C) and (D) mango baited trap at Opetekwei.

3.5 Insect identification using Taxonomic keys.

Kirk-Spriggs & Sinclair, (2017) gave clear keys for the morphological identification of Muscidae, Sarcophagidae and Calliphoridae (Kirk-Spriggs & Sinclair, 2017). The primary dipterous families' diagnosis and keys used by Sawaby *et al.* (2018) (appendix 2) were also used in the identification of insects into their respective families.

3.6 Insect swabbing

Swabbing of the insects was done at the Target malaria laboratory. The samples were grouped according to the sample sites, months of collection and family. Twenty-four pools were gathered for each sample site making a total of ninety-six (96) pools. All insects in a pool were swabbed and treated as a single unit.

3.7 Media preparation

For this study, media was prepared for four pathogens; *Salmonella* spp., *shigella* spp., *Vibrio cholerae* and *Escherichia coli*. 150 glass petri dishes were washed and autoclaved at 161°C degrees Celsius for an hour.

3.7.1 Modified Tryptone Soya Broth (TSB).

16.5 grams was added to 500 ml of distilled water and mixed well. The medium was sterilised by autoclaving at 121°C. It was cooled to 50°C and poured into 96 vials to 5ml mark with code names that consisted of acronyms of the various sample sites with numbers.

3.7.2 Salmonella, Shigella (S.S) Agar.

66 grams of S.S agar powder was added to 1 litre of distilled water and allowed to soak for 10 minutes. The mixture was swirled and brought to a boil. It was allowed to cool to 47°C and

poured into 47 petri dishes. The prepared medium was incubated overnight at 37°C for bacteria culture.

3.7.3 Thiosulfate citrate bile salts sucrose T.C.B. S Agar.

88 grams of T.C.B.S agar powder was added to 1 litre of distilled water and allowed to soak for 10 minutes. It was mixed well and brought to a boil. It was cooled at 47°C and poured into 50 petri dishes to set. The medium was incubated overnight at 37°C.

3.7.4 Chromogenic UTI medium Agar.

21.5 grams of chromogenic UTI Agar powder was added to 500ml of distilled water and missed well. It was sterilised by autoclaving at 121°C for 15 minutes. It was cooled to approximately 50°C and poured into 25 sterile petri dishes, it was incubated overnight at 37°C degrees Celsius.

3.7.5 Brilliance UTI Clarity Agar

18.5 grams of Brilliance UTI Clarity agar powder was added to 500ml of distilled water and missed well. It was sterilised by autoclaving at 121°C for 15 minutes. It was cooled to approximately 50°C and poured into 20 sterile petri dishes, it was incubated overnight at 37°C.

3.7.6 Nutrient Agar

14 grams of nutrient Agar powder was added to 500ml of distilled water and allowed to soak for 10 minutes. It was swirled to mix properly and autoclaved for 15 minutes at 121°C. it was cooled at 47°C and poured into 25 plates to set.

Figure 13. Bacteria media. (A) T.C.B.S medium, (B) S.S Agar, (C) TSB, (D) CHROMO UTI, (E) Brilliance UTI Agar, (F) Nutrient Agar.

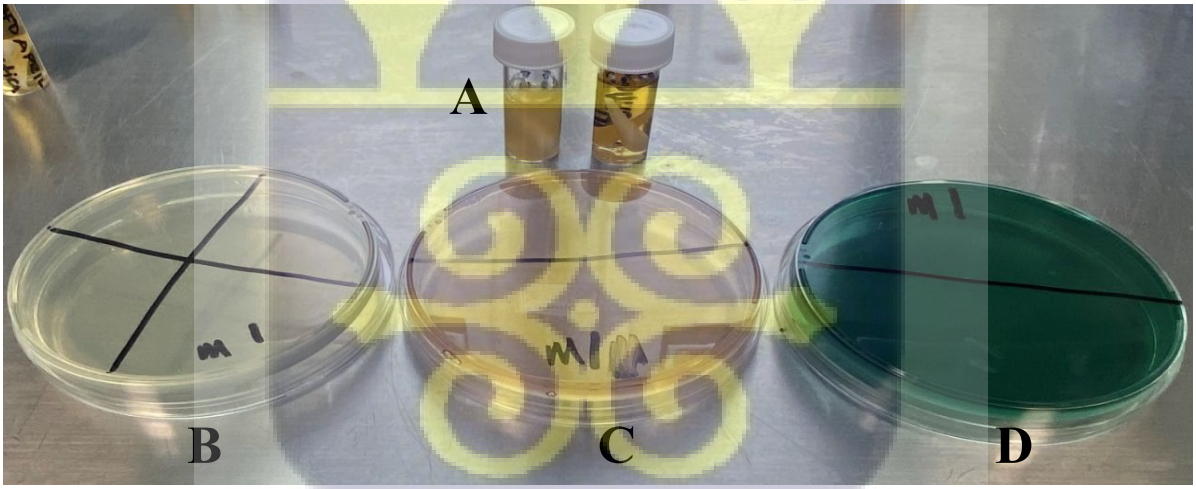

Figure 14. Growth media used for bacteria culture. (A), TSB with swabs. (B), Chromogenic UTI Agar. (C), S.S Agar. (D), T.C.B.S Agar.

3.8 Bacteria culture (streaking)

Samples were placed in the Tryptone Soya Broth and were placed into the incubator at 37°C for 24 hours. The samples were cultured in a clean bench or Hood to prevent contamination during streaking. Sterile inoculation loops were dipped into TSB and Immediately, using a back-and-forth motion, streaking the inoculating loop across a quarter of the S.S Agar, T.C.B. S Agar, Chromogenic UTI medium Agar plates. The inoculated plates were incubated at a temperature of 37°C for 24 hours.

3.8.1 Bacteria Sub-culture.

After twenty-four hours the plates were removed and checked for bacteria growth. Some plates had more than one bacteria colony growing on one medium. This was observed mostly on the S.S agar and the chromogenic UTI medium agar plates. Suspected *E. coli* bacteria colonies were isolated from the chromogenic UTI medium agar and inoculated onto Brilliance UTI Clarity Agar. Suspected *Salmonella* spp. and *Shigella* spp. bacteria were also sub cultured on S.S Agar. Using close parallel streaks, an isolated colony from the agar plate culture was distributed throughout the first quadrant (about 1/4 of the plate) to get pure bacteria colonies. These bacteria were sub cultured on nutrient agar after which identification was done.

Figure 15. Streaking done in a clean bench (Hood) to prevent contamination.

Figure 16. Streaking inoculated TSB on Chromogenic UTI Agar.

3.9 Bacteria identification

Bacteria are routinely identified using morphological and biochemical tests, with further testing such as serotyping and antibiotic inhibition patterns performed as appropriate. Newer molecular techniques make it possible to identify species based on their genetic sequences, which can sometimes be done immediately from a clinical specimen (Baron *et al.,* 1996).

3.9.1 Oxidase test for *Vibrio cholerae.*

Fresh growth from any non-carbohydrate-containing media was used for the oxidase test. Growth from thiosulfate citrate bile salts sucrose (TCBS) agar was not used. The oxidase test protocol was obtained from CDC Laboratory Methods for the Diagnosis of *Vibrio cholerae* manual. In a petri dish, 2 to 3 drops of oxidase reagent (1 % tetramethyl-p-phenylenediamine) was dropped onto a piece of filter paper. Using a sterile inoculation loop, a colony was smeared on moist paper (Thacker *et al.,* 2012). Within 10 seconds of a good reaction, the bacterial growth is supposed to turn dark purple (positive), the test culture showed no visible colour change indicating that the bacteria tested was not *Vibrio cholerae*.

3.9.2 Gram staining

A drop of distilled water was placed on a slide. A sterile inoculation tool was used to pick a colony and smeared on the slide with the water. It was laid out to air dry. After it had air dried a drop of gram crystal violet was added to it for one (1) minute. The slide was washed gently in flowing stream of tap water for about two (2) minutes. A drop of Gram Iodine was dropped on the slide. A drop of Gram safranin was added for a minute then washed. An immersion oil was dropped on the slide observed under ×100 magnification.

3.9.3 Indole Test for *E. coli*

Bacteria samples were placed in 1.5ml tubes, 1 ml of T.S.B was poured into the tubes and mixed well. 2 to 3 drops of Kovac's reagent were added, pink colour change indicates the presence of *E. coli*, the colourless reaction is negative.

3.9.4 API test

Colonies of bacteria were picked from the various plates and plated on 5 ml of peptone broth. An aliquot was distributed into API 20E test kits and incubated for 24 hours. TDA, IND, VP 1 and VP 2 were added to the respective cells for the final reading. The colour change was read according to a chart book or software that aids the identification of the bacteria.

Values of 1, 2 and 4 are awarded if the corresponding cells are positive according to the API 20E chart. The sum of the values was from each group of cells were put together to produce seven profile identification code numbers. This 7-digit profile number is inputted in API 20E software for the identification of bacteria or the code is read from the API 20E bacteria identification book.

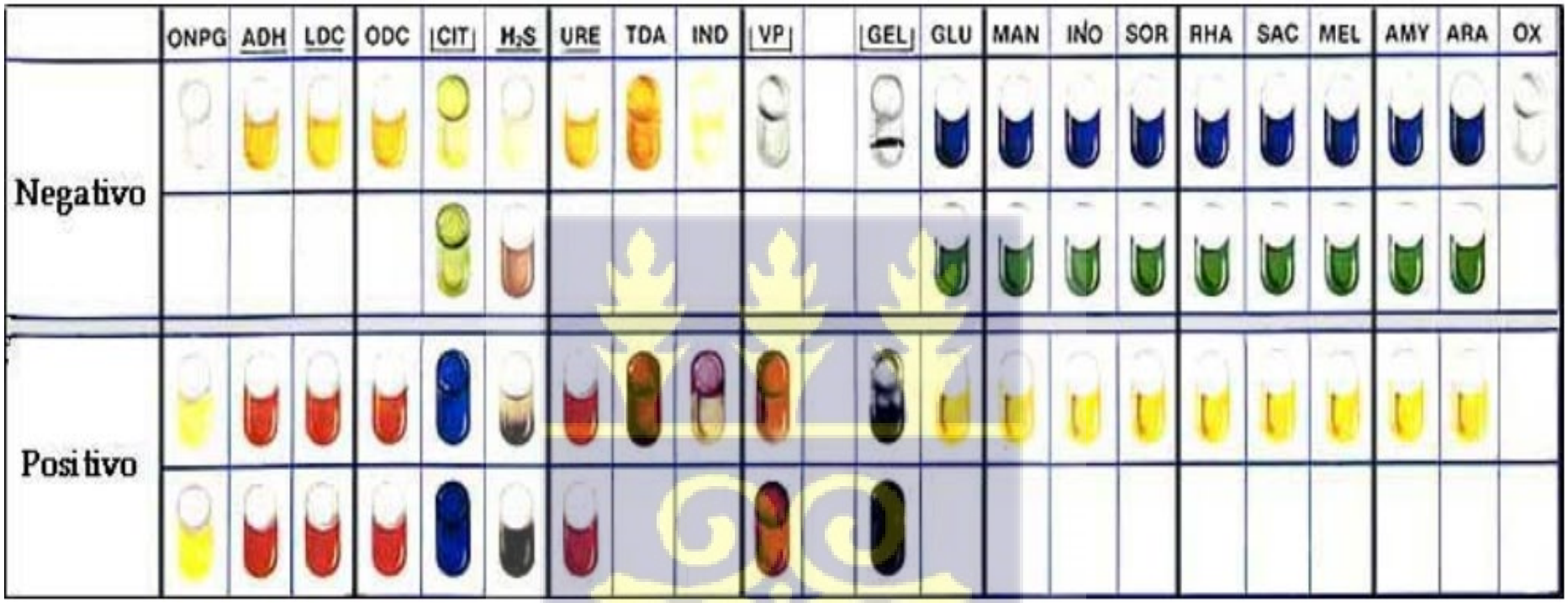

Figure 17. API results chart.

3.10 Data Analyses.

Recorded Data from the four study sites were arranged according to study sites, months, insect family and recorded environmental factors. The data was pooled and organised in Microsoft Excel spread sheet. The Maps of the study sites were mapped out using QGIS v 3.16.11.

The data was imported into SPSS v25 and checked for normality then a correlation test which measures the relationship or association between two continuous variables was carried out. If the data is normality distributed a parametric Pearsons correlation (r) test will be carried out. If the data is not normally distributed a non-parametric Spearman's rank correlation (rho).

The data for this study was not normally distributed and a Spearman's rank correlation (rho) was performed to analyse the correlation relationship between the recorded population of the insects collected and the recorded environmental factors.

The Shannon diversity index (*H*) was developed using the sampled data and calculations of species diversity, richness, and evenness.

Sanitary conditions of the study sites will be classified into three groups (a) good - high standard sanitary condition (presence of waste bins, no to less littering, good drainage system, well planned building layout), b) moderate -fair sanitary conditions (some presence of waste bins, some drainage system planned building layout and c) poor – bad sanitary condition (no waste bins, littering, bad drainage system and poor building layout.

CHAPTER FOUR

4.0 RESULTS

4.1 Percentage of insects from the study sites.

During the process of insect collection, it was noted that some of the traps used were lost, damaged or emptied of their content. This was occurred in all four study areas. A total of 8817 of insects were collected from all four communities. Figure 18 represents the total percentage number of flies collected from all four study areas. Anyako which was 45% was the highest, followed by Anyanui (22%), Mumford (21%) and Opetekwei (12%).

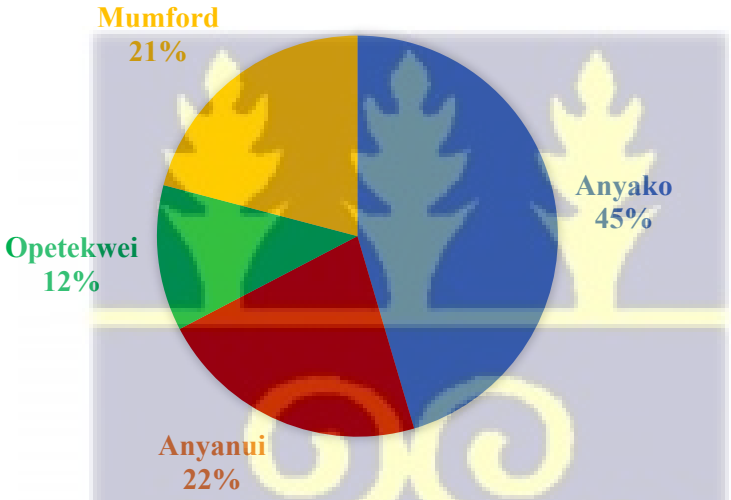

Figure 18. Percentage number of flies collected from each of the study sites from January to June.

4.1.1 Shannon diversity index and Shannon Equitability Index.

No significant results were recorded for diversity (H= 0.45) and Evenness (E= 0.28) of insect fauna in the study sites. The lower the value of H and E, the lower the diversity and species evenness.

Table 1. Analysis of Shannon diversity indices and Evenness of collected insect fauna

Family	Number	pi	*In*pi	pi**In*pi
Muscidae	7680	0.871045	-0.13806	-0.12026
Sarcophagidae	176	0.019961	-3.91395	-0.07813
Calliphoridae	940	0.106612	-2.23856	-0.23866
Hymenoptera	18	0.002042	-6.19407	-0.01265
Coleoptera	3	0.00034	-7.98582	-0.00272
			H	0.45
			E	0.28

$H = -\Sigma p_i * ln(p_i)$

- **Σ:** sum
- ***ln*:** Natural log
- p_i**:** The proportion of the entire community made up of species *i*

$E = H / ln(S)$

4.2 Observed environmental (sanitary) conditions.

During data collection, the sanitary conditions of the study sites were observed from January to December. These conditions, though subjective, were categorised into three; a) good -high standard sanitary condition, b) moderate -fair sanitary conditions and c) poor – bad sanitary condition.

In Anyanui, the environmental condition was very good; the community had Zoomlion bins at designated points. Residents also dug holes to burn waste products. The sandy nature of the soil enabled water do be absorbed quickly, preventing creation of puddles of water. The buildings were widely distributed and fairly urbanised.

In Mumford, the sanitary condition observed was fairly good. Some parts of Mumford were clean and others were littered with both animal and human waste. The community had

Zoomlion bins at designated points but some areas were littered with pigs feeding on these wastes. This was observed from January to December.

In Anyako, it was noted that the banks of the lagoon served as dumping sites for household waste, animal waste and other waste products from January to December. Sheep, goats and pigs were observed feeding along the banks of the river. A number of the residents were interviewed about the practises and they believed it helped retained the land. Because Anyako is an island the buildings are clustered and slummed with only walkways between buildings.

Opetekwei had the estate part and a slummed area. The estate area was clean with well-planned buildings. The houses in the estate had household bins for waste disposal. The slum part of Opetekwei had the worst sanitation condition. The building plan was poor, slummed and clustered with choked gutters. The shore was littered with dead animals, animal and human waste. Sewage flowed into the sea where fishing was taking place.

4.3 Morphological description of flies.

Muscidae made up about 87.10% (7680), Sarcophagidae contributed about 2 % (176) and Calliphoridae made up about, 10.66% (940) for the target insects. Non-target insects were also collected during sample collection. These were Hymenopterans and Coleopterans. These insects accounted for 0.2% (18) and 0.03% (3) respectively of the total number of flies collected. The target families mainly Muscidae, Sarcophagidae and Calliphoridae were prepared for identification using taxonomic keys.

Musca domestica (Muscidae) was observed to have a lighter brown abdomen, gray thorax with four longitudinal strips along the thorax. The wings observed to have an anal wing vein (A1) that was not curved forward on a trajectory that would intersect vein anterior cubitus and posterior cubitus (CuA+CuP) if extended; anal wing vein (A1) was not curved forward on a

trajectory that would intersect vein CuA+CuP if extended. The Muscidae wing has the anal veins A1 and A2 nearly parallel or diverging.

Family Sarcophagidae, the body was not metallic or shiny, the lower calypter lacked setulae on the dorsal surface and the thorax had four notopleural setae. Most species were either black or grey, with the dorsum of thorax often striped (and abdomen chequered, maculate or fasciate), antennal arista (ar) bare or plumose in basal 2/3, and 2–4 notopleural setae.

The body of the Calliphoridae family is generally metallic green, blue, or copper, with a metallic shine on the abdomen, and many species are yellow to brown. On the dorsal surface of the lower calypter, there are setulae or not. If the abdomen is grey and black, the thorax is covered with yellow crinkly hair-like setae. Two notopleural setae on the thorax. Antennal arista (ar) is plumose from tip to tip.

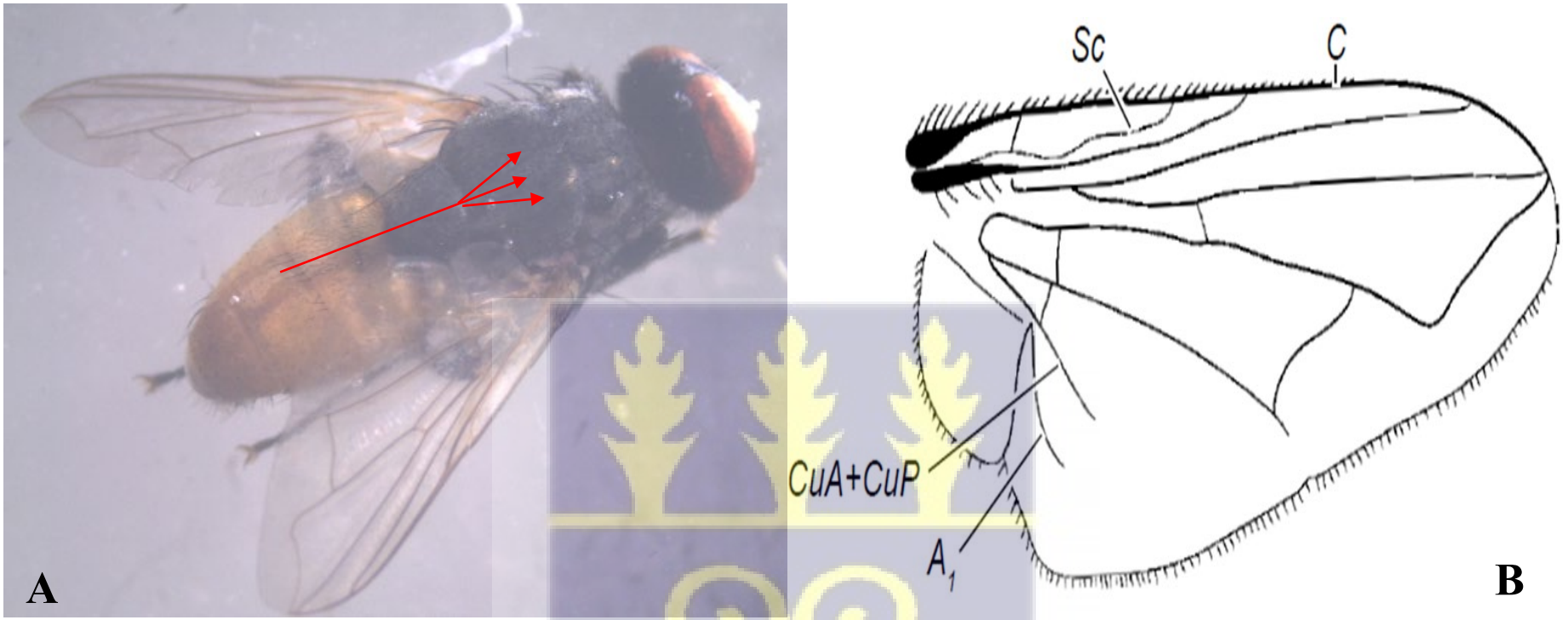

Figure 19. (A) Identified Muscidae (*Musca domestica*) with red arrows pointing to thorax with longitudinal strips. (B) Wing venation of Muscidae family showing the anal wing (A_1) not curved forward towards anterior cubitus vein and posterior cubitus vein (CuA+CuP). Source: (Kirk-Spriggs & Sinclair, 2017).

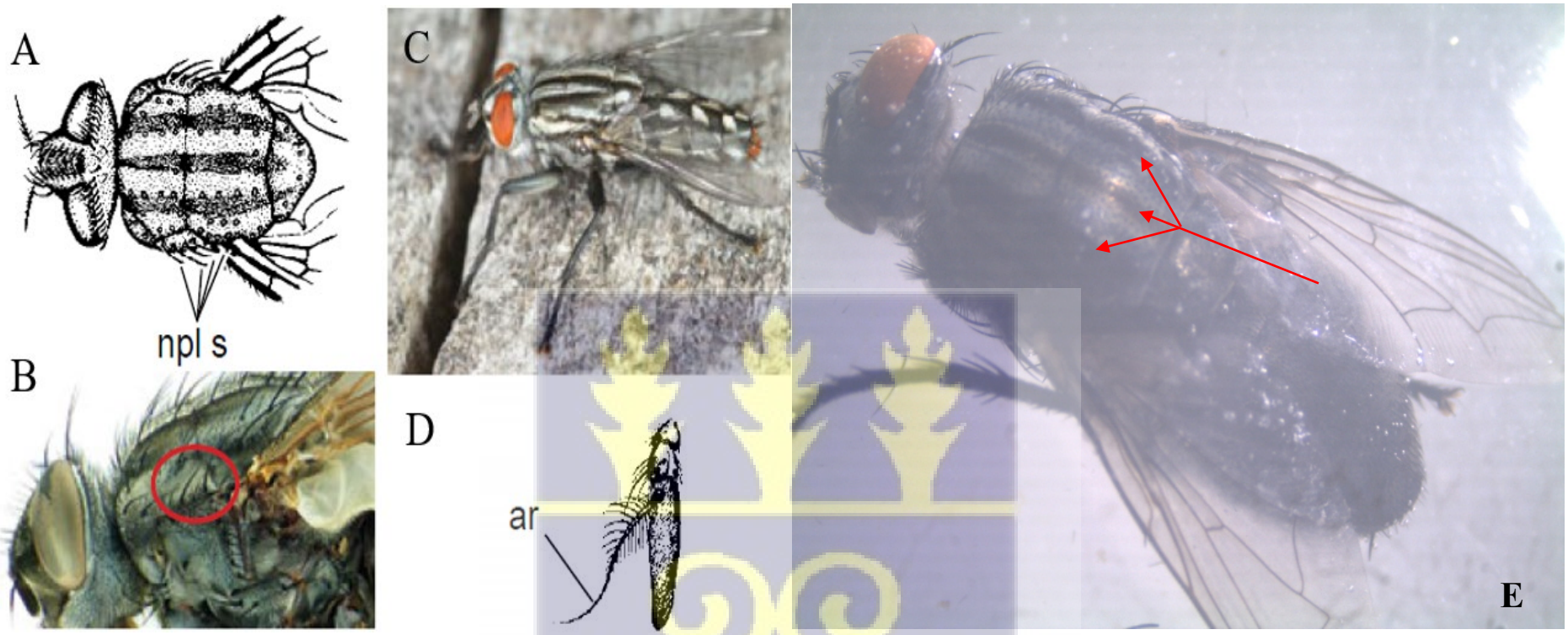

Figure 20. Family Sarcophagidae (A) and (B), Thorax has four notopleural setae. (C), The body is not metallic or shiny but checkered. (D), Antennal arista (ar) bare or plumose in basal 2/3. (E), red arrows showing strips on thorax of identified *Sarcophaga* spp. Source :(Kirk-Spriggs & Sinclair, 2017).

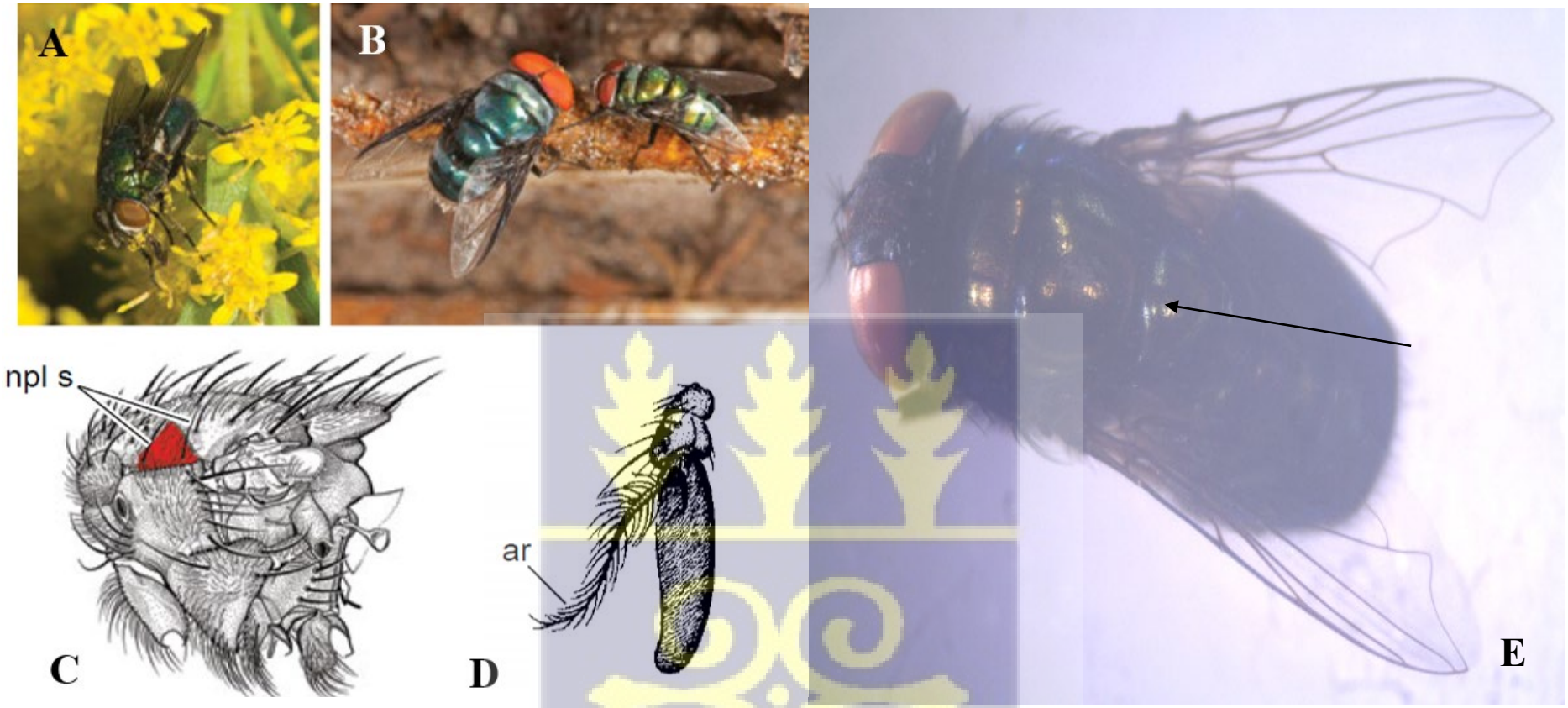

Figure 21. Family Calliphoridae (A) and (B). Body is metallic green and blue. (C). thorax with two notopleural setae (npl s in red). (D). Antenna arista (ar) usually plumose to tip, (E) black arrow pointing to metallic green thorax of identified *Chrysomya* spp. Source: (Kirk-Spriggs & Sinclair, 2017)

4.3.1 Anyako.

Out of the 3984 adult flies collected for the study at Anyako, 3838 samples (96.34%) were morphologically identified to be Muscidae, 73 samples (1.83%) were Sarcophagidae, 61 samples (1.53%) were Calliphoridae. Many non-target insects were also collected and these contributed 0.28% and 0.03% of the number collected, corresponding to 11 Hymenopterans and 1 Coleoptera respectively. Figure 22 shows the trend analysis of the mean numbers of flies collected from January to June

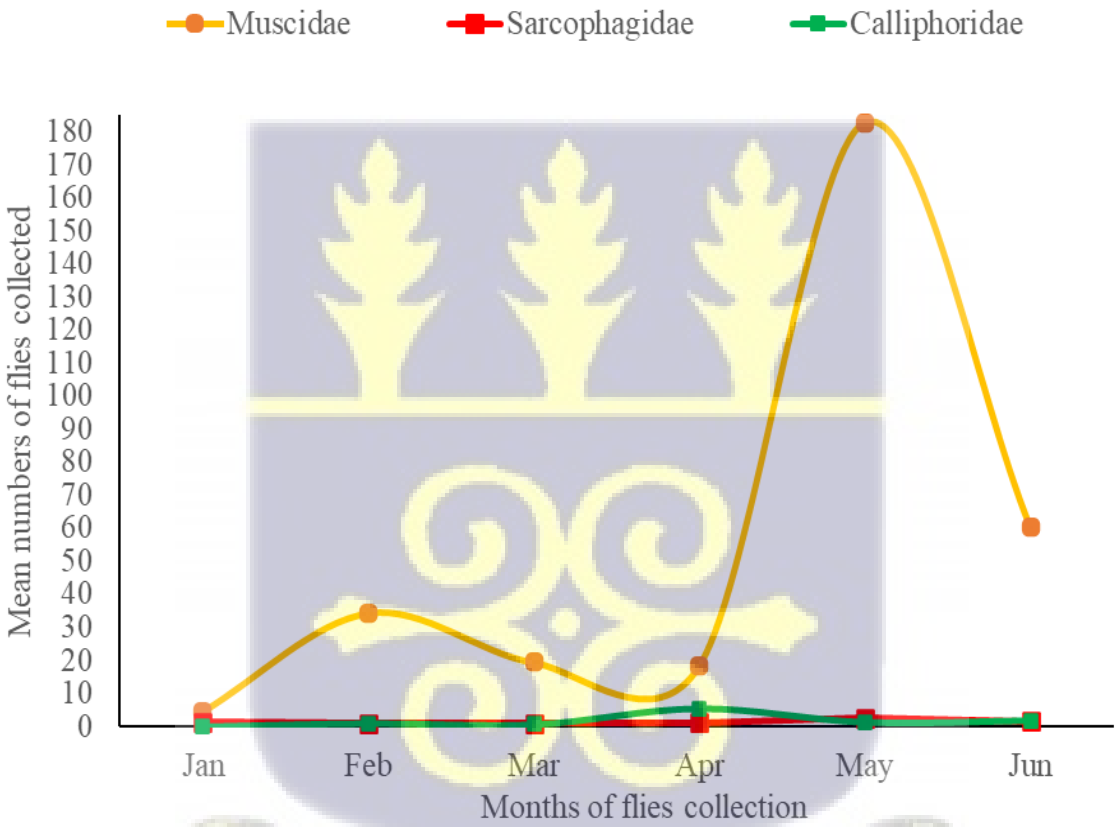

Figure 22. Graph showing insect family composition and abundance from January - June in Anyako.

4.3.2 Anyanui

Figure 23, shows the line graph of the average numbers of the diarrhoeal vectors from January to June. Out of the 1921 adult flies collected from January to June. Muscidae contributed to 1863 individuals (97%) Sarcophagidae 2 samples (0.1%) and Calliphoridae 56 samples (2.9%). In Anyanui no non-target insects were collected.

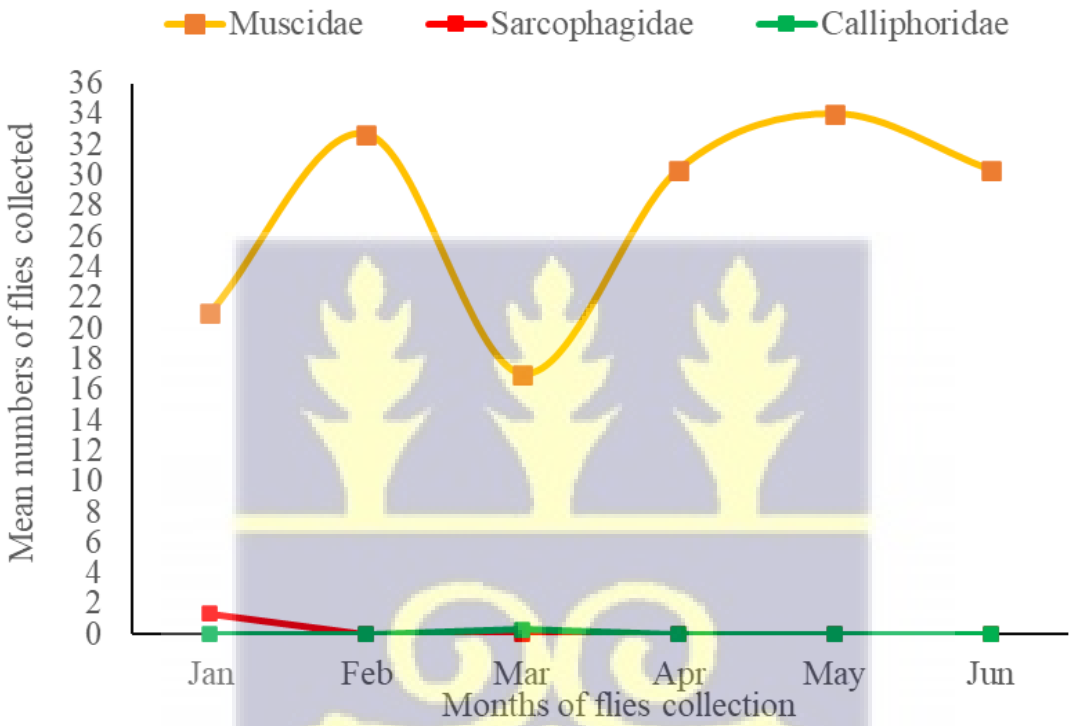

Figure 23. Graph showing insect family composition and abundance from January - June in Anyanui.

4.3.3 Opetekwei

Of the total of 1031 samples collected, Calliphoridae was most abundant contributing to 77.8% (802), followed by Muscidae contributing to 168 samples (16.3%); next was Sarcophagidae of which 54 samples (5.2%) were recorded. Non-target insects like hymenoptera were recorded, contributing 7 samples (0.7%) of the total catch.

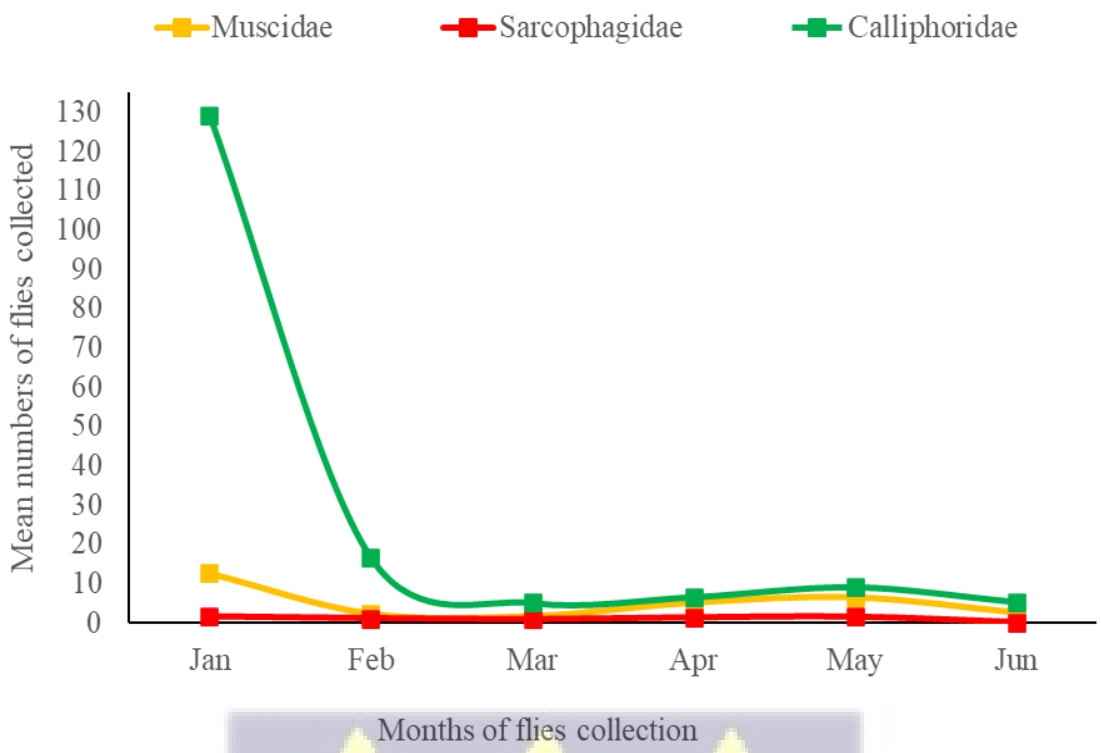

Figure 24. Graph showing insect family composition and abundance from January - June in Opetekwei

4.3.4 Mumford

From Figure 25, the trend analysis shows the fluctuation of vector population from January to June. Of 1833 samples collected, Muscidae was most abundant -contributing 96.3% (1766), followed by Sarcophagidae contributing to 44 samples (2.4%). Next in abundance was Calliphoridae, of which 21 samples (1.1 %) were recorded. Non-target insects like Coleopteran were also recorded, contributing 2 samples (0.1%) of the total catch.

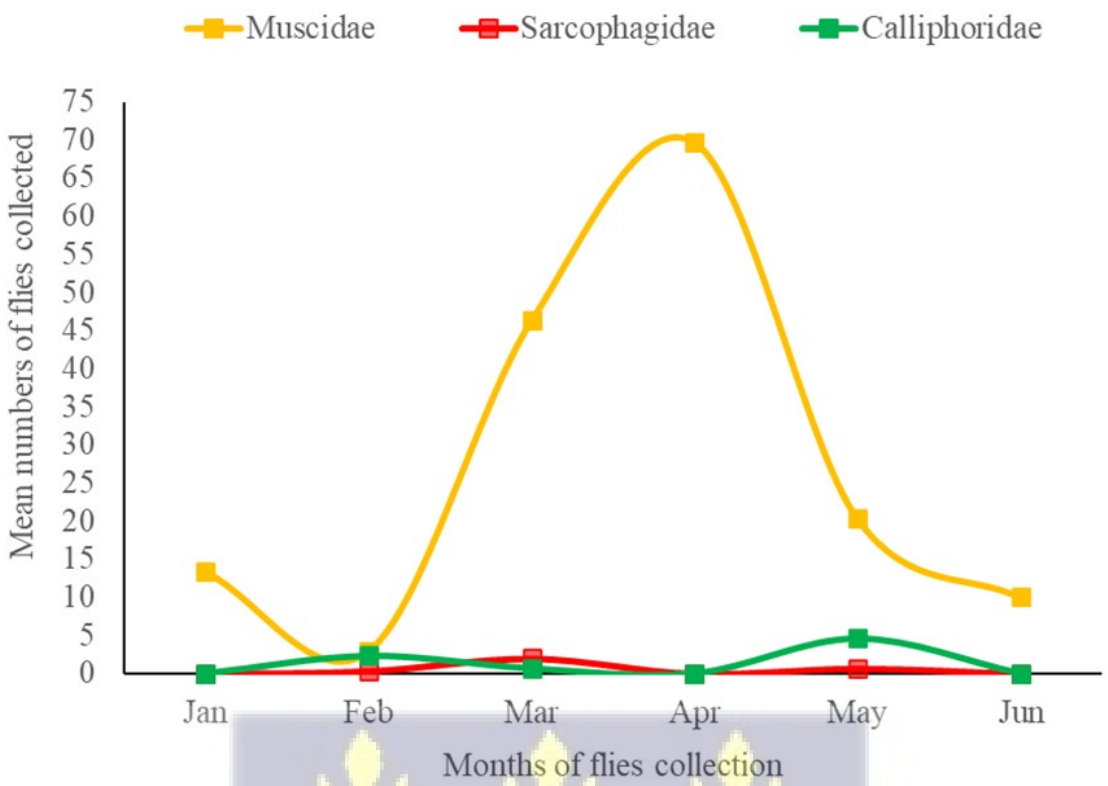

Figure 25. Graph showing insect family composition and abundance from January - June in Anyanui Mumford.

4.4. Relationship between the Environmental factors (Temperature, Humidity, Precipitation) and the insect population in Anyako.

During sample collection, environmental conditions were recorded for all communities. In the months of January, February and March, the highest and lowest temperature recorded was 36°C and 27°C respectively. For Precipitation the highest and lowest recordings were 18% and 1% respectively. Humidity was 93% for highest and 18% for the lowest recorded value. In the wet months (April, May and June), 34°C was recorded as the highest temperature and 24°C as the lowest. Increasing precipitation was also recorded. The highest precipitation value was 97% and the lowest recorded value was 4%. The highest Relative humidity value was 95% and the lowest value was 3%.

From Figure 26, it was observed that the numbers of Calliphoridae and Sarcophagidae were quite low and with that, no relationship was observed between Calliphoridae, Sarcophagidae and the environmental factors. From April, the number of insects collected increased from and peaked in June. The number of Calliphoridae recorded also had a slight increase from April to June. For Muscidae, a relation could be observed from the trend presented in Figure 26. From January to March (dry season), the numbers were low while the temperature increased. The temperature began to drop from April to June (wet season) whiles the number of insects increased with the highest being in May.

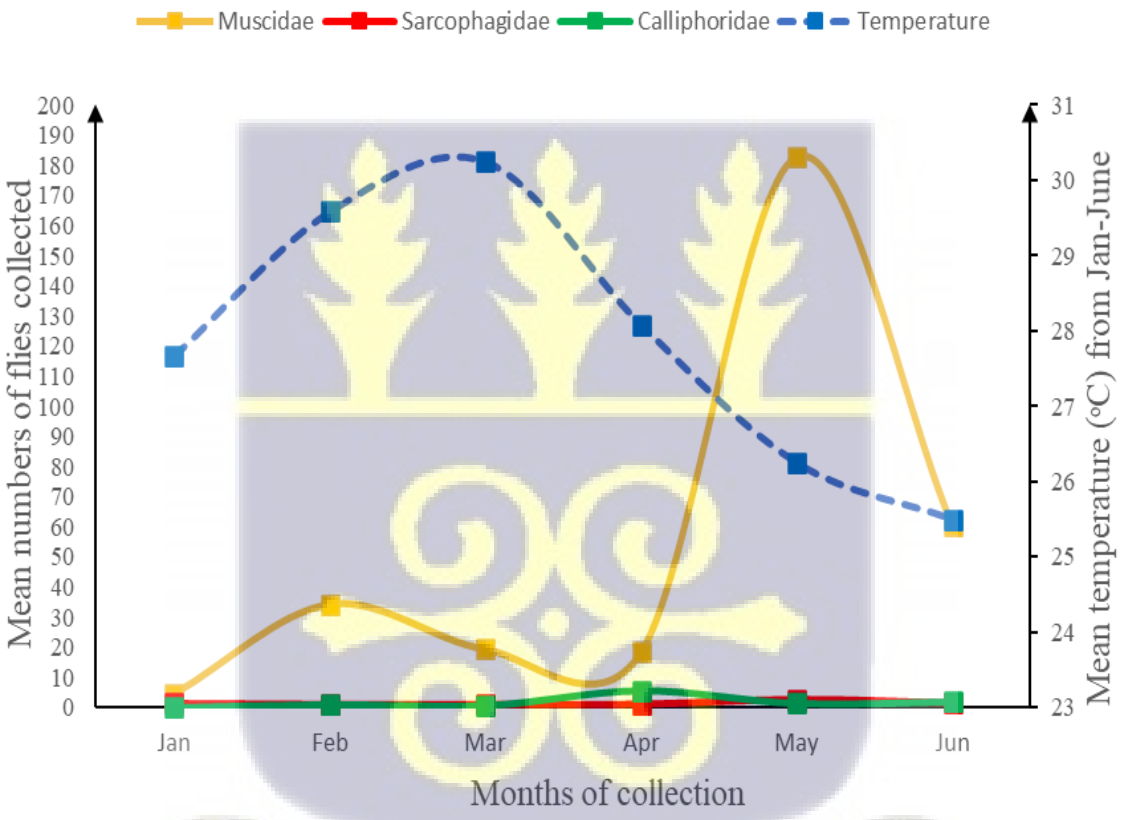

Figure 26. Relationship between temperature and the numbers of Muscidae, Sarcophagidae and Calliphoridae trapped in Anyako during the study period.

Figure 27, shows the influence of humidity on Muscidae, Sarcophagidae and Calliphoridae populations in Anyako. It was observed that the number of these insects, especially Muscidae numbers fluctuated with humidity. This pattern is shown in the graph that depicts the numbers

of Muscidae increasing when relative humidity decreases in the dry season (January to March) (Figure 27). In the wet season (April to June), a steady increase in humidity was observed whiles the population of Muscidae increased in April and then decreases in June. A slight increase in the number of Calliphoridae was recorded in April and dropped in May and June.

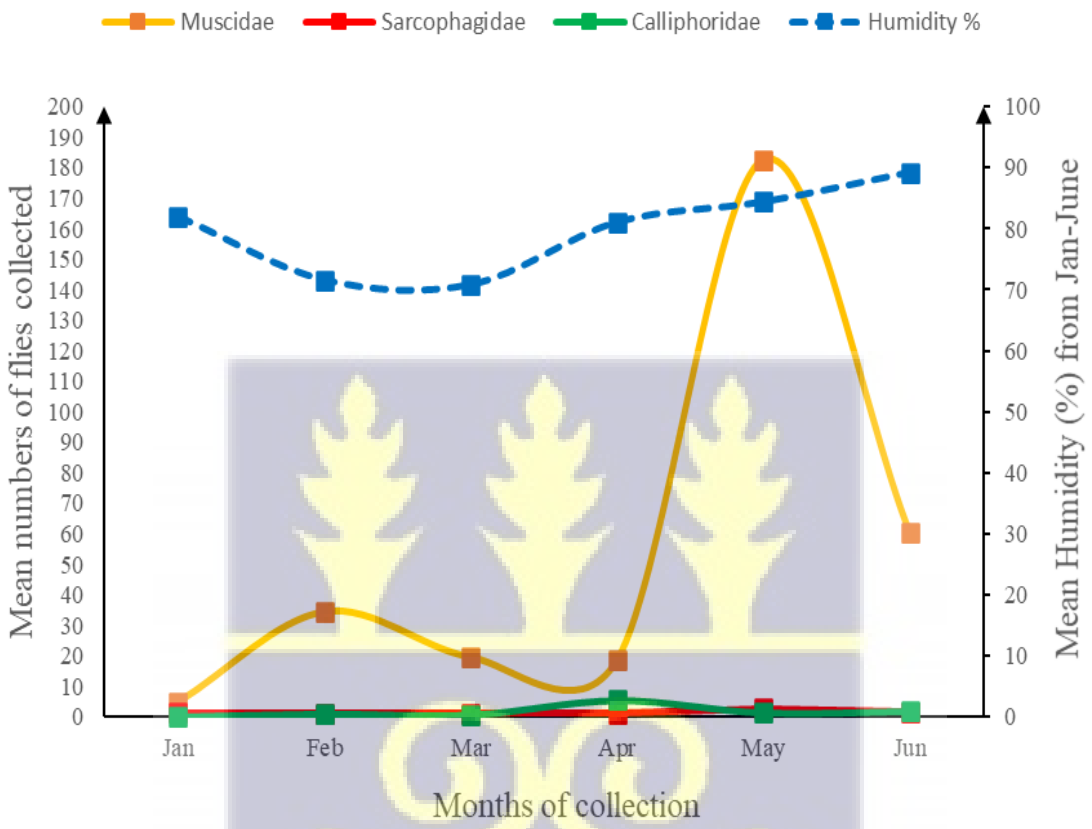

Figure 27. Relationship between humidity and numbers of Muscidae, Sarcophagidae and Calliphoridae trapped in Anyako during the study period.

Precipitation in Anyako from January to June had a steady increase (Figure 28). The trend of precipitation observed was similar to the number of Muscidae collected from January to June. Both the number of Muscidae and pattern of precipitation fluctuated in the dry season (Figure 28), with both volume of precipitation and the number of Muscidae increasing significantly in the wet season, from April to June (Figure 28). The numbers of Sarcophagidae and Calliphoridae only had a minor increase in April, dropping off in May and June.

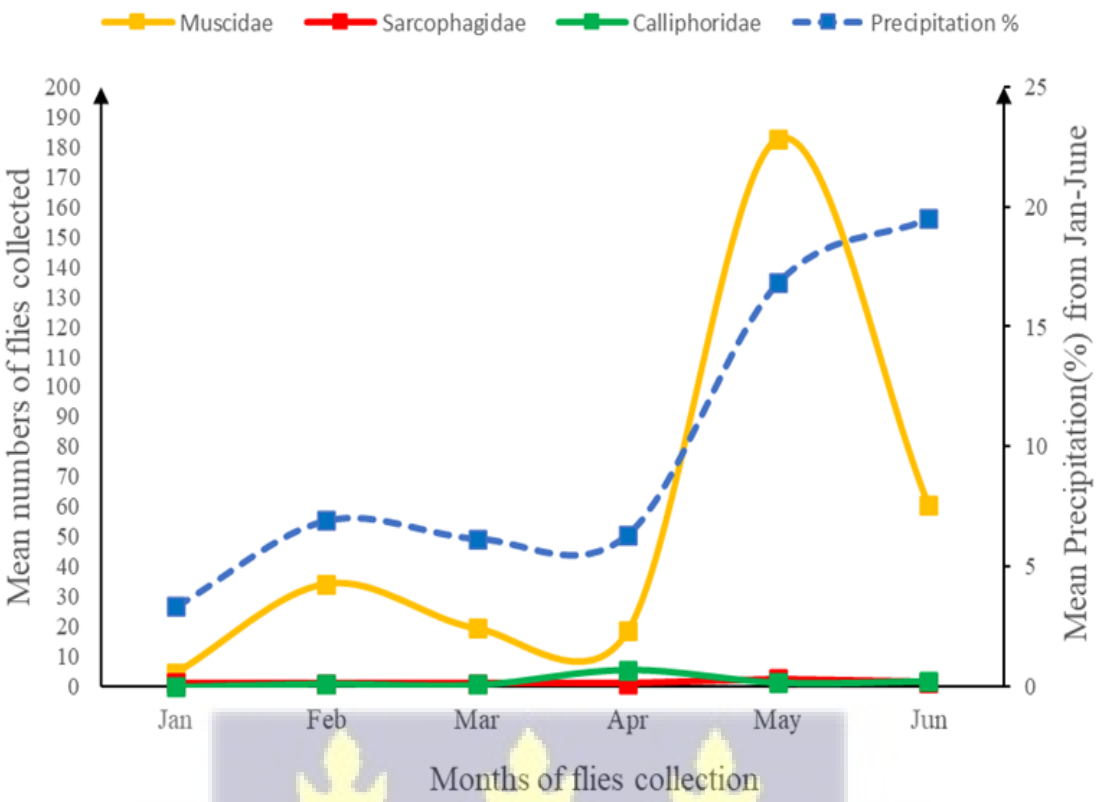

Figure 28. Relationship between precipitation and numbers of Muscidae, Sarcophagidae and Calliphoridae trapped in Anyako during the study period.

4.4.1. Relationship between Environmental factors (Temperature, Humidity, Precipitation) and insect population in Anyako.

The data was analysed to check for normality. Data was not normally distributed. A non-parametric test, Spearman's correlation (rho) analysis using SPSS version 25 was performed. From the analysis (Table 1), page 60. The relationship between Muscidae abundance and temperature, with sample size (n) of 68 sampling days and a degree of freedom (df) of 3 had a weak negative correlation coefficient (r) of -0.196 which was not significant. A moderate positive correlation coefficient (r) of 0.313 was observed for Muscidae and humidity which was significant with a *p*-value less than $p < 0.01$. The relationship between the number of

Muscidae and Precipitation had a positive correlation coefficient (r) was 0.159 but was not significant.

A negative correlation coefficient (r) -0.201, was observed for the relationship between the number of Sarcophagidae and temperature with a sample size (n) of 68 sampling days and a degree of freedom (df) of 3, however this was not significant. On the other hand, the relationship between the abundance of Sarcophagidae and humidity was a moderate positive correlation coefficient (r) of 0 .263 which was significant (p <0.05).

Generally, for the abundance of Calliphoridae, the relationship with temperature had a moderate negative correlation coefficient (r) -0.225. A moderate positive correlation coefficient (r) 0.195 was observed for the number of Calliphoridae and humidity. Between the numbers of Calliphoridae and levels of precipitation, the relationship was a moderate positive correlation coefficient (r) 0.373 which was significant (p< 0.01).

Table 2. Summary of correlation analysis between environmental factors and vectors of diarrhoea in Anyako.

Variables	M	SD	(Temp)	(Hum)	(PP)	(Mu)	(S)	(C)
Temperature (Temp)	28.01	2.674						
Humidity (Hum)	79.54	16.508	-.731**					
Precipitation (PP)	10.41	16.483	-.299*	0.215				
Muscidae (Mu)	58.75	131.325	-0.196	.313**	0.159			
Sarcophagidae (S)	1.28	2.442	-0.201	.263*	0.103	.473**		
Calliphoridae (C)	1.85	5.379	-0.225	0.195	.373**	.272*	.352**	

**p< 0.01 (2-tailed), *p<0.05 (2-tailed), N=68, M= Mean, SD= Standard Deviation

4.5 Relationship between Environmental factors (Temperature, Humidity, Precipitation) and the insect population in Anyanui.

It was observed that the numbers of Calliphoridae and Sarcophagidae were low, therefore no relationship was observed these flies and the environmental factors (Figure 29). A slight increase in the number of Calliphoridae was recorded in March, which plateaued from April to June. The numbers of Sarcophagidae trapped were highest in January only and decreased from February to June. As shown by the temperature trend line in figure 29, a positive correlation relationship was observed for the number of Muscidae collected over the study period. The number of Muscidae fluctuated with the increase and decrease in temperature; as the temperature decreased, the population increased and vice versa; this was seen from January through June.

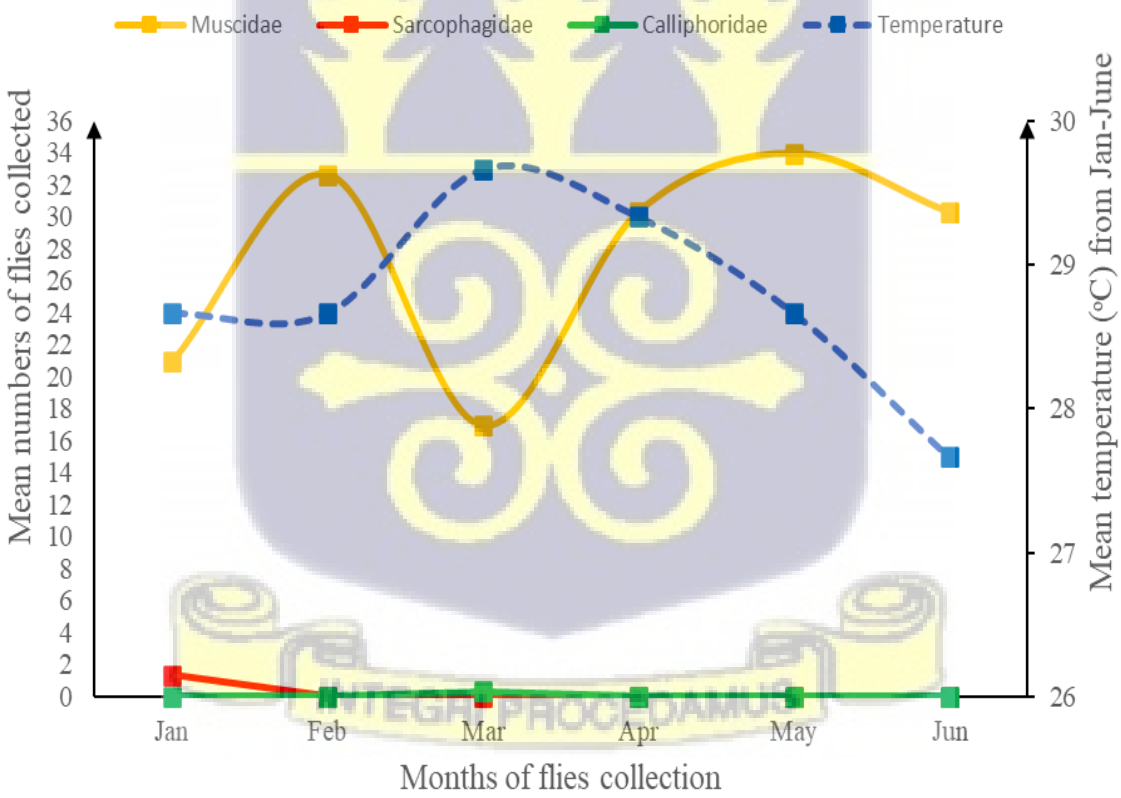

Figure 29. Relationship between temperature and numbers of Muscidae, Sarcophagidae and Calliphoridae trapped in Anyanui during the study period.

In figure 30, the influence of humidity on the numbers of Muscidae, Sarcophagidae and Calliphoridae collected in Anyanui is shown. The number of these insects especially that of Muscidae fluctuated with humidity. There was a steady rise in relative humidity from January to June. The number of Muscidae sampled increased with humidity in January and February, but decreased in March and April when humidity increased. Muscidae numbers increased as humidity decreased in May. In June, an increase in humidity corresponded to a decrease in the number of Muscidae. For Sarcophagidae and Calliphoridae the numbers were very low and this trend was throughout the months of collections. A negative relationship was observed between Sarcophagidae and relative humidity; from January to June, as relative humidity increased the numbers of Sarcophagidae plummeted. With Calliphoridae, a low capture frequency was observed from January to February, which increased in March as relative humidity increased and then decreased from April to June as relative humidity steadily increased.

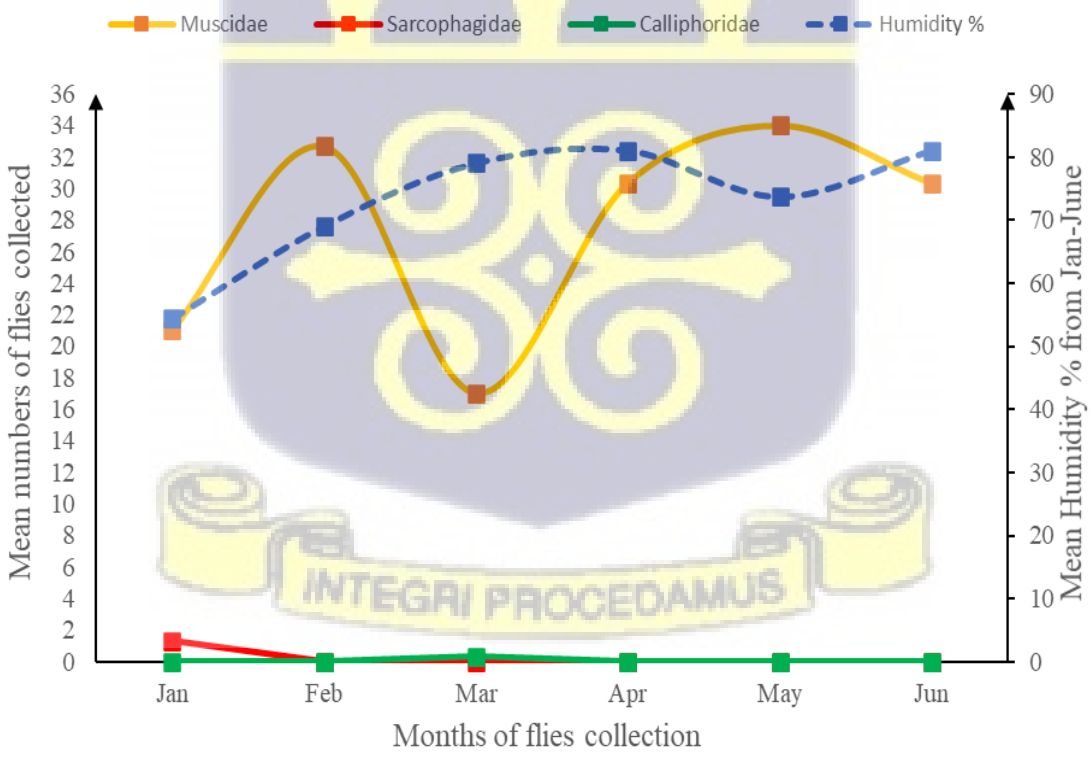

Figure 30. Relationship between humidity and numbers of Muscidae, Sarcophagidae and Calliphoridae trapped during the study in Anyanui.

Levels of precipitation were generally quite low from January to April, with a sharp increase in May and June (Figure 31). Precipitation levels seemed to have a relationship with the number of Muscidae trapped, such that, as precipitation increased, their numbers also increased. However, a deviation from this was observed in June, when the precipitation levels were at their highest but the Muscidae numbers decreased.

For Sarcophagidae and Calliphoridae the population numbers were very low. Precipitation fluctuated from January to June, causing a decrease in Sarcophagidae. Calliphoridae numbers were low from January to February and increased in March. Calliphoridae numbers decreased with decreasing precipitation from April to May.

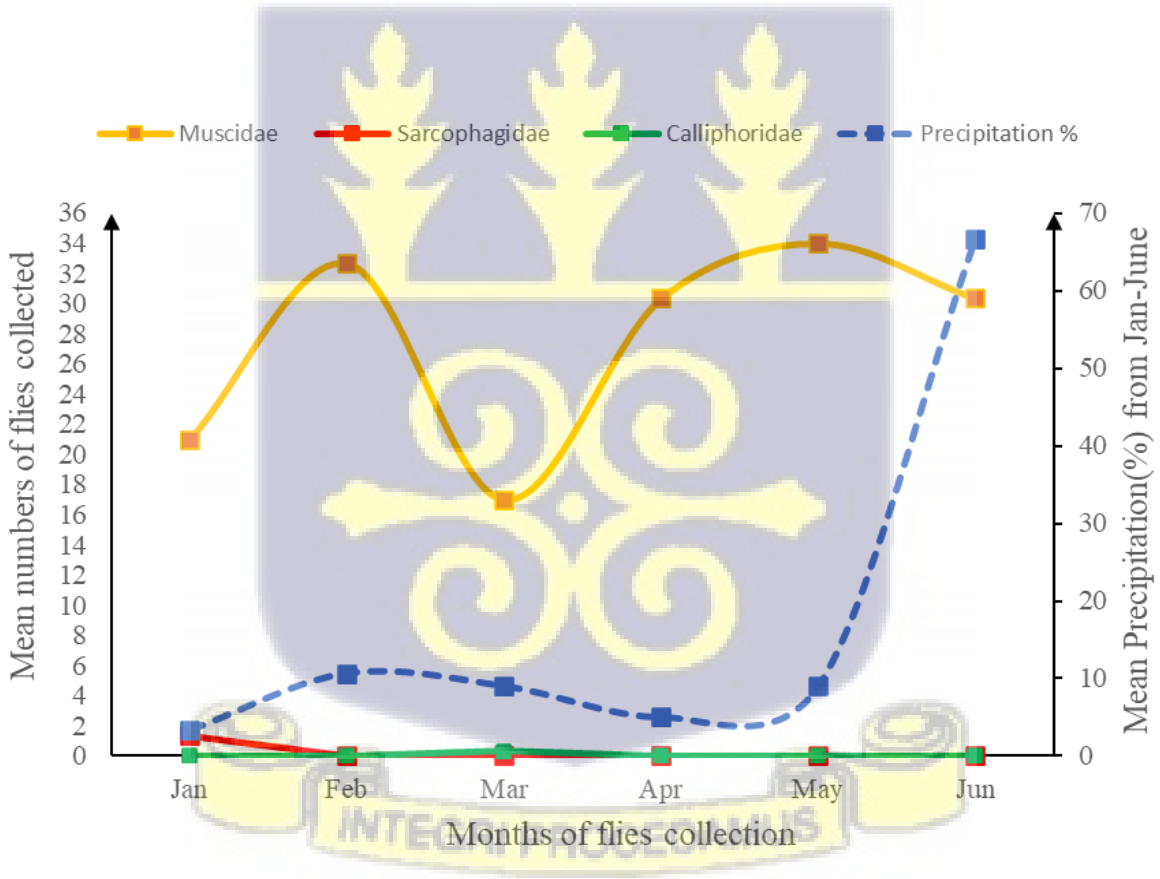

Figure 31. Relationship between precipitation and numbers of Muscidae, Sarcophagidae and Calliphoridae trapped during the study in Anyanui.

4.5.1 Relationship between Environmental factors (Temperature, Humidity, Precipitation) and insect population in in Anyanui.

The data was analysed to check for normality. Data was not normally distributed. A non-parametric test, Spearman's correlation (rho) analysis using SPSS version 25 was performed. From Table 2, page 64, the abundance of Muscidae with a sample size (n) of 68 sampling days and a degree of freedom (df) 3, had a moderate positive correlation coefficient (r) 0.302 with temperature which was significant with a *p*-value of $p < 0.05$. Muscidae had a low negative correlation coefficient (r) -0.023 with humidity which is not significant. The relationship between Muscidae and temperature shows a positive correlation coefficient (r) of 0.302 which was significant with a *p* value of $p < 0.05$.

With a sample size (n) of 68 and a degree of freedom (df) 3, the relationship between Sarcophagidae and ttemperature, humidity and precipitation showed low positive correlation coefficient (r) 0.003, low negative correlation coefficient (r) -0.104 and low negative correlation (r) -0.137 respectively which had no significant *p* values. There were no significant *p* values recorded for the relationship between Calliphoridae, temperature, humidity and precipitation which had low positive correlation (r) 0.016, low positive correlation (r) 0.104 and a low negative correlation (r) -0.137 respectively with a degree of freedom (df) 3 and a sample size (n) of 68 sampling days.

Table 3. Summary of correlation analysis between environmental factors and vectors of diarrhoea in Anyanui.

Variables	M	SD	(Temp)	(Hum)	(PP)	(Mu)	(S)	(C)
Temperature (Temp)	28.69	1.083						
Humidity (Hum)	75.85	8.847	-.277*					
Precipitation (PP)	17	23.01	-0.223	.486**				
Muscidae (Mu)	27.85	12.339	.302*	-0.023	0.173			
Sarcophagidae (S)	0.06	0.34	0.003	-0.072	-0.118	-0.12		
Calliphoridae (C)	0.82	2.443	0.016	0.104	-0.137	-.250*	.471**	

**$p < 0.01$ (2-tailed), *$p < 0.05$ (2-tailed), N=68, M= Mean, SD= Standard Deviation

4.6 Relationship between Environmental factors (Temperature, Humidity, Precipitation) and the insect population in Opetekwei.

From Figure 32, the temperature in Opetekwei was observed to have increased from January to April and then decreased from May to June. The influence of temperature trends can be seen on Calliphoridae and Muscidae numbers. As the temperature increased, the population trend decreased from January to February. Both insects maintained a linear population trend with a slight increase in May when the temperature dropped. As shown in Figure 32, the Sarcophagidae population trend was steady from January to June with no visible influence from temperature

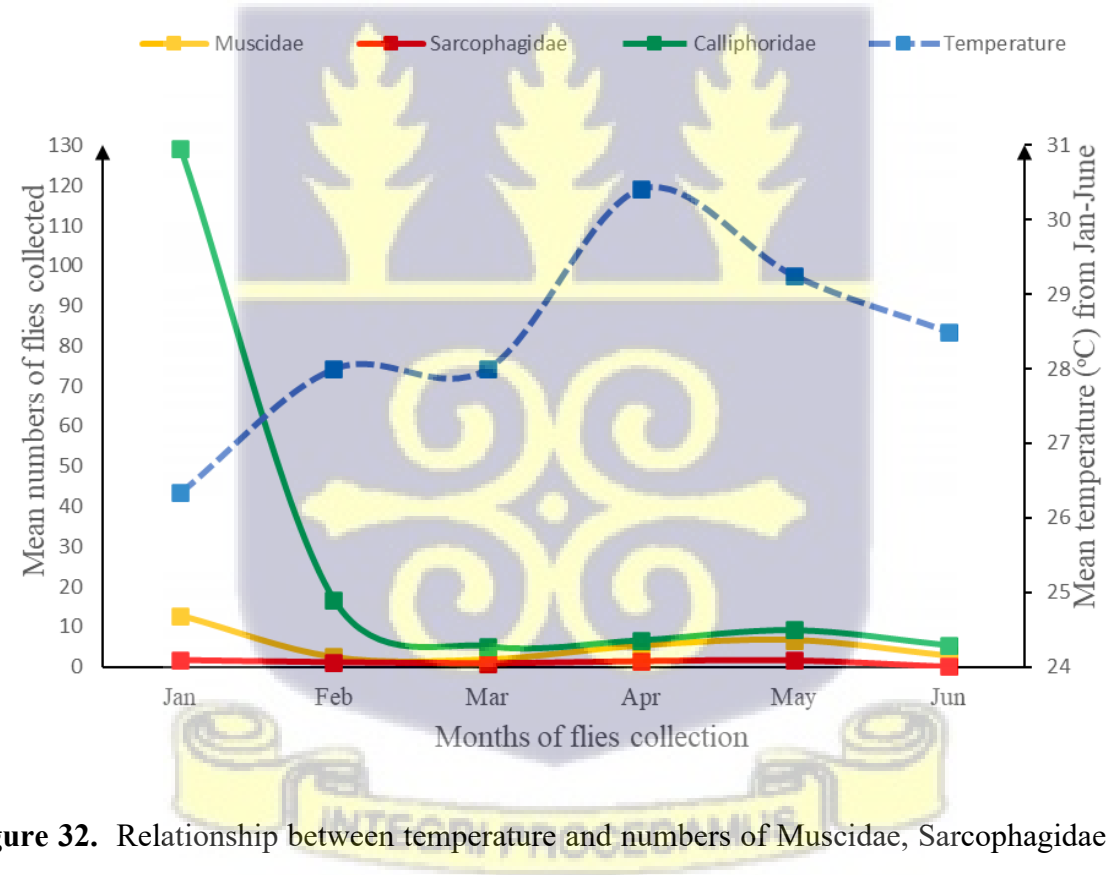

Figure 32. Relationship between temperature and numbers of Muscidae, Sarcophagidae and Calliphoridae trapped in Opetekwei during the study period.

Figure 33 shows the relative humidity trend increasing from January to March (dry season), followed by a decrease in April and a steady increase from April to June. A visible relationship can be seen between Muscidae and Calliphoridae population trends. As relative humidity increases, these two population trends (Muscidae and Calliphoridae) decreases. A slight increase was observed with Muscidae and Calliphoridae population trend in April and May and a decrease in June when humidity trend was in it's second ascent from April to June.

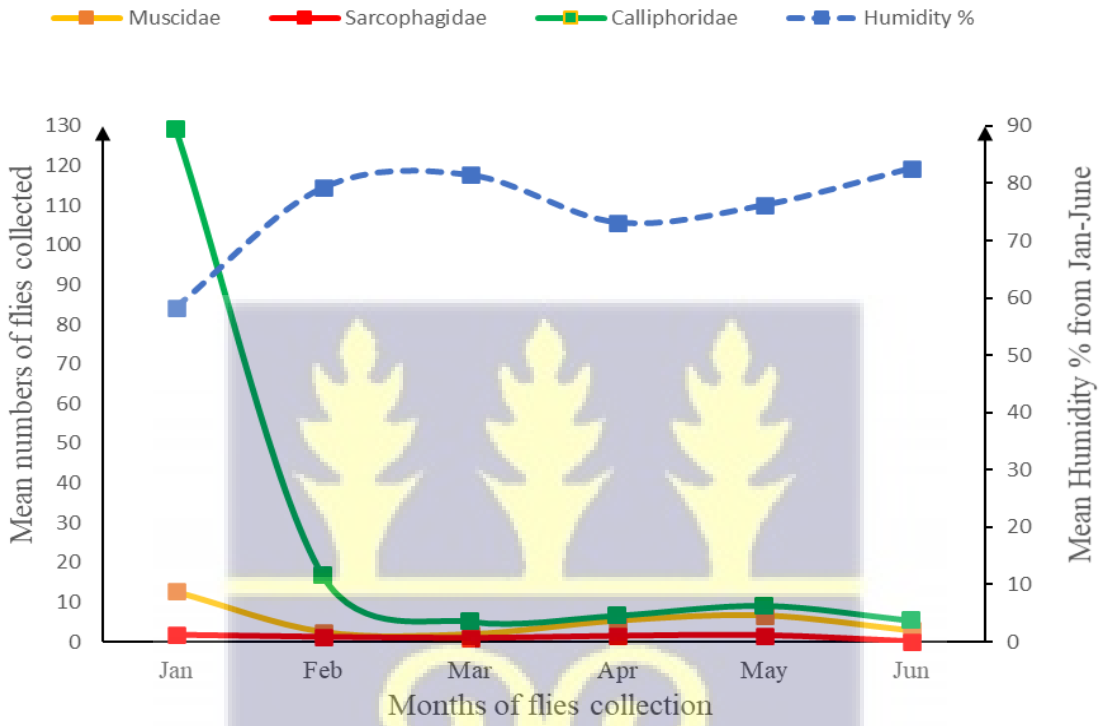

Figure 33. Relationship between humidity and numbers of Muscidae, Sarcophagidae and Calliphoridae trapped in Opetekwei during the study period.

In Figure 34, the precipitation trend decreases with the Muscidae and Calliphoridae population trends from January to February. The precipitation trend fluctuated from March to June with a slight increase. In the last three (3) months, a relationship was observed between all insect populations and precipitation trends. These trends are in synchronization; as precipitation increases, the insect population increases. Insect population decreased with decreasing precipitation.

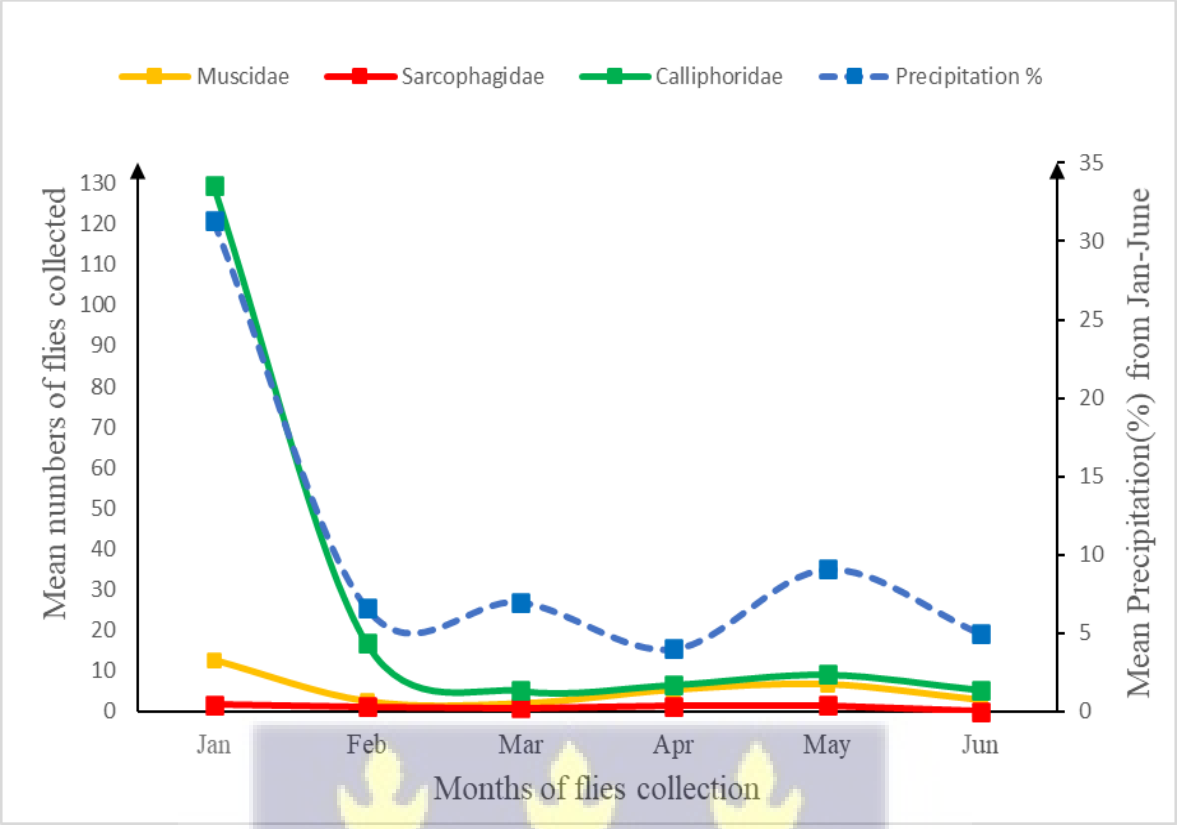

Figure 34. Relationship between precipitation and numbers of Muscidae, Sarcophagidae and Calliphoridae trapped in Opetekwei during the study period.

4.6.1 Relationship between Environmental factors (Temperature, Humidity, Precipitation) and insect population in Opetekwei.

Collected data from Opetekwei was checked for normality. The data was not uniformly distributed; therefore, a Spearman's correlation analysis was carried out using SPSS version 25. From Table 3, page 68, it was observed that the relationship between temperature, humidity, precipitation and Muscidae population trend with a sample size (n) of 68 sampling days and a degree of freedom (df) of 3 was low negative correlation coefficient (r) of -0.043, low positive coefficient (r) of 0.044 and low negative coefficient (r) of -0.008 respectively, resulting in an insignificant *p* value of $p < 0.05$. Sarcophagidae numbers had a sample size (n) of 68 sampling days and a degree of freedom (df) of 3. Between Sarcophagidae numbers and the environmental

factors, there was a low negative coefficient (r) of -0.015 and -0.069 with temperature and humidity, respectively. A moderate positive coefficient (r) of 0.225 was associated with precipitation. There were no significant *p* values. The correlation between Calliphoridae numbers having a sample size (n) of 68 sampling days and a degree of freedom (df) of 3 and temperature, humidity, and precipitation were low with a negative coefficient (r) of -0.167, a low positive coefficient (r) of 0.097, and a low positive coefficient (r) of 0.084, respectively, with no significant p values.

Table 4. Summary of correlation analysis between environmental factors and vectors of diarrhoea in Opetekwei.

Variables	M	SD	(Temp)	(Hum)	(PP)	(Mu)	(S)	(C)
Temperature (Temp)	28.71	1.425						
Humidity (Hum)	77.63	14.082	-.525**					
Precipitation (PP)	7.51	11.163	-.331**	0.221				
Muscidae (Mu)	3.97	4.962	-0.043	0.044	-0.008			
Sarcophagidae (S)	1.01	1.816	-0.015	-0.069	0.225	.302*		
Calliphoridae (C)	13.41	36.933	-0.167	0.097	0.084	.445**	.361**	

**p< 0.01 (2-tailed), *p<0.05 (2-tailed), N=68, M= Mean, SD= Standard Deviation

4.7 Relationship between Environmental factors (Temperature, Humidity, Precipitation) and the insect population in Mumford.

In Figure 35, the Muscidae population trend is in synchronization with the temperature trend, with both trends increasing in the first 3 months and decreasing in the later months. Very little relationship can be seen between the recorded temperature and Sarcophagidae and Calliphoridae population trends. Their trend fluctuated from January to June with no discernible influence from the temperature trend.

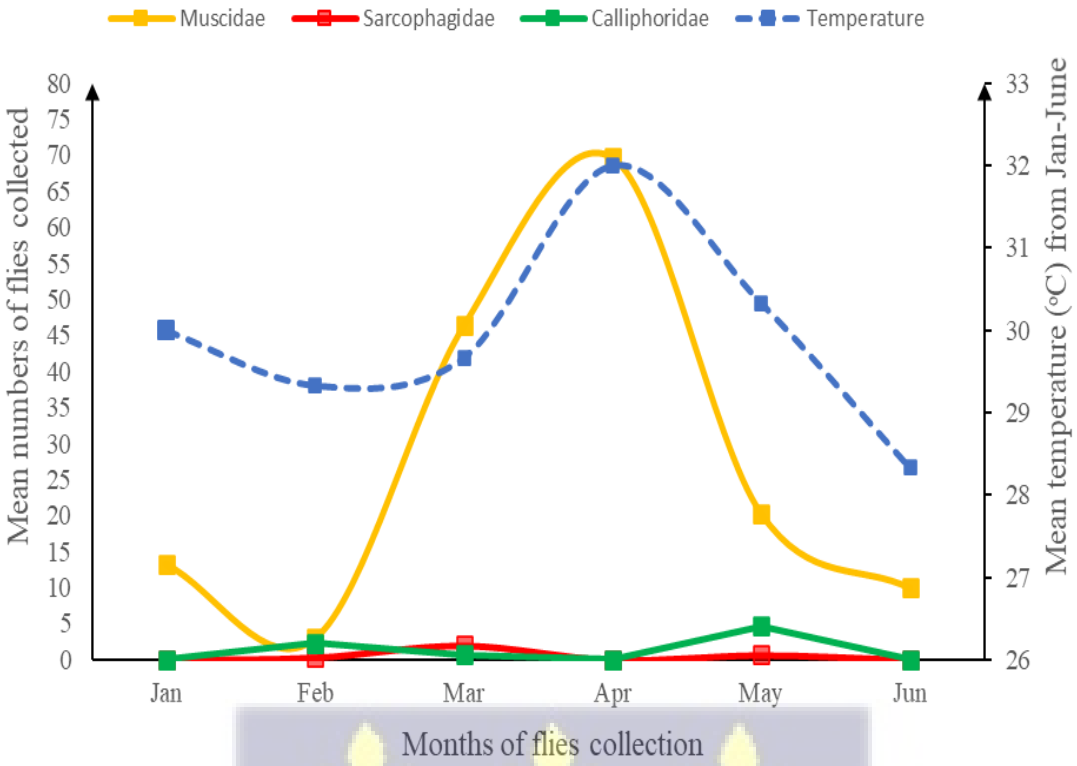

Figure 35. Relationship between Temperature and numbers of Muscidae, Sarcophagidae and Calliphoridae population in Mumford.

From Figure 36, not much relationship can be seen between Sarcophagidae and Calliphoridae population trends and humidity trend. Their trend fluctuated from January to June with no discernible influence from the humidity trend. The Muscidae population trend increased rapidly from January to April as humidity also increased from January to March, followed by a decrease in April and an increase in May. The Muscidae population trend decreased with increasing humidity in May.

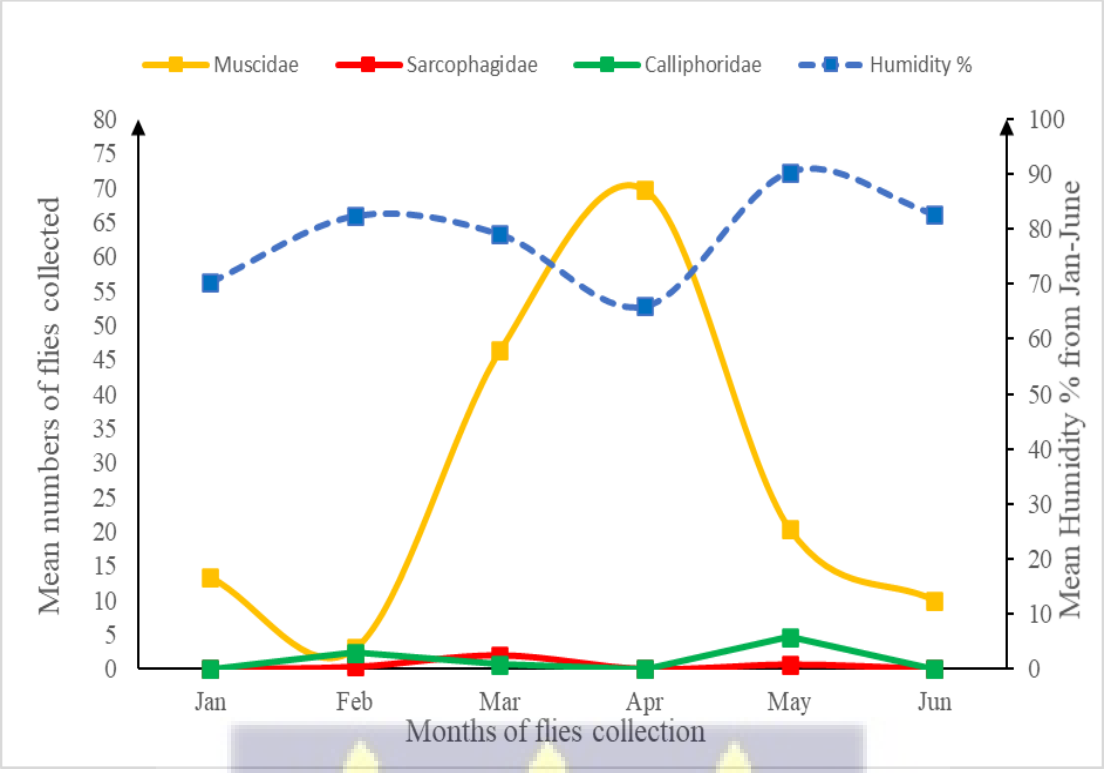

Figure 36. Relationship between Humidity and numbers Muscidae, Sarcophagidae and Calliphoridae population in Mumford.

Figure 37 shows the precipitation trend alternating with the Muscidae population trend. As precipitation increased, Muscidae decreased and increased with decreasing precipitation trend. This is seen from January to June. No clear correlation was seen between the precipitation trend and the Calliphoridae and the Sarcophagidae population trend.

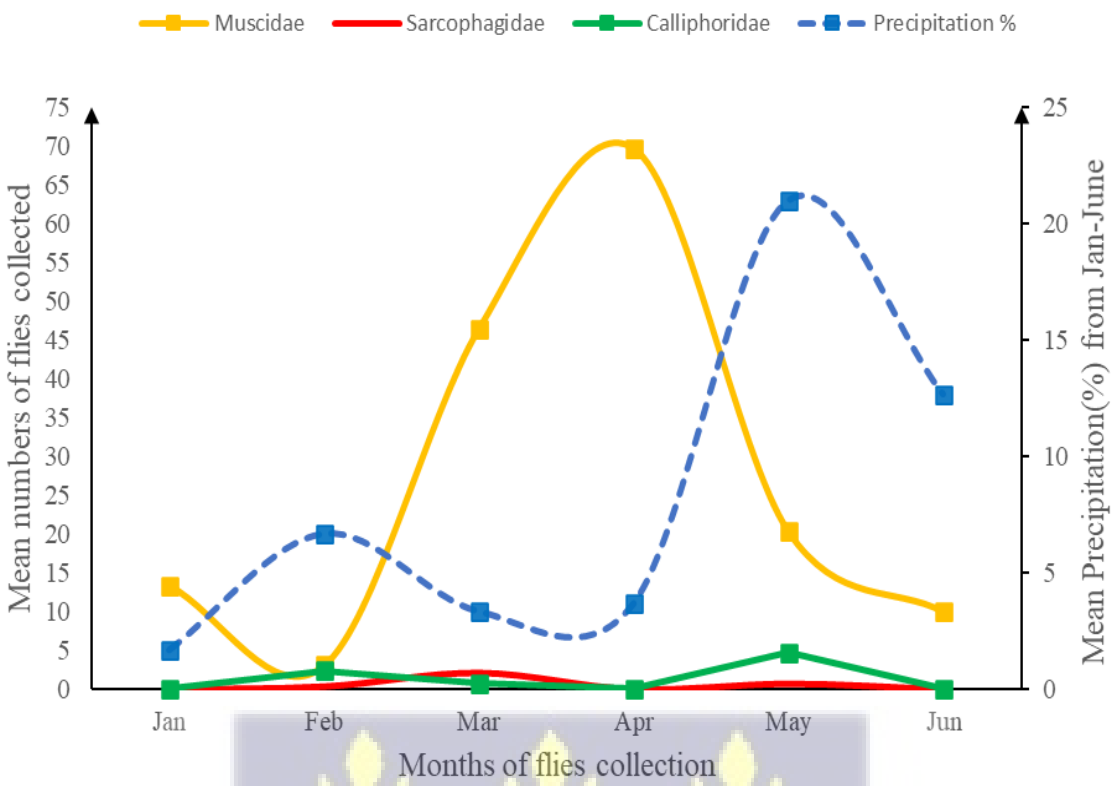

Figure 37. Relationship between Precipitation and numbers of Muscidae, Sarcophagidae and Calliphoridae population in Mumford.

4.7.1 Relationship between Environmental factors (Temperature, Humidity, Precipitation) and insect population in Mumford.

A normality test was performed for data collected from Mumford. The data was not normally distributed. A Spearman's correlation (rho) was performed using SPSS version 25. From Table 4. Page 72, a significant p value of $p < 0.01$ and a positive correlation coefficient (r) of 0.368 were seen between Muscidae numbers with a sampling size (n) of 68 sampling days and a degree of freedom (df) of 3. Besides this, Muscidae had a negative correlation coefficient (r) of 0.163 and -0.015 with humidity and precipitation, respectively, with no significant p values. Between Sarcophagidae with a sampling size (n) of 68 sampling days and a degree of freedom (df) of 3, the environmental factors were low positive correlation coefficient (r) of 0.015 and

0.01 with temperature and humidity, respectively, and low negative correlation coefficient (r) of -0.166 with precipitation. The correlation between Calliphoridae with a sampling size (n) of 68 sampling days and a degree of freedom (df) of 3 and temperature, humidity, and precipitation were a low positive correlation coefficient (r) of 0.074, a low positive correlation coefficient (r) of 0.052 and a low negative correlation coefficient (r) of -0.106, respectively. Correlations for the families Sarcophagidae and Calliphoridae and the environmental factors had insignificant p values.

Table 5. Summary of correlation analysis between environmental factors and vectors of diarrhoea in Mumford

Variables	M	SD	(Temp)	(Hum)	(PP)	(Mu)	(S)	(C)
Temperature (Temp)	29.38	2.654						
Humidity (Hum)	79.71	7.863	-.334**					
Precipitation (PP)	8.1	6.913	-.411**	.495**				
Muscidae (Mu)	31.69	29.239	.368**	-0.163	-0.015			
Sarcophagidae (S)	1.01	2.098	0.015	0.01	-0.166	-0.104		
Calliphoridae (C)	1.46	2.888	0.074	0.052	-0.106	-.240*	0.129	

**p< 0.01 (2-tailed), *p<0.05 (2-tailed), N=68, M= Mean, SD= Standard Deviation

4.8 Bacteria identified on insect vectors.

All the insects were swabbed based on study sites, insect family and months. Four main diarrhoea causing pathogens namely *Vibrio cholerae*, *E. coli*, *Salmonella* spp and *Shigella* spp. The appropriate culture media used were T.C.B.S for *Vibrio cholerae*, Chromogenic UTI for *E. coli*, S.S agar for *Salmonella* spp and *Shigella* spp. and Nutrient agar to support growth of these bacteria. Various identification methods like biochemical tests and gram staining were employed in the identification of the bacteria cultured.

4.8.1 Oxidase test for *Vibrio cholerae*

All samples tested for *Vibrio cholerae* were negative. The oxidase test showed no sign of purple colour when tested.

4.8.2 Gram staining

Samples were stained with crystal violet and Gram's iodine to help in the identification, in this process *Enterococci* spp. and *Enterobacter* spp. were identified (Figure 38).

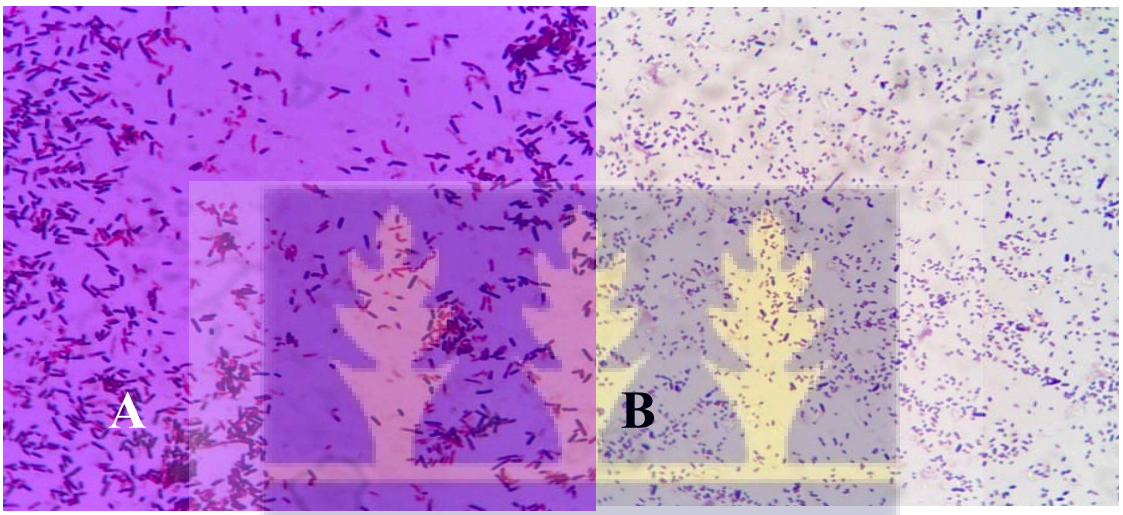

Figure 38. Gram staining. (A) Gram-negative *Enterobacter* spp., (B) Gram-positive *Enterococci* spp.

4.8.3 Indole test for *E. coli*

For the indole test in Figure 39, the presence of pink colour change indicates the presence of *E. coli*, the colourless reaction is negative.

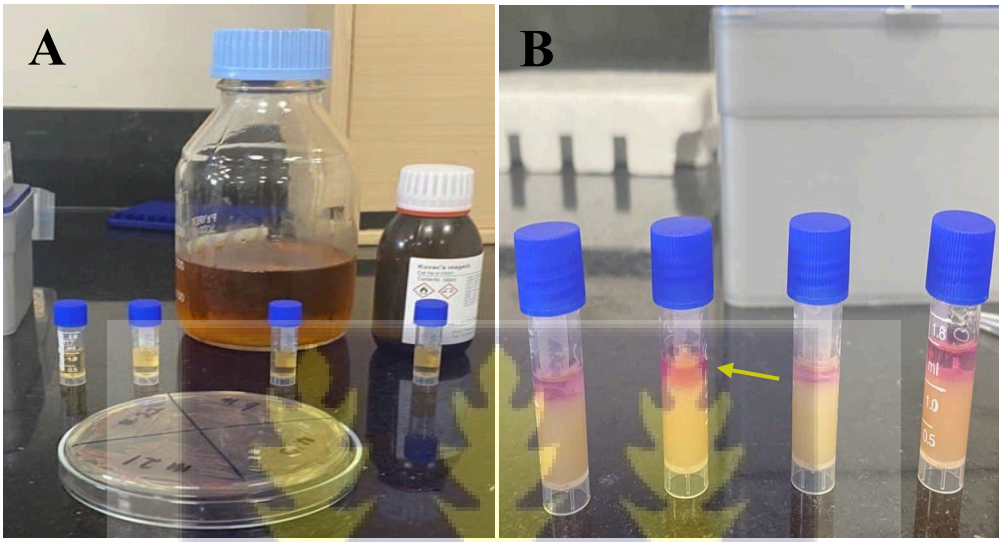

Figure 39. (A). General setup of Indole test for *E. coli*. (B). Yellow arrows showing pink rings after Indole test confirming the presence of *E. coli* from insects.

4.8.4 API Test.

In Figure 40, a colour a pink layer is visible in the IDN (indole) well in the API 20E test kits after incubation for twenty-four (24) hours. This confirmed the presence of *E. coli*. After the test, *E. coli* and *Citrobacter freundii* were identified.

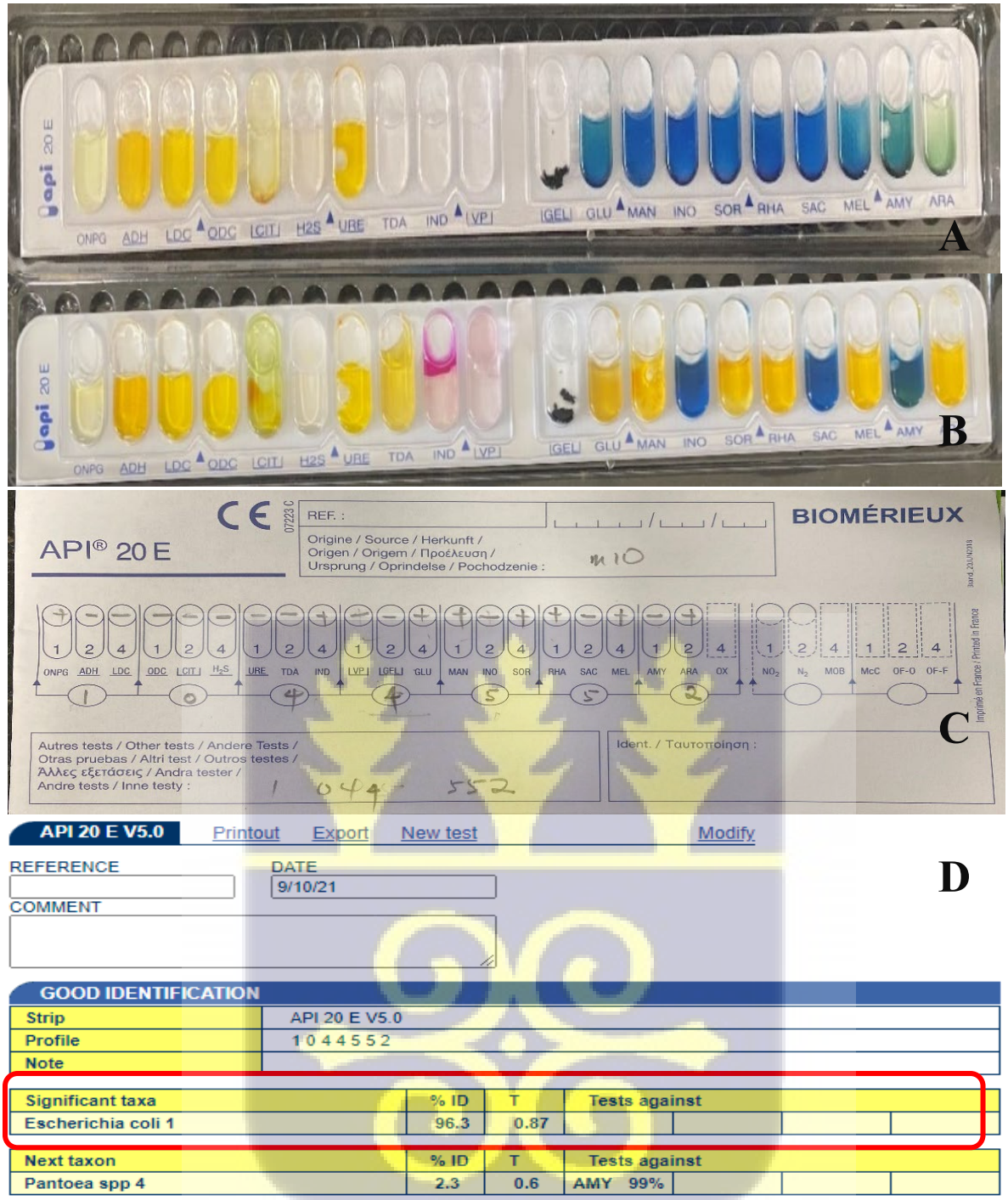

Figure 40. API results for *E. coli* . (A), test kit before incubation. (B), test kit after incubation. (C), Bacteria Profile number booklet. (D), API software for bacteria identification.

4.8.5 Identified bacteria from insect families from the study sites.

The insects were tested for the target species of bacteria namely *E. coli*, *V. cholerae*, *Salmonella* spp. and *Shigella* spp. In all a total of six (6) bacteria namely *E. coli*, *coliform*, *Enterococci* spp., *Enterobacter* spp., *Proteus* spp. and *Citrobacter freundii* were cultured from the insects collected from the study sites (Figure 41).

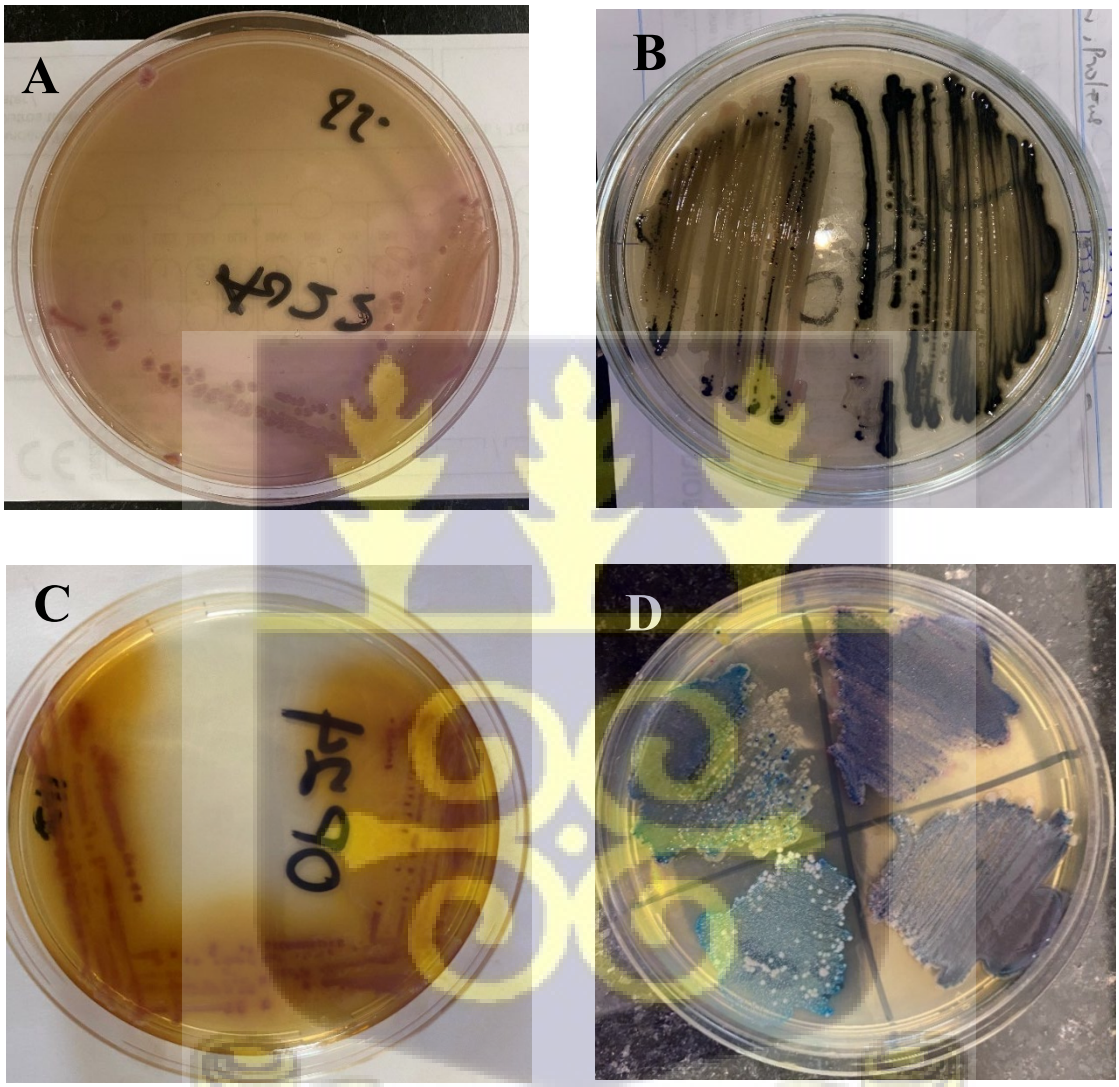

Figure 41. Identified bacteria from the communities. (A), *Enterobacter* spp. on S.S Agar. (B), *Citrobacter freundii* on S.S Agar. (C), *E. coli* on Chromogenic UTI clarity Agar. (D), *Enterococcus* spp. (Turquoise or blue-green) and Coliforms (Dark blue or purple).

4.8.5.1 Bacteria cultured from Anyako.

In Anyako a total of six (6) bacteria were cultured and identified, all the insect families had at least one bacterium present (Table 6, page 83). In the dry season starting from January to March only four out of the six bacteria were present and a majority was found on Sarcophagidae and Muscidae which are *Escherichia coli*, *Coliform*, *Enterococci* spp., *Enterobacter* spp. On Calliphoridae three (3) bacteria (*Coliform*, *Enterococci* spp., *Enterobacter* spp.) were identified.

In the wet season (April to June) more bacteria were identified. In addition to the bacteria identified in the dry season, two more bacteria were identified namely *Proteus* spp. and *Citrobacter freundii*. *Proteus* spp. was also found on Sarcophagidae. On Calliphoridae *E. coli* and *Proteus* spp. were identified.

Table 6. Bacteria identified from insect families collected from Anyako from January-June.

Months	Pool/Family	*Escherichia coli*	*V. cholerae*	*Shigella* spp.	*Salmonella* spp.
January	1(M)	✗	✗	✗	✗
	2(S)	✗	✗	✗	✗
February	3(M)	✗	✗	✗	✗
	4(M)	✓	✗	✗	✗
	5(M)	✗	✗	✗	✗
	6(S)	✓	✗	✗	✗
	7(C)	✗	✗	✗	✗
March	8(M)	✓	✗	✗	✗
	9(M)	✓	✗	✗	✗
	10(S)	✗	✗	✗	✗
	11(C)	✗	✗	✗	✗
April	12(M)	✓	✗	✗	✗
	13(S)	✗	✗	✗	✗
	14(C)	✓	✗	✗	✗
May	15(M)	✗	✗	✗	✗
	16(M)	✓	✗	✗	✗
	17(M)	✗	✗	✗	✗
	18(S)	✗	✗	✗	✗
	19(C)	✗	✗	✗	✗
June	20(M)	✗	✗	✗	✗
	21(M)	✓	✗	✗	✗
	22(S)	✓	✗	✗	✗
	23(C)	✗	✗	✗	✗

✓	present
✗	absent
M	Muscidae
S	Sarcophagidae
C	Calliphoridae

4.8.5.2 Bacteria cultured from Anyanui.

The first three months (January to March) had several bacteria cultured from the three insect families. On Muscidae, *Proteus* spp., Coliform, *E. coli*, *Enterobacter* spp. and *Enterococci* spp. were identified. Coliform and Enterobacter were found on Sarcophagidae and Calliphoridae.

In the wet season (April to June), Muscidae was linked to *Citrobacter freundii, Proteus* spp., *E. coli*, *Enterobacter* spp. and *Enterococci* spp. These bacteria were found on Calliphoridae except for *Proteus* spp. There were no Sarcophagidae samples collected in the wet season (Table 7, page 85).

Table 7. Bacteria identified from insect families collected from Anyanui from January-June.

Months	Pool/Family	*Escherichia coli*	*V. cholerae*	*Shigella* spp.	*Salmonella* spp.
January	1(M)	✗	✗	✗	✗
	2(S)	✗	✗	✗	✗
February	3(M)	✗	✗	✗	✗
	4(M)	✓	✗	✗	✗
	5(M)	✗	✗	✗	✗
March	6(M)	✓	✗	✗	✗
	7(M)	✗	✗	✗	✗
	8(M)	✗	✗	✗	✗
	9(C)	✗	✗	✗	✗
April	10(M)	✗	✗	✗	✗
	11(M)	✗	✗	✗	✗
	12(M)	✗	✗	✗	✗
	13(C)	✗	✗	✗	✗
May	14(M)	✓	✗	✗	✗
	15(M)	✗	✗	✗	✗
	16(M)	✗	✗	✗	✗
	17(C)	✓	✗	✗	✗
June	18(M)	✓	✗	✗	✗
	19(M)	✗	✗	✗	✗
	20(M)	✗	✗	✗	✗
	21(M)	✓	✗	✗	✗
	22(M)	✓	✗	✗	✗
	23(C)	✗	✗	✗	✗

✓ present
✗ absent
M Muscidae
S Sarcophagidae
C Calliphoridae

4.8.5.3 Bacteria cultured from Opetekwei

E. coli, Proteus spp., *Enterobacter* spp. and *Citrobacter freundii* were found on Muscidae. Sarcophagidae was linked to three (3) bacteria; *E. coli*, Coliform and *Enterobacter* spp. Calliphoridae had *E. coli, Enterococci* spp., *Enterobacter* spp., Coliform, *Proteus* spp. and *Citrobacter freundii* in the dry season.

Bacteria identified in the wet season (April to June) were similar to those identified in the dry season. *Enterobacter* spp., Coliform, *Enterococci* spp. and *Citrobacter freundii* on Muscidae. *Enterobacter* spp., Coliform, *Enterococci* spp. and *Citrobacter freundii* were cultured from Sarcophagidae. *E. coli*, Coliform, *Enterobacter* spp. and *Proteus* spp.

Table 8. Bacteria identified from insect families collected from Opetekwei from January-June.

Months	Pool/Family	*Escherichia coli*	*V. cholerae*	*Shigella* spp.	*Salmonella* spp.
January	1(M)	✓	✗	✗	✗
	2(S)	✓	✗	✗	✗
	3(C)	✓	✗	✗	✗
	4(C)	✗	✗	✗	✗
	5(C)	✓	✗	✗	✗
February	6(M)	✗	✗	✗	✗
	7(S)	✗	✗	✗	✗
	8(C)	✗	✗	✗	✗
	9(C)	✗	✗	✗	✗
	10(C)	✗	✗	✗	✗
	11(C)	✗	✗	✗	✗
March	12(M)	✓	✗	✗	✗
	13(S)	✓	✗	✗	✗
	14(C)	✗	✗	✗	✗
	15(C)	✗	✗	✗	✗
April	16(M)	✗	✗	✗	✗
	17(S)	✗	✗	✗	✗
	18(C)	✓	✗	✗	✗
May	19(M)	✗	✗	✗	✗
	20(S)	✗	✗	✗	✗
	21(C)	✗	✗	✗	✗
June	22(M)	✗	✗	✗	✗
	23(C)	✗	✗	✗	✗
	24(C)	✓	✗	✗	✗

✓ present
✗ absent
M Muscidae
S Sarcophagidae
C Calliphoridae

4.8.5.4 Bacteria cultured from Mumford.

In the dry months (January to March) *E. coli*, *Enterobacter* spp., Coliform, *Enterococci* spp. on Muscidae. *Escherichia coli* and *Enterobacter* spp. were cultured from Sarcophagidae. *Escherichia coli* and *Citrobacter freundii* were cultured from Calliphoridae.

In the wet months Muscidae was linked to *Enterococci* spp., *E. coli*, Coliform and *Enterobacter* spp. were found on Muscidae. *E. coli*, *Enterobacter* spp., *Enterococci* spp. and coliform were cultured from Sarcophagidae and Calliphoridae.

Table 9. Bacteria identified from insect families collected from Mumford from January-June.

sMonths	Pool/Family	*Escherichia coli*	*V. cholerae*	*Shigella* spp.	*Salmonella* spp.
January	24(M)	✓	✗	✗	✗
February	23(M)	✗	✗	✗	✗
	22(M)	✓	✗	✗	✗
	21(S)	✓	✗	✗	✗
	20(C)	✗	✗	✗	✗
March	19(M)	✓	✗	✗	✗
	18(M)	✗	✗	✗	✗
	17(S)	✗	✗	✗	✗
	16(C)	✗	✗	✗	✗
April	15(M)	✗	✗	✗	✗
	14(M)	✗	✗	✗	✗
	13(M)	✓	✗	✗	✗
	12(M)	✗	✗	✗	✗
	11(M)	✗	✗	✗	✗
	10(S)	✓	✗	✗	✗
	9(C)	✓	✗	✗	✗
May	8(M)	✓	✗	✗	✗
	7(M)	✗	✗	✗	✗
	6(M)	✗	✗	✗	✗
	5(M)	✓	✗	✗	✗
	4(S)	✗	✗	✗	✗
	3(C)	✗	✗	✗	✗
June	2(M)	✗	✗	✗	✗
	1(M)	✓	✗	✗	✗

✓	present
✗	absent
M	Muscidae
S	Sarcophagidae
C	Calliphoridae

CHAPTER FIVE

5.0 DISCUSSION

5.1 General overview.

Generally, flies were encountered throughout the collection period (January to June). Not much diversity was observed from the collected samples in the wet season (January -March) and dry season (April – June) from the various study sites. The major species identified were *Musca domestica*, *Sarcophaga* spp. and *Chrysomya* spp. Majority of the samples were collected in the wet season (April to June). Out of the aforementioned target bacteria, only *E. coli* was successfully cultured. Five other non-target bacteria species namely *Proteus* spp., Coliform, *Enterobacter* spp., *Citrobacter freundii* and *Enterococci* spp. The presence of *E. coli* on all insect families regardless of season, implies that these flies are capable of diarrhoeal disease transmission all year round.

5.2 Seasonal variations in species composition and abundance of dipteran vectors of diarrhoeal diseases.

Based on the calculated Shannon diversity index (H= 0.45) and Evenness (E = 0.28) of the insect fauna collected, the numbers of these sampled insects showed no numerical variations for the different families. The higher the value of H, the higher the diversity of insect families. The lower the value of H, the lower the diversity. A value of H = 0.45 indicated less diversity. Relative abundance and of insects and diversity over the sampling period was not consistent which could be due to type of bait used, sanitation conditions or trap disturbance (Boonchu *et al*., 2003; Getachew *et al*., 2007).

The number of Muscidae trapped gradually increased from the dry season to the wet season and peaked in May when the most collection was made in Anyako. A similar trend was

observed for Sarcophagidae and Calliphoridae (except in Opetekwei). These environmental factors were noted having an influence on the vector abundance in all the communities, this could be due to the physiological changes and shorter generation time resulting in an increase in insect population. As the climate changes and influences the physiology and biology of these insects it can lead to the increase in diarrhoeal bacteria transmission. In a study carried out by Figueiró *et al*. (2014), the seasonal variations of black fly taxocenoses from the Brazilian savannah were studied and it was observed that the species richness and abundance of black flies were significantly higher in the wet season. A positive correlation was observed between the black flies and precipitation during the wet season and this could result in their abundance and being widely distributed (Figueiró et al., 2014). Though black flies are not muscoids, Calliphoridae or Sarcophagidae, insects are generally affected by environmental factors regardless of their distribution.

Previous studies of medical and veterinary importance evidently showed how seasonal variation affects vector species composition and abundance. In Thailand, *Chrysomya megacephala* and *Chrysomya rufifacies* population fluctuated similarly in accordance to seasonal variation. Calliphoridae monthly abundance were greatest in January, September and June which coincided with rainfall (Phasuk *et al*., 2013). The abundance of *Chrysomya megacephala* grew with the start of the rainy season and subsequently decreased throughout the dry hot season in India (Wall et al., 2001).

The Calliphoridae abundance at Opetekwei was highest in January (dry month) and declined towards the rainy months. This is noted in a study by Marinho *et al*. (2006). Calliphoridae numbers were most abundant in May, June, September and January in Brazil when there was little rainfall. The data analysis revealed a significant negative correlation between temperature and relative humidity for Calliphoridae population as seen in Opetekwei. Seasonal fluctuations

in Calliphoridae abundance and distribution are commonly influenced by environmental factors such as rainfall, temperature, humidity, flora, fauna, human disturbance and breeding sites (Marinho *et al*., 2006).

5.3 Correlation between Environmental factors (Temperature, Humidity, Precipitation) on insect population in study sites.

Forty-five (45%) per cent of all the insects were collected from Anyako a fishing community which is an island separated from the surrounding land. Majority of the insects collected was Muscidae, followed by Sarcophagidae, Calliphoridae. Significant positive correlation was observed between the Calliphoridae insect population and precipitation in Anyako, this phenomenon was supported by *C. megacephala* abundance in India which grew with the start of the rainy season and subsequently decreased throughout the dry hot season (Wall *et al.,* 2001). Environmental factors always affect the developmental stages of insects, these factors also affect the activities of insects in the environment (Hagstrum & Hagstrum, 1970). From January to June the insect population for Calliphoridae and Sarcophagidae were quite low which could be attributed to disturbance of the traps installed in the community, the geographical location of the Anyako. The sanitary condition of the community can lead to competitive interactions from other baits in the community and urbanization-related processes might also promote the composition and structure of necrophagous assemblages leading to human action altering their composition and structure (Kavazos & Wallman, 2012). For example, Leong & Grace, (2009) *Chrysomya megacephala* was found in huge numbers in food waste, while *Chrysomya rufifacies* was found in abundance at butchery sites and outdoor latrines (Getachew *et al.,* 2007).

Anyanui had the second-highest insect count, Muscidae activity and population had a significant positive correlation with the temperature only, humidity and Precipitation didn't

have any effect on the Muscidae activity and population, these factors could be attributed to the other activities in the community like low active space of the scent of the baits. Boonchu *et al.* (2003) conducted a study in which they found that pork viscera baits were particularly appealing to adults of *C. megacephala* and that the wind direction aided in the dispersion of the bait scent (Pickens *et al.,* 1994). This could mean other meat sources in the community had more attractant power that that of the meat used in the traps in Anyanui. Sarcophagidae activity was not influenced significantly by all three environmental factors.

Mumford had the third-highest collection with Muscidae, Sarcophagidae then Calliphoridae, these flies' activities had a negative correlation with precipitation though was not significant. Relative humidity and temperature did not influence the insect activity on Sarcophagidae and Calliphoridae except for temperature influencing Muscidae activity.

Opetekwei recorded more Calliphoridae followed by Muscidae than Sarcophagidae. No significant correlation was established between the flies and environmental factors. The high abundance of Calliphoridae could be due to habitat settlement structure, and environmental sanitation. Opetekwei has two parts the estate part was quite clean and fewer insects were collected compared to the slum part which is closer to the shore is littered with pig pens, sheep, rotten fruit, leaking public sewage and dead animals.

Muscidae being the most collected in the three (3) out of four (4) of the study sites could be due to it being highly polyphagous found in rural and urban areas and feeding on all three baits used. Muscidae is highly associated with human dwelling, therefore, feed on garbage, animal and human faeces. Although Calliphoridae and Sarcophagidae (are relatively uncommon in houses) they are necrophagous insects feeding mainly on rotten animal flesh or meat and other substances such as decaying vegetable and fruits matter. The fish and meat bait seem not to be an efficient attractant, so this discrepancy could have been mostly due to bait selection or

competitive interactions with other flies like Muscidae (Phasuk *et al.,* 2013). The low abundance of Sarcophagidae could be a result of the trap's design, which is harmed by strong winds. Strong wind is known to impede blow flies from flying, and salinity in the sediment may function as an unfavourable factor in the development of immature flies (Mulieri *et al.,* 2011).

As Temperature increased the population and insect activities of these families increased though there was no significant correlation, implying that as the temperature dropped from the dry season (January to March) to wet season (April to May) fly activities and abundance was not affected. The positive and significant correlation between Muscidae, Sarcophagidae and relative humidity suggests that fly activity was greatly affected in the seasons, increase in relative humidity from the dry season to the wet season influenced the increase in fly activity and abundance of Muscidae and Sarcophagidae. Calliphoridae abundance was influenced by Precipitation, the relationship was positive giving empirical evidence of flies' higher activities and abundance in raining seasons (Mello *et al.,* 2007).

5.4 Identified bacteria on Vectors of Diarrhoea.

The most common bacteria cultured from the flies were *Enterobacter* spp. and Coliform. These were found on all families of vectors of diarrhoea in all six (6) months. It was most frequently associated with the insects found in both dry and wet seasons. *Proteus* spp., Coliform, *Enterobacter* spp., *E. coli*, *Citrobacter freundii* and *Enterococci* spp. were all associated with Muscidae, Calliphoridae and Sarcophagidae. Comparing the frequencies of bacteria occurrence in wet and dry seasons, more bacteria appear more often in the wet season. This trend was seen in Anyako and Anyanui. As temperature decreased, humidity and precipitation increased and more bacteria were associated with the flies. It was observed that the species increased with increase with fly abundance and the bacteria were found throughout the collection period.

In Opetekwei and Mumford, bacteria frequency was equally distributed in both seasons, this seems to be associated with low and fluctuating fly abundance. Microorganism growth slows and practically stops when temperatures drop below 18°C, studies have shown that when temperatures drop below -18°C, microbes such as bacteria stop growing completely. To reproduce and grow, bacteria require varying amounts of water. The majority of these bacteria necessitate a relative humidity (RH) of 60% or higher. In the case of locations of these study sites, the highest relative humidity being 90% and lowest being 54% these ranges are optimum for bacteria growth with a temperature between 32°C (highest) and 26°C (lowest). Hoeksma *et al.* (2015) studied the influence of temperature (T) and relative humidity (RH) on the survival of *Escherichia coli*, *Enterococcus mundtii*. These bacteria were examined at T = 10, 20 and 30°C and RH = 40, 60 and 80%. At the end it was observed that the tested bacteria survive between the temperature (10-30°C) and relative humidity (40-80%) ranges. This conditions were optimum for the bacteria to survive long enough to be transported over a long distance implying temperature, humidity all have an impact on growth (Hoeksma *et al.,* 2015).

E. coli infections are severe and life-threatening, especially for small children, the elderly, and people with weaker immune systems, due to the low infectious dosage and high pathogenicity of the bacteria. *E. coli* has been associated with all three (3) insect families and was found in all the months most associated with Muscidae, Sarcophagidae and Calliphoridae. The Primary sources of *E. coli* are raw undercooked meat, contaminated water, raw milk, vegetables contaminated with faeces. These sources are predominantly visited by the flies and when these come into contact the bacteria can be found on the insects, through this interaction *E. coli* is transmitted to non-contaminated substances (Robert *et al.,* 2021). Robert *et al.* (2021) studied water throughout the year in Burkina Faso and found the water was normally tainted by bacteria of faecal origin, although this was more during the rainy season. Monthly epidemiological data for instances of diarrhoea were also obtained from three health centres and compared to

microbiological and environmental data. In surface waters, there was a positive association between *E. coli* and precipitation, showing that *E. coli* is a reliable predictor of faecal pollution in this area. The presence of *E. coli* and diarrheal illnesses was highly linked to monsoonal precipitation. Diarrheal illnesses, as well as *E. coli*, were substantially linked to these factors. During the rainy season, the population's susceptibility to diarrhoea increases (Robert *et al.*, 2021). During the rainy season, from June to September, the microbiological health risk is greater (Robert *et al.,* 2021).

Enterococci spp. are frequent, commensal members of mammalian and bird gut populations, but they are also opportunistic pathogens responsible for millions of human and animal illnesses each year. Because they are shed in human and animal faces, are easily culturable, and predict human health concerns from exposure to polluted recreational waters, they are used as surrogates for waterborne pathogens and as faecal indicator bacteria (FIB) in research and water quality assessment all over the world. However, evidence from decades of research reveals that enterococci can exist in high densities even in the absence of obvious faecal sources and that environmental reservoirs of this FIB are key sources and sinks with the potential to contaminate water (Byappanahalli *et al.,* 2012). Cystitis, pyelonephritis, catheter-associated urinary tract infections (UTI), endocarditis, and mixed-organism infections of the abdomen and pelvis are all illnesses caused by *Enterococcus* spp. (Sood *et al.,* 2008). Chaiwong *et al.* (2014) Robert *et al*. (2021) conducted a study were 700 adult flies were collected from fresh-food markets, waste dumps, restaurants, school cafeterias, and rice paddy fields in Thailand. A total of 120 *Enterococcus* spp. isolates were obtained from a number of 67 *M. domestica* and 53 *C. megacephala* (Robert *et al.*, 2021). Robert *et al.* (2021) observed a significant relationship between *E. coli* and *Enterococci* spp. which was predicted by precipitation. This is evident as both *Enterococci* spp. and *E. coli* were both sampled from Muscidae, Sarcophagidae and Calliphoridae in the later months.

Insects that operate as mechanical vectors, such as flies, can carry and spread enterobacteria (Enterobacteriaceae) that cause intestinal illnesses (Cadavid-Sanchez *et al.,* 2015). *Enterobacter cloacae*, *E. aerogenes*, and *E. agglomerans* are the most common species (Ramirez & Giron, 2020). Bacteraemia, lower respiratory tract infections, skin and soft-tissue infections, urinary tract infections (UTIs), endocarditis, intra-abdominal infections, septic arthritis, osteomyelitis, CNS infections, and eye infections are only some of the illnesses that can occur. Of the conditions caused by *Enterobacter* spp. enterobacter infections can result in extended hospitalization, a plethora of imaging exams and laboratory testing, a variety of surgical and nonsurgical procedures, and the use of potent and costly antimicrobial medications (Ramirez & Giron, 2020). In Anyako, Opetekwei and Mumford where sanitation is quite poor, the inhabitants may have a higher risk of infection compared to inhabitants of Anyanui which is relatively cleaner.

A study by Cadavid-Sanchez *et al.* (2015) linked synanthropic flies to enterobacteria that were isolated and identified. Cadavid-Sanchez *et al.* (2015) also suggested that *Musca domestica*, *Chrysomya megacephala*, *Lucilia cuprina*, *Chrysomya albiceps*, and *Chrysomya albiceps* is of high risk of transmitting diarrhoea causing pathogens. This connection poses a significant threat to public health since they are carriers of enteropathogenic bacteria that can be transmitted to food, because prevalence of *Enterobacter* spp. is in the intestinal tracts of animals which results in their widespread distribution in soil, water, and sewage.

Citrobacter freundii, *Enterobacter* spp., Coliform and *Proteus* spp. bacteria can be found in the environment (for example, in soil) and in the intestines of animals (Feng *et al.,* 2002). *Citrobacter freundii* have been isolated from *Musca domestica* (Kababian *et al.,* 2020) Calliphoridae and Sarcophagidae (Boiocchi *et al.,* 2019). *Citrobacter freundii* is most

commonly linked to gastroenteritis, new-born meningitis, and septicaemia. It has been linked to infections of the urinary system and respiratory tract in patients receiving medical care (Murray *et al.,* 2010). *Citrobacter freundii*, *Enterobacter* spp., Coliform and *Proteus* spp. has been linked to Muscidae, Sarcophagidae and Calliphoridae (Kababian *et al.,* 2020) and also confirmed and documented in this study. Because of the relationship between the bacteria and these flies, the bacteria can be transmitted from contaminated objects to food and vice versa. *Proteus* spp. can be found in large quantities in soil, decaying meat and water, and while it is a natural element of human gut flora, it has been known to cause significant illnesses in humans like urinary tract infections (UTIs) (Flores-Mireles *et al.,* 2015).

As the inhabitants are actively involved in fishing at the beaches they come into contact with sand. Most children in the study communities play outside in the sand and along the beaches, and through this interaction *Citrobacter freundii*, and *Enterobacter* spp can be contracted leading to UTIs in these communities. *Citrobacter* spp., *Enterobacter* spp. and *Proteus* spp. were cultured from adults with UTIs in Accra (Donkor et al., 2019). Though these bacteria are not associated with diarrhoea, they can cause serious urinary tract infections in the stucommunities. These bacteria are known as indicator organisms because their presence in food indicates that the environment is favourable for the growth of enteric pathogens and may indicate sanitary difficulties. (Akpor *et al.,* 2014).

CHAPTER SIX

6.0 CONCLUSION, RECOMMENDATIONS AND LIMITATIONS OF THE STUDY

6.1 Conclusion

Musca domestica, *Sarcophaga* spp. and *Chrysomya* spp. were widely distributed and collected throughout the months of sampling with the most collections made in the wet season (April – June).

Environmental factors such as temperature, precipitation and humidity were positively correlated with the abundance of the insects; increase in fly population and activity were prominent during the wet season (April, May and June), as temperature (°C) dropped and precipitation (%) and relative humidity (RH) increased. All identified bacteria on the flies were associated with all three insect families. When comparing the frequency of bacteria that occurred in the wet and dry seasons, the rainy season had a higher bacteria load on the flies. In Anyako and Anyanui only, as the temperature dropped, humidity rose, and precipitation fell, more bacteria load was associated with the flies, this was not observed in the other study sites. The number of different bacteria species found the captured flies increased as the number of flies grew, but remained equally distributed in all six (6) months in Mumford and Opetekwei. In Anyanui and Anyako, more bacteria were identified in the raining months (April-June). Although seasonal changes in insect abundance may contribute to changes in insect abundance, these insects may be present at any time of year when food debris, garbage, decaying plants, dead animals, and manure are available to sustain growing larvae (maggots), which may facilitate diarrhoea transmission as the numbers of these vectors increase.

The results of the study revealed that the Dipteran families; Muscidae, Sarcophagidae and Calliphoridae were the major vectors that may be transmitting diarrhoea in the selected coastal communities. These families of flies were found carrying varied pathogenic loads of several

bacteria species including *E. coli*, which is one of the major causative organisms of diarrhoea. *Escherichia coli* infections are severe and life-threatening, especially for small children, the elderly, and people with weaker immune systems. Other bacteria, though not mainly known for causing diarrhoea, cause other ailments such as UTIs, intestinal infection and meningitis; these are *Enterobacter* spp., *Enterococci* spp., *Proteus* spp., *Citrobacter freundii* and Coliforms.

6.2 Recommendations

- Based on the findings of the study, it is recommended that measures like setting of traps in both dry and wet reasons can help control fly population since these insects are found in both seasons carrying *E. coli*.
- It is recommended that genomic sequencing be carried out on the DNA of *E. coli* to enable comparison with other sequences of DNA of *E. coli* that other researchers have found, because there are different strains of *E. coli* and this can help create a distribution map of different *E. coli* strains.

6.3 Limitation

- Several bacteria culture media such as blood agar, MacConkey and Eosin methylene blue agar, could have been used in the current study to give a broader picture of the bacteria colonising the exterior of the flies. Because of limited funds and logistics, it was not possible.
- The sampling period should have span at least one (1) year or longer to allow for sample collection across the seasons to gain a better understanding of the relationships between the seasonality of the different fly abundance and monthly meteorological data for temperature, rainfall, and relative humidity.

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APPENDIX 1

Table 1. Sampling and Data collection sheet.

Serial Number	Collection Date	Collection locality	GPS Coordinates	Insect species	Bacteria present					
					V. cholerae	*E. coli*	*Salmonella* spp.	*Shigella* spp.	*Campylobacter* spp.	Other(s)
1										
2										
3										
4										
5										
6										

APPENDIX 2

1- Antennal flagellum with four or more segments which are usually uniform in shape and size, without apical stylus or arista; palpi usually with 3–5 segments .....Psychodidae

– -Antennal flagellum consists of single-segment, with apical stylus or arista; palpi with two or less segments…….....2

2- Ptilinal suture and lunule present…….…….....3

– Ptilinal suture and lunule absent……..……12

3- Antennal pedicel with a complete dorsal seam; vibrissae usually present; greater ampulla present below wing base; calypters usually present ……..……...…..……4

– Antennal pedicel usually without or with incomplete dorsal seam; vibrissae absent; greater ampulla absent; calypters usually small or undeveloped…….…..……7

4- Meron with a vertical row of bristles……5

– Meron without bristles…….…..…6

5- Abdomen and usually thorax metallic blue or green in colour……...**Calliphoridae**

– Abdomen dull gray, brown or black in colour, sometimes shiny black ……...……...……..…**Sarcophagidae**

6- Wing with anal veins A1 and A2 convergent or nearly intersecting ...……...Fanniidae

– Wing with anal veins A1 and A2 nearly parallel or diverging…….……**Muscidae**

APPENDIX 3

Descriptive Statistics for Anyako

	N	Minimum	Maximum	Mean	Std. Deviation
Temperature°C	68	24	36	28.01	2.674
Humidity %	68	3	95	79.54	16.508
Precipitation %	68	0	97	10.41	16.483
Muscidae	68	0	902	58.75	131.325
Sarcophagidae	68	0	12	1.28	2.442
Calliphoridae	68	0	41	1.85	5.379
Valid N (listwise)	68				

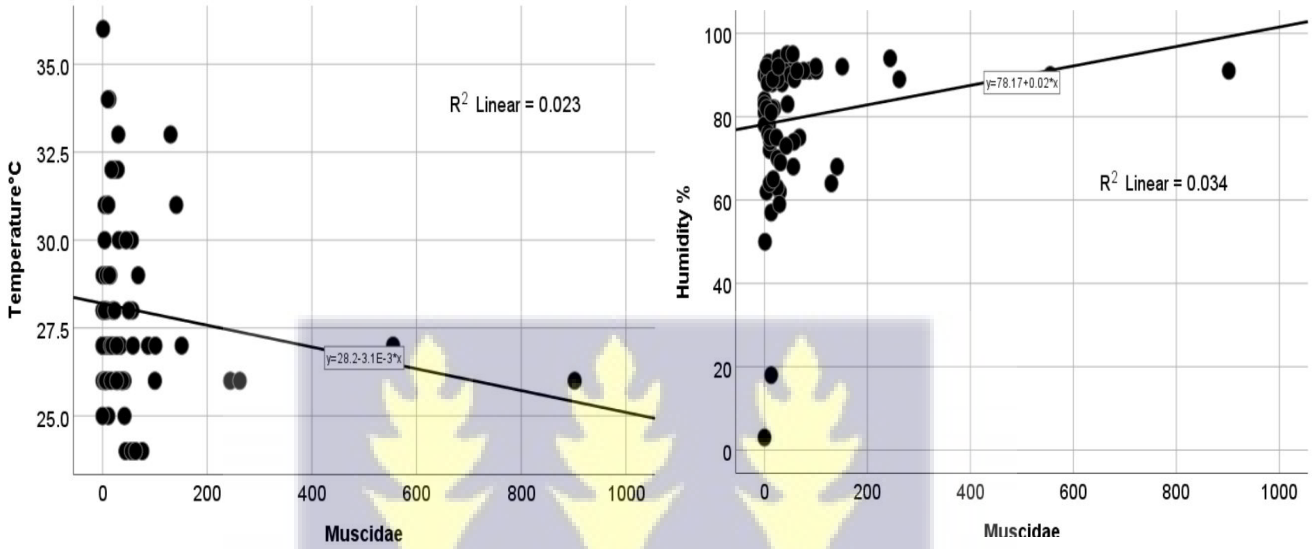
Nonparametric Correlations for Anyako

			Temperature°C	Humidity %	Precipitation %	Muscidae	Sarcophagidae	Calliphoridae
Spearman's rho	Temperature°C	Correlation Coefficient	1.000	-.731**	-.299*	-.196	-.201	-.225
		Sig. (2-tailed)	.	.000	.013	.109	.101	.065
		N	68	68	68	68	68	68
	Humidity %	Correlation Coefficient	-.731**	1.000	.215	.313**	.263*	.195
		Sig. (2-tailed)	.000	.	.078	.009	.030	.110
		N	68	68	68	68	68	68
	Precipitation %	Correlation Coefficient	-.299*	.215	1.000	.159	.103	.373**
		Sig. (2-tailed)	.013	.078	.	.194	.402	.002
		N	68	68	68	68	68	68
	Muscidae	Correlation Coefficient	-.196	.313**	.159	1.000	.473**	.272*
		Sig. (2-tailed)	.109	.009	.194	.	.000	.025
		N	68	68	68	68	68	68
	Sarcophagidae	Correlation Coefficient	-.201	.263*	.103	.473**	1.000	.352**
		Sig. (2-tailed)	.101	.030	.402	.000	.	.003
		N	68	68	68	68	68	68
	Calliphoridae	Correlation Coefficient	-.225	.195	.373**	.272*	.352**	1.000
		Sig. (2-tailed)	.065	.110	.002	.025	.003	.
		N	68	68	68	68	68	68

**. Correlation is significant at the 0.01 level (2-tailed).

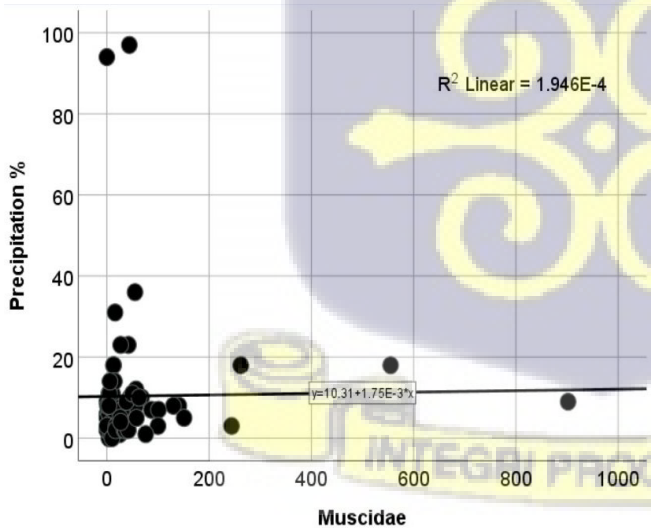
*. Correlation is significant at the 0.05 level (2-tailed).

Correlation between Environmental factors (Temperature °C, Humidity (%), Precipitation (%)) and Vectors of diarrhoea sampled from Anyako. (A) correlation of Muscidae and Temperature. (B) correlation of Muscidae and Humidity (%). (C) correlation of Muscidae and Precipitation (%).



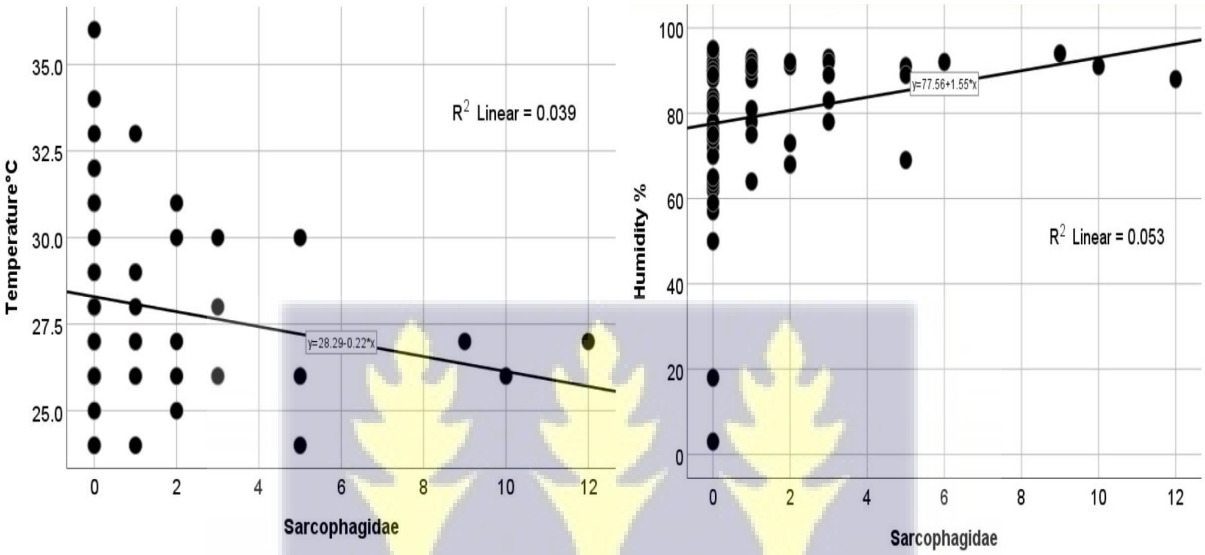
A

B



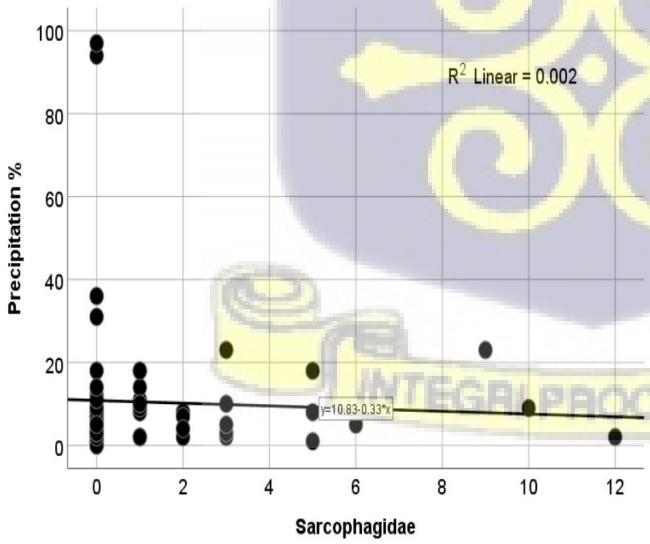
C

Correlation between Environmental factors (Temperature °C, Humidity (%), Precipitation (%)) and Vectors of diarrhoea sampled from Anyako. (A) correlation of Sarcophagidae and Temperature. (B) correlation of Sarcophagidae and Humidity (%). (C) correlation of Sarcophagidae and Precipitation (%).



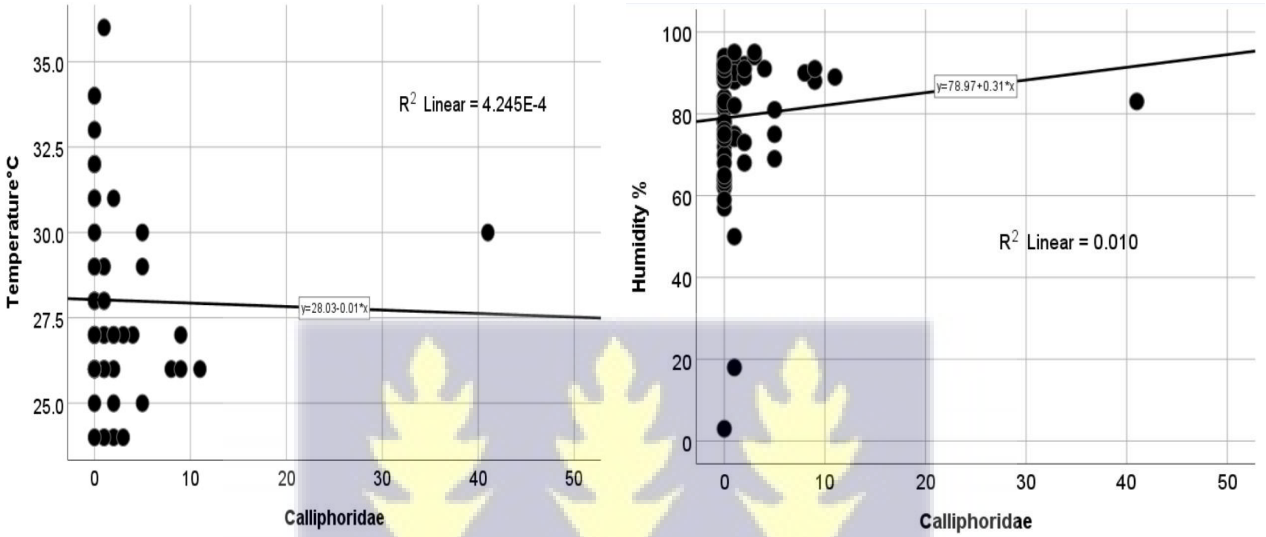
A

B



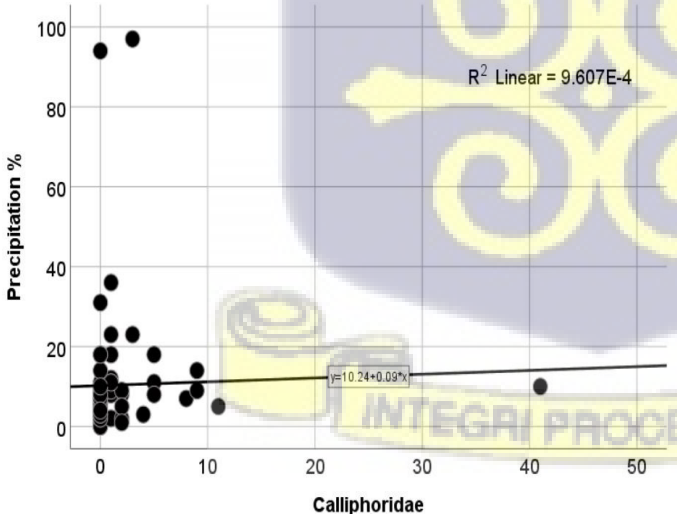
C

Correlation between Environmental factors (Temperature °C, Humidity (%), Precipitation (%)) and Vectors of diarrhoea sampled from Anyako. A correlation of Calliphoridae and Temperature. B, correlation of Calliphoridae and Humidity (%). C, correlation of Calliphoridae and Precipitation (%).



A

B



C

APPENDIX 4

Descriptive Statistics for Anyanui

	N	Minimum	Maximum	Mean	Std. Deviation
Temperature°C	68	24	36	28.01	2.674
Humidity %	68	3	95	79.54	16.508
Precipitation %	68	0	97	10.41	16.483
Muscidae	68	0	902	58.75	131.325
Sarcophagidae	68	0	12	1.28	2.442
Calliphoridae	68	0	41	1.85	5.379
Valid N (listwise)	68				

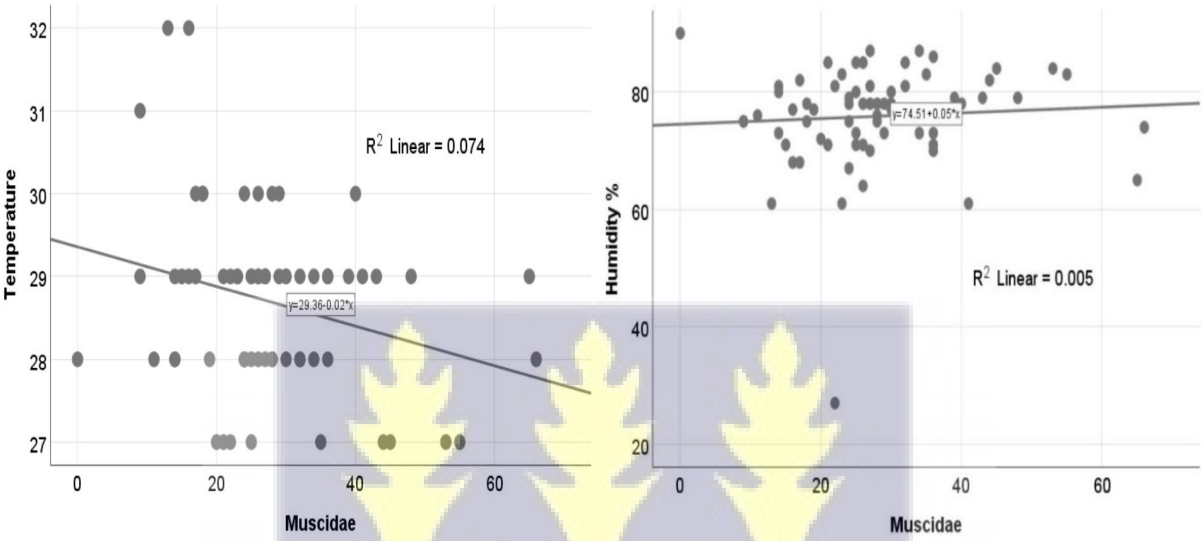
Nonparametric Correlations Anyanui

		Temperature	Humidity %	Precipitation %	Muscidae	Sarcophagidae	Calliphoridae
Temperature	Pearson Correlation	1	-.042	-.354**	-.273*	-.112	-.134
	Sig. (2-tailed)		.733	.003	.024	.363	.277
	N	68	68	68	68	68	68
Humidity %	Pearson Correlation	-.042	1	.195	.067	-.631**	.090
	Sig. (2-tailed)	.733		.112	.585	.000	.466
	N	68	68	68	68	68	68
Precipitation %	Pearson Correlation	-.354**	.195	1	.396**	-.091	.074
	Sig. (2-tailed)	.003	.112		.001	.458	.547
	N	68	68	68	68	68	68
Muscidae	Pearson Correlation	-.273*	.067	.396**	1	-.076	-.189
	Sig. (2-tailed)	.024	.585	.001		.537	.124
	N	68	68	68	68	68	68
Sarcophagidae	Pearson Correlation	-.112	-.631**	-.091	-.076	1	-.059
	Sig. (2-tailed)	.363	.000	.458	.537		.632
	N	68	68	68	68	68	68
Calliphoridae	Pearson Correlation	-.134	.090	.074	-.189	-.059	1
	Sig. (2-tailed)	.277	.466	.547	.124	.632	
	N	68	68	68	68	68	68

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Correlation between Environmental factors (Temperature (°C), Humidity (%), Precipitation (%)) and Vectors of diarrhoea sampled from Anyanui. A correlation of Muscidae and Temperature. B, correlation of Muscidae and Humidity (%). C, correlation of Muscidae and Precipitation (%).



A

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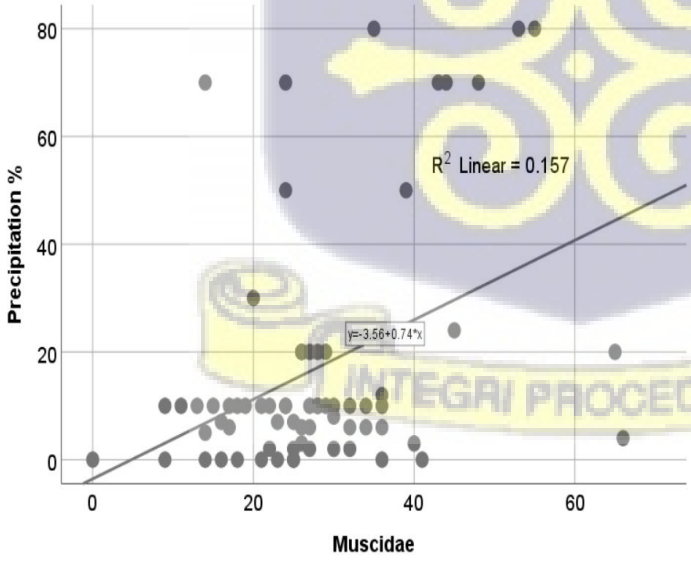
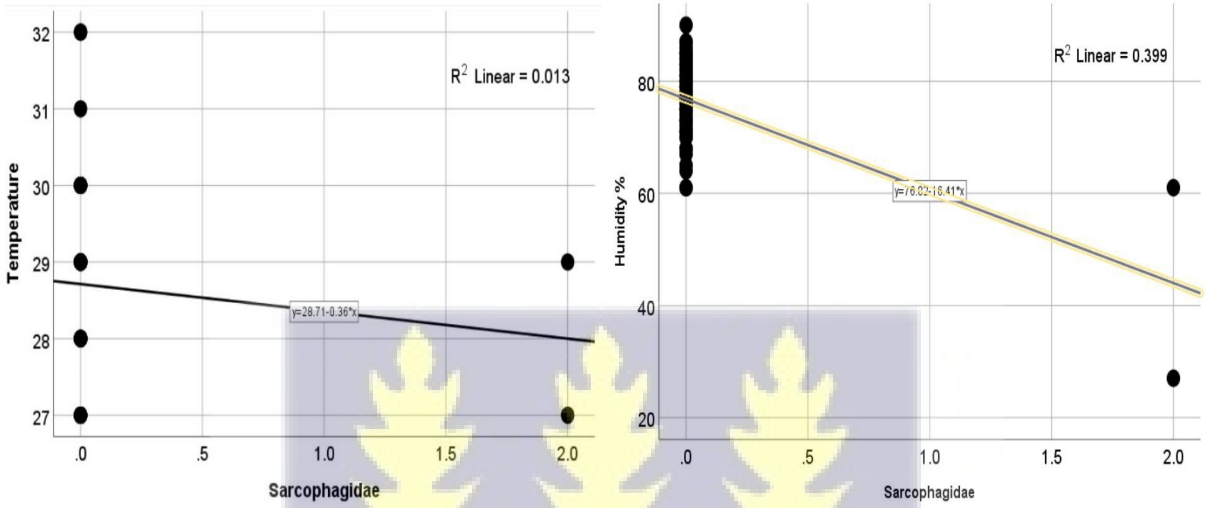


C

Correlation between Environmental factors (Temperature °C, Humidity (%), Precipitation (%)) and Vectors of diarrhoea sampled from Anyanui. (A) correlation of Sarcophagidae and Temperature. (B) correlation of Sarcophagidae and Humidity (%). (C) correlation of Sarcophagidae and Precipitation (%).



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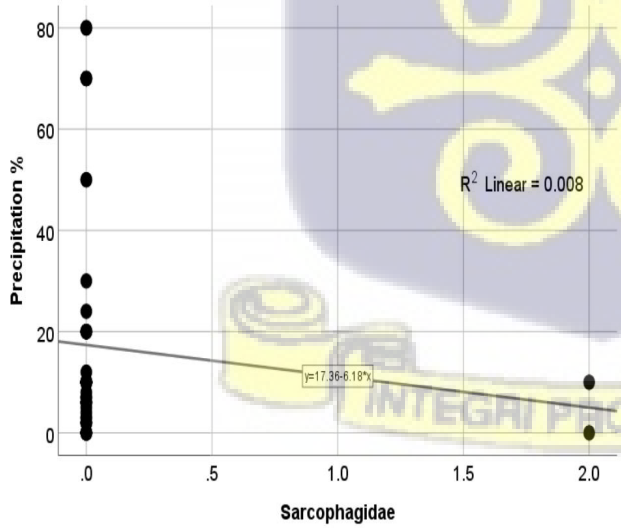
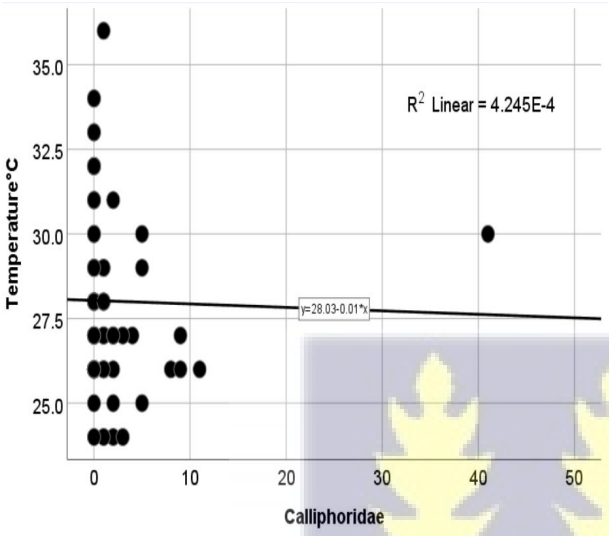


C

Correlation between Environmental factors (Temperature °C, Humidity (%), Precipitation (%)) and Vectors of diarrhoea sampled from Anyanui. (A) correlation of Calliphoridae and Temperature. (B) correlation of Calliphoridae and Humidity (%). (C) correlation of Calliphoridae and Precipitation (%).



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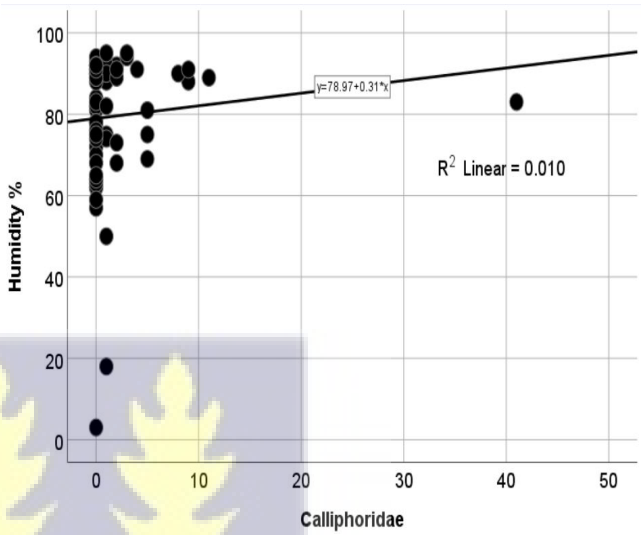

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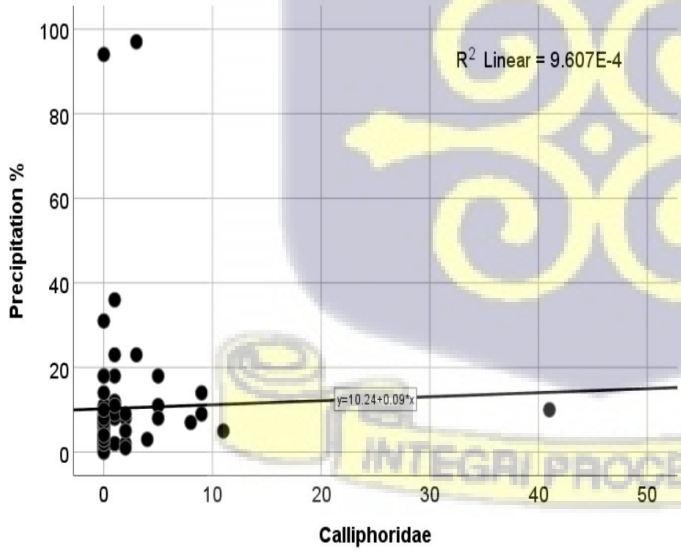


C

APPENDIX 5

Descriptive Statistics for Opetekwei

	N	Minimum	Maximum	Mean	Std. Deviation
Temperature °C	68	25	31	28.71	1.425
Humidity %	68	4	93	77.63	14.082
Precipitation %	68	1	82	7.51	11.163
Muscidae	68	0	19	3.97	4.962
Sarcophagidae	68	0	10	1.01	1.816
Calliphoridae	68	0	286	13.41	36.933
Valid N (listwise)	68				

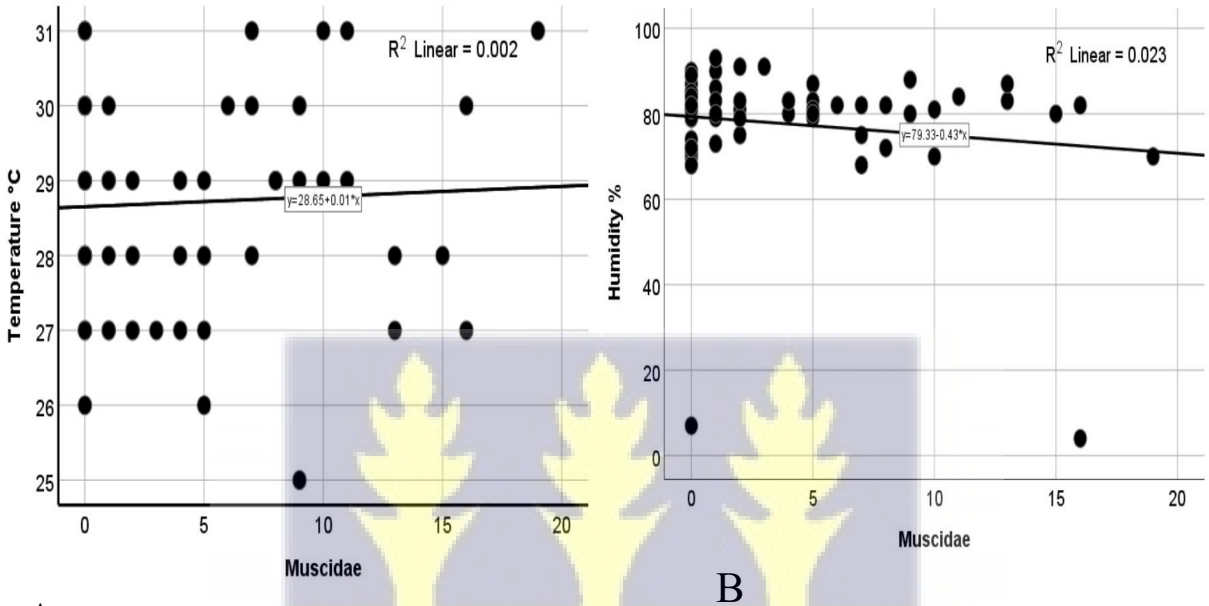
Nonparametric Correlations Opetekwei

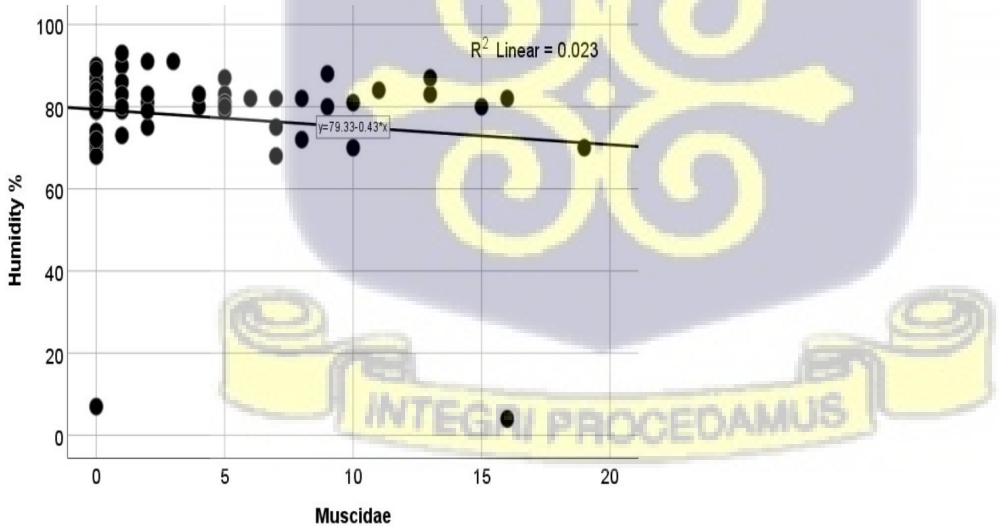
			Temperature °C	Humidity %	Precipitation %	Muscidae	Sarcophagidae	Calliphoridae
Spearman's rho	Temperature °C	Correlation Coefficient	1.000	-.525**	-.331**	-.043	-.015	-.167
		Sig. (2-tailed)	.	.000	.006	.730	.902	.172
		N	68	68	68	68	68	68
	Humidity %	Correlation Coefficient	-.525**	1.000	.221	.044	-.069	.097
		Sig. (2-tailed)	.000	.	.069	.724	.577	.429
		N	68	68	68	68	68	68
	Precipitation %	Correlation Coefficient	-.331**	.221	1.000	-.008	.225	.084
		Sig. (2-tailed)	.006	.069	.	.948	.065	.494
		N	68	68	68	68	68	68
	Muscidae	Correlation Coefficient	-.043	.044	-.008	1.000	.302*	.445**
		Sig. (2-tailed)	.730	.724	.948	.	.012	.000
		N	68	68	68	68	68	68
	Sarcophagidae	Correlation Coefficient	-.015	-.069	.225	.302*	1.000	.361**
		Sig. (2-tailed)	.902	.577	.065	.012	.	.002
		N	68	68	68	68	68	68
	Calliphoridae	Correlation Coefficient	-.167	.097	.084	.445**	.361**	1.000
		Sig. (2-tailed)	.172	.429	.494	.000	.002	.
		N	68	68	68	68	68	68

**. Correlation is significant at the 0.01 level (2-tailed).

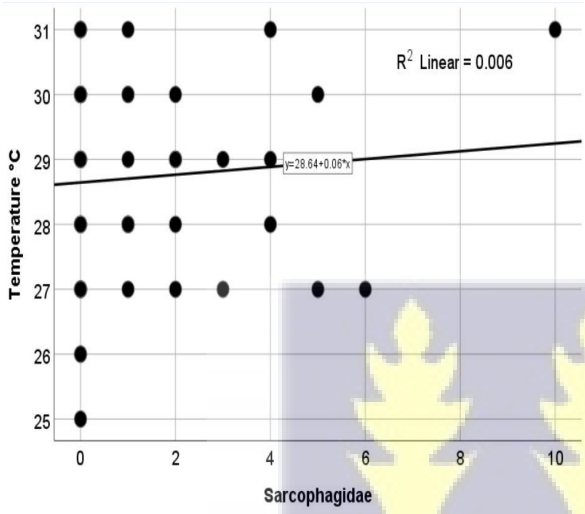
*. Correlation is significant at the 0.05 level (2-tailed).

Correlation between Environmental factors (Temperature (°C), Humidity (%), Precipitation (%)) and Vectors of diarrhoea sampled from Opetekwei. (A) correlation of Muscidae and Temperature. (B) correlation of Muscidae and Humidity (%). (C) correlation of Muscidae and Precipitation (%).





Correlation between Environmental factors (Temperature (°C), Humidity (%), Precipitation (%)) and Vectors of diarrhoea sampled from Opetekwei. (A) correlation of Sarcophagidae and Temperature. (B) correlation of Sarcophagidae and Humidity (%). (C) correlation of Sarcophagidae and Precipitation (%).



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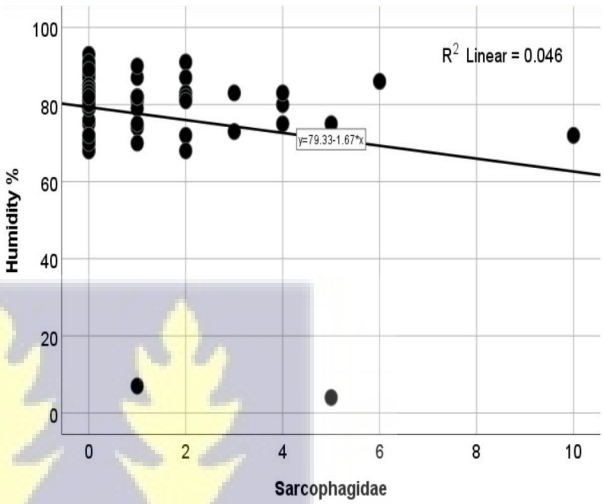

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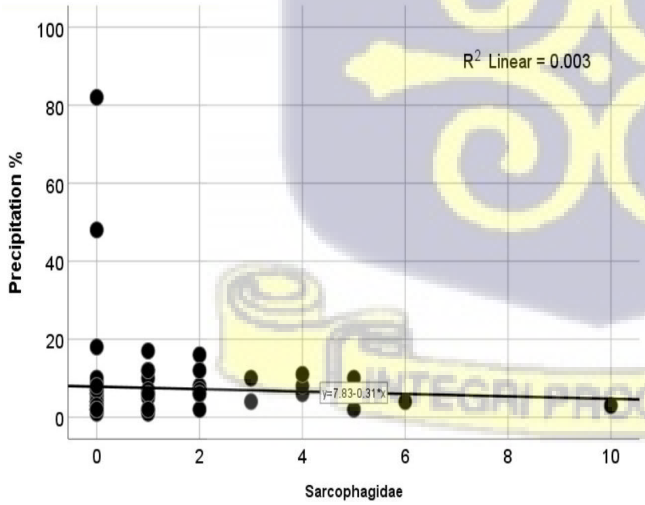
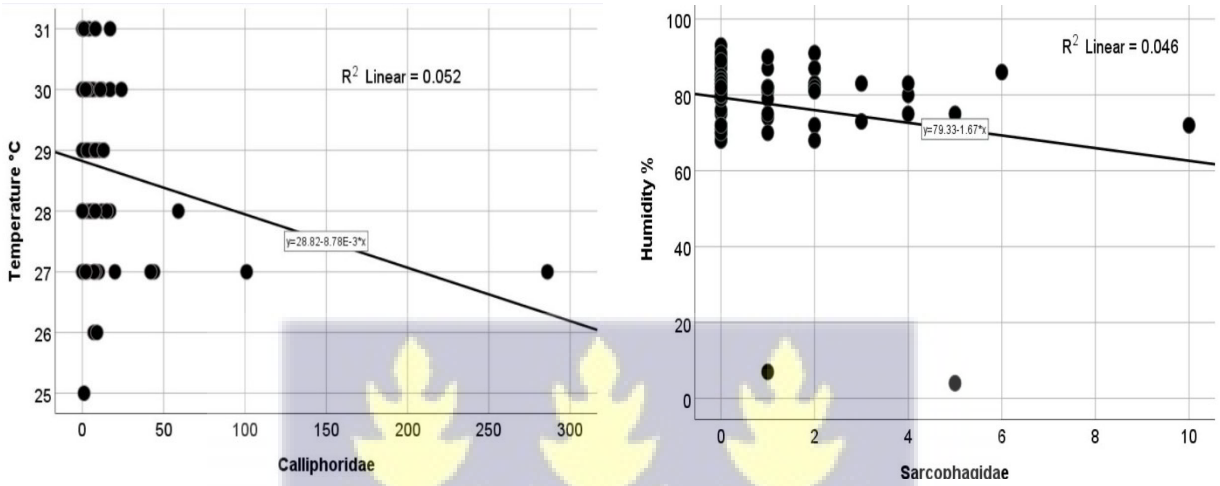


C

Correlation between Environmental factors (Temperature °C, Humidity (%), Precipitation (%)) and Vectors of diarrhoea sampled from Opetekwei. (A) correlation of Calliphoridae and Temperature. (B) correlation of Calliphoridae and Humidity (%). (C) correlation of Calliphoridae and Precipitation (%).



A

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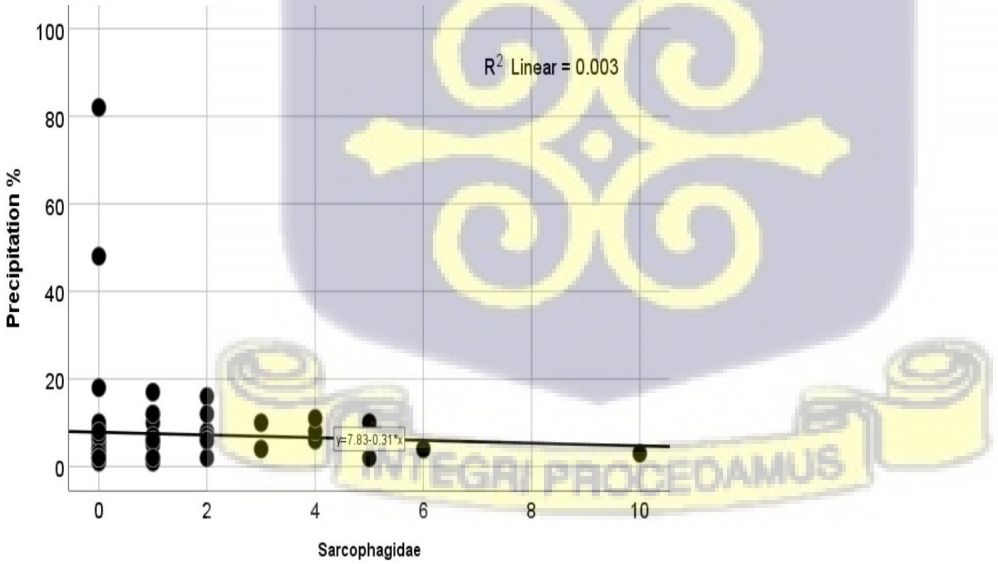


C

APPENDIX 6

Descriptive Statistics for Mumford

	N	Minimum	Maximum	Mean	Std. Deviation
Temperature	68	23	35	29.38	2.654
Humidity %	68	62	94	79.71	7.863
Precipitation %	68	1	24	8.10	6.913
Muscidae	68	0	111	31.69	29.239
Sarcophagidae	68	0	11	1.01	2.098
Calliphoridae	68	0	14	1.46	2.888
Valid N (listwise)	68				

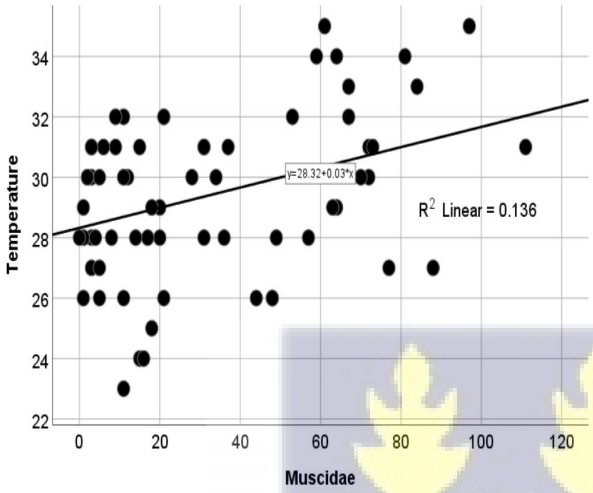
Nonparametric Correlations for Mumford

			Temperature	Humidity %	Precipitation %	Muscidae	Sarcophagidae	Calliphoridae
Spearman's rho	Temperature	Correlation Coefficient	1.000	-.277*	-.223	.302*	.003	.016
		Sig. (2-tailed)	.	.022	.068	.012	.980	.895
		N	68	68	68	68	68	68
	Humidity %	Correlation Coefficient	-.277*	1.000	.486**	-.023	-.072	.104
		Sig. (2-tailed)	.022	.	.000	.852	.561	.398
		N	68	68	68	68	68	68
	Precipitation %	Correlation Coefficient	-.223	.486**	1.000	.173	-.118	-.137
		Sig. (2-tailed)	.068	.000	.	.159	.338	.266
		N	68	68	68	68	68	68
	Muscidae	Correlation Coefficient	.302*	-.023	.173	1.000	-.120	-.250*
		Sig. (2-tailed)	.012	.852	.159	.	.329	.039
		N	68	68	68	68	68	68
	Sarcophagidae	Correlation Coefficient	.003	-.072	-.118	-.120	1.000	.471**
		Sig. (2-tailed)	.980	.561	.338	.329	.	.000
		N	68	68	68	68	68	68
	Calliphoridae	Correlation Coefficient	.016	.104	-.137	-.250*	.471**	1.000
		Sig. (2-tailed)	.895	.398	.266	.039	.000	.
		N	68	68	68	68	68	68

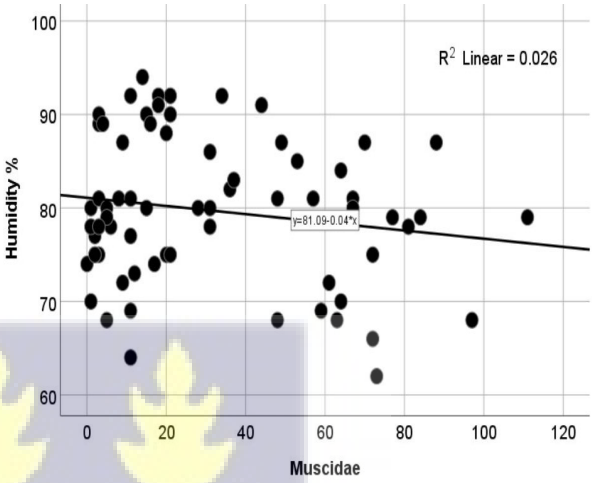
*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Correlation between Environmental factors (Temperature (°C), Humidity (%), Precipitation (%)) and Vectors of diarrhoea sampled from Mumford. (A) correlation of Muscidae and Temperature. (B) correlation of Muscidae and Humidity (%). (C) correlation of Muscidae and Precipitation (%).



A



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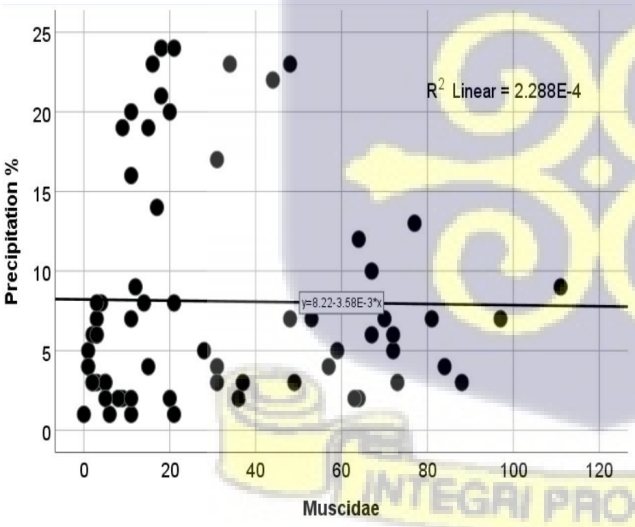
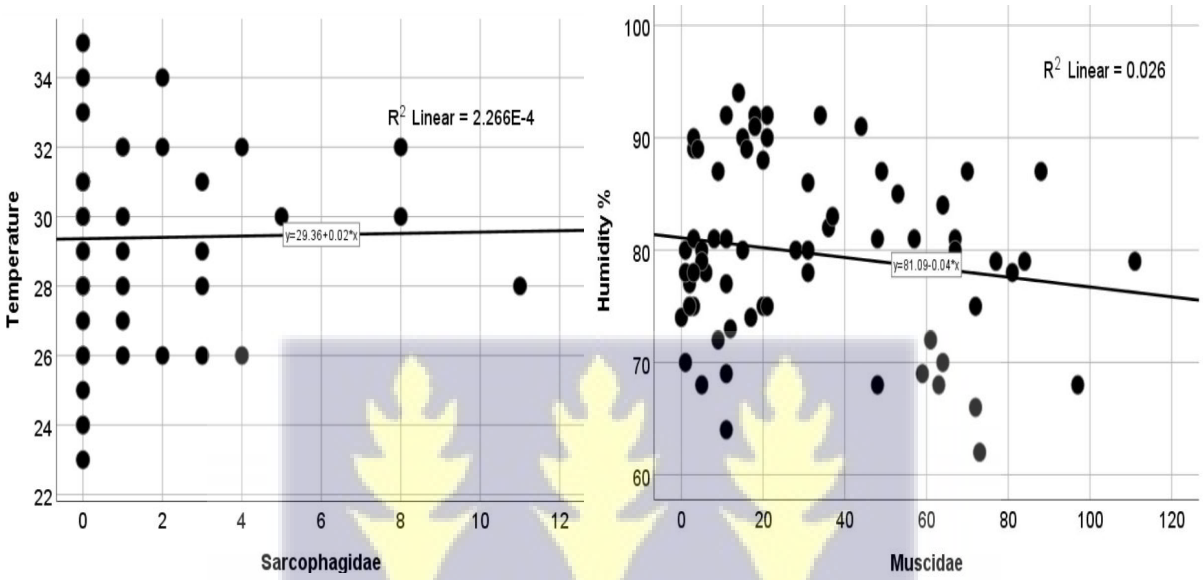
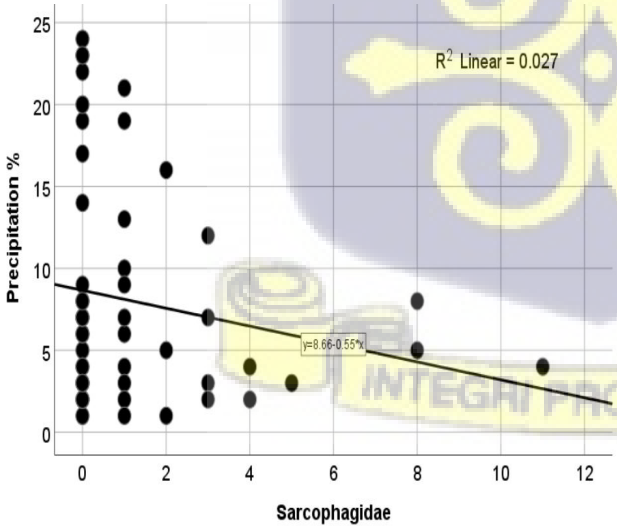


C

Correlation between Environmental factors (Temperature (°C), Humidity (%), Precipitation (%)) and Vectors of diarrhoea sampled from Mumford. (A) correlation of Sarcophagidae and Temperature. (B) correlation of Sarcophagidae and Humidity (%). (C) correlation of Sarcophagidae and Precipitation (%).

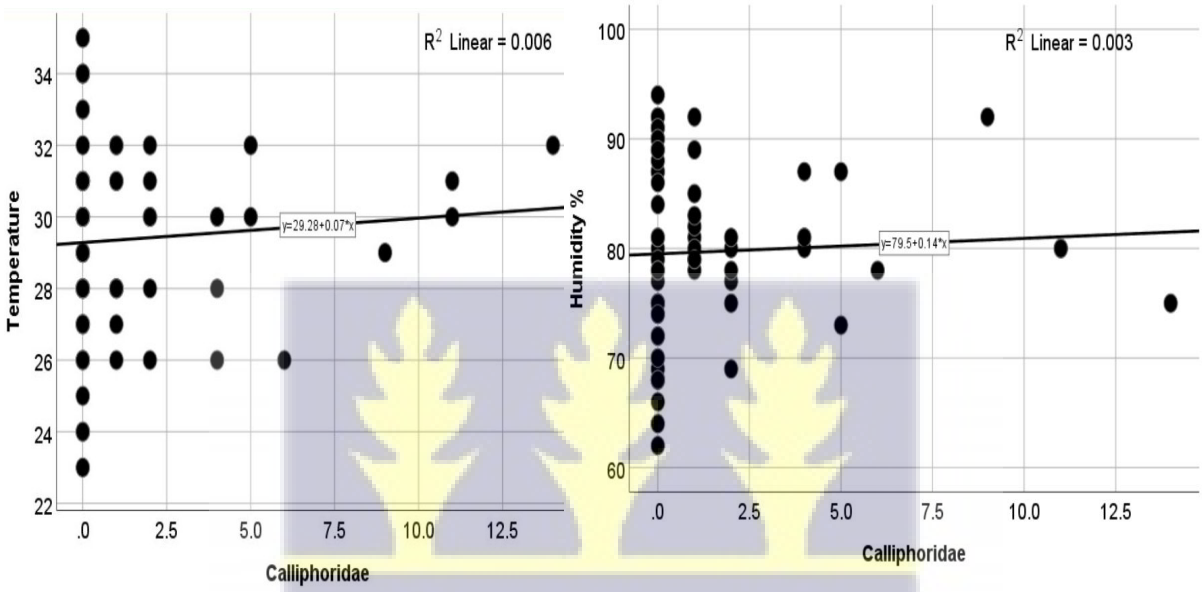


A B



C

Correlation between Environmental factors (Temperature °C, Humidity (%), Precipitation (%)) and Vectors of diarrhoea sampled from Mumford. (A) correlation of Calliphoridae and Temperature. (B) correlation of Calliphoridae and Humidity (%). (C) correlation of Calliphoridae and Precipitation (%).



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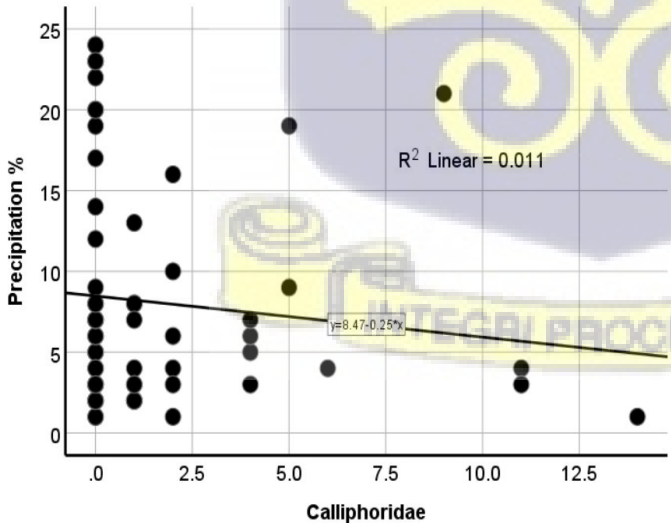

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