WATER QUALITY AND HEAVY METAL CONCENTRATION IN ZOOPLANKTON AND SEDIMENT WITHIN TIDAL ZONE OF SUNGAI PERAI

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by

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LIST OF SYMBOLS

^{o}C	Degree Celsius
m	Meter
m^3	Cubic Meter
mg/L	Milligram Per Liter
mg/kg	Milligram Per Kilogram
µS/cm	Microsiemens Per Centimeter
µg/L	Microgram Per Liter
µg/kg	Microgram Per Kilogram
Al	Aluminium
As	Arsenic
Mn	Manganese
Pb	Lead
NH_3	Ammonia
NO_2	Nitrite
PO_4^{3-}	Orthophosphate

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
BOD	Biochemical oxygen demand
DO	Dissolved oxygen
EC	Electrical Conductivity
pH	Potential of Hydrogen
TDS	Total dissolved solids
TSS	Total suspended solids
USM	Universiti Sains Malaysia

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KUALITI AIR DAN KEPEKATAN LOGAM BERAT DALAM ZOOPLANKTON DAN SEDIMEN DI ZON PASANG SURUT SUNGAI PERAI

ABSTRAK

Kajian ini meneliti kualiti air dan pengumpulan logam berat dalam sedimen dan zooplankton di Sungai Perai. Sampel air, sedimen, dan zooplankton telah diambil dari lapan kawasan sepanjang sungai semasa pasang surut anak dan pasang surut perbani pada bulan Januari, Februari, April, dan Mei 2024. Untuk kualiti air, konduktiviti elektrik (EC), jumlah pepejal terlarut (TDS), saliniti (Sal), oksigen terlarut (DO), suhu (Temp), dan kedalaman cakera Secchi diukur secara in situ. Permintaan oksigen biokimia (BOD), jumlah pepejal terampai (TSS), ortofosfat (PO_4), nitrit (NO_2), ammonia (NH_3), dan klorofil *a* (Chl *a*) dianalisis di makmal menggunakan kaedah piawai. Zooplankton dikumpulkan dengan menapis 40 liter air melalui jaring plankton bersaiz 35µm. Menggunakan Ekman Grab, sampel sedimen diambil dari setiap kawasan persampelan. Logam berat yang dianalisis dalam sedimen dan zooplankton menggunakan Spektroskopi Serapan Atom (AAS) ialah: aluminium (Al), arsenik (As), plumbum (Pb), dan mangan (Mn). Parameter kualiti air didapati berubah mengikut ruang dan masa, dipengaruhi oleh kitaran pasang surut dan perubahan musim. Kebanyakan tapak diklasifikasikan sebagai Kelas II atau III mengikut Standard Kualiti Air Negara (NWQS) yang menunjukkan keadaan bersih hingga sedikit tercemar. Semasa pasang surut perbani, parameter air seperti EC, TDS, dan saliniti biasanya lebih tinggi berbanding pasang surut anak. Ini mungkin disebabkan oleh peningkatan aliran air dan pencampuran semasa pasang surut perbani yang boleh menggerakkan pencemar dan meningkatkan parameter tersebut. Sebanyak 67 taksa zooplankton direkodkan, termasuk 44 Rotifera, 12 Cladocera, dan 11 Copepoda. Analisis sedimen menunjukkan kepekatan As dan Pb melebihi nilai kerak benua,

mencadangkan input antropogenik yang signifikan, manakala Al dan Mn berada di bawah tahap latar belakang. Urutan purata kepekatan logam berat dalam zooplankton adalah: Al > Mn > Pb > As. Nilai Igeo untuk As menunjukkan pencemaran sederhana hingga kuat, manakala nilai Igeo untuk Pb menunjukkan pencemaran sederhana. Nilai faktor pencemaran (CF) untuk As menggambarkan tahap pencemaran tinggi dalam sedimen, manakala nilai CF untuk Pb menunjukkan pencemaran sederhana Pb dalam sedimen. As dan Pb memberikan ancaman pencemaran sederhana hingga tinggi dalam sedimen dengan sumber berpotensi daripada industri dan urbanisasi sepanjang kawasan tadahan sungai serta pelepasan dari aktiviti pertanian hulu.

WATER QUALITY AND HEAVY METAL CONCENTRATION IN ZOOPLANKTON AND SEDIMENT WITHIN TIDAL ZONE OF SUNGAI PERAI

ABSTRACT

This study investigates the water quality and accumulation of heavy metals in sediments and zooplankton of Sungai Perai. Water, sediment, and zooplankton samples were collected from eight sites along the river during the ebb neap and spring tides of January, February, April, and May 2024. For water quality, electrical conductivity (EC), total dissolved solids (TDS), salinity (Sal), dissolved oxygen (DO), temperature (Temp), and Secchi disk depth were measured *in situ*. Biochemical oxygen demand (BOD), total suspended solids (TSS), orthophosphate (PO_4), nitrite (NO_2), ammonia (NH_3), and chlorophyll *a* (Chl *a*) were analysed in the lab using standard methods. The zooplankton were collected by filtering 40 litres of water through a 35μm plankton net. Using an Ekman Grab, sediment samples were taken from each sampling site. The heavy metals analysed in the sediment and zooplankton using Atomic Absorption Spectroscopy (AAS) were: aluminium (Al), arsenic (As), lead (Pb), and manganese (Mn). Water quality parameters were found to be varied spatially and temporally, influenced by tidal cycles and seasonal changes. Most sites were classified as Class II or III according to the National Water Quality Standards (NWQS), indicating clean to slightly polluted conditions. During spring tides, water parameters such as EC, TDS, and salinity were generally higher compared to neap tides. This is likely due to increased water flow and mixing during spring tides, which can mobilize pollutants and elevate these parameters. A total of 67 zooplankton taxa were recorded, including 44 Rotifera, 12 Cladocera, and 11 Copepoda. Sediment analysis revealed that As and Pb concentrations exceeded continental crust values, suggesting significant anthropogenic input, while Al and Mn were below background levels. The order of mean concentration of the heavy metals in zooplankton was: Al >Mn>Pb>As. Igeo values for As indicate moderate to strong pollution, while Igeo values for Pb indicate moderate pollution. The contamination factor (CF) values for As depict a high degree of contamination in the sediment, while the CF values of Pb indicate moderate pollution of Pb in the sediment. Arsenic and lead provide moderate to high contamination threats in the sediment with potential sources from industries and urbanization along the river catchments and discharge from upstream agricultural activities.

CHAPTER 1
INTRODUCTION

1.1 Introduction

The need for water is growing exponentially as developing countries experience economic revolutions and see an increase in living standards. The use of the environment for production and consumption leads to the destruction of the biosphere and water supplies (Grizzetti & Poikane, 2024). With 69 percent of all yearly water withdrawals going toward irrigation, animal husbandry, and aquaculture, agriculture is the world's largest user of water. Households make up 12 percent of the total, and industry, including power generation, accounts for 19 percent; however, these percentages differ significantly between nations and continents (FAO, 2021). One of the most endangered ecosystems on Earth is the freshwater ecosystem, such as rivers and inland lakes (Alahuhta et al., 2019). Because of retention effects and comparatively longer water residence durations, sediments in inland waters have the capacity to accumulate nutrients and contaminants. (Michalec & Cupak, 2021). Heavy metals generated by industry and residential processes frequently contaminate aquatic systems in the natural world. This contamination can have severe consequences on the ecological equilibrium of the environment and the various aquatic organisms that inhabit it (Farombi et al., 2007). Agricultural practices significantly contribute to heavy metal pollution in rivers primarily by the application of metal-laden agrochemicals, irrigation with contaminated water, soil amendments, and runoff caused by erosion (Angon et al., 2024). Zooplankton and fish, inhabitants of aquatic environments, are unable to escape the harmful effects of pollutant (Olaifa et al., 2004). Heavy metals which are harmful to the environment and human health are passed from phytoplankton and zooplankton to fish, people, and other piscivorous animals through aquatic food webs (C. Y. Chen et al., 2000). Floods, contaminated rivers, and interruptions to water supplies are all frequent problems in Malaysia. Pollutants often cause unplanned disruptions, which can be harmful. Scheduled water cuts are necessary to perform maintenance and repairs on the water supply system. River pollution in Malaysia can be classified into two: point source pollution and non-point source pollution (NPS). Point source pollution is pollution that is attributed to a single identifiable source, such as discharge pipes from factories or industries. On the other hand, non-point source (NPS) pollution emanate from multiple sources, including rainwater runoff from night markets that carry refuse, food waste, and oils into nearby rivers and storm drains.(*Much Ado about Malaysia's Water Woes*, 2023).

1.1.1 Background of Study

Rivers are continuously being subjected to anthropogenic effects due to improper release of treated and untreated waste from various sources, such as wastewater from domestic activities, mining operations, industrial discharge, or even just agricultural areas runoff that contains heavy metals, polycyclic aromatic hydrocarbons, parasites, and agrochemicals (Bae et al., 2017). Since several pathological processes, such as reproductive effects and carcinogenesis are linked to many environmental pollutants that affect DNA directly or indirectly, these contaminants have paramount toxicological importance because their effects extend beyond the individual and are passed down to succeeding descendants (Bolognesi & Cirillo, 2014). The most prevalent contaminants that have an impact on aquatic habitats are heavy metals. These metals include copper (Cu), zinc (Zn), and chromium (Cr), which are necessary for life but can also be hazardous in concentrated forms. Even in trace concentrations, non-essential metals like lead (Pb), cadmium (Cd), and mercury (Hg) are toxic to health (Rathi et al., 2021). Assessing the concentrations of heavy metal tissues of residing species provides a site-specific metal bioavailability picture that is integrated and ecologically appropriate, together with biomarker responses (De Jonge et al., 2015). Water contaminated by heavy metals can be harmful to human, animal, and plant health by bio-concentrating in various tissues and organs (Ndome et al., 2014).

Because zooplankton have a solid inclination to concentrate heavy metals, they are useful as markers of metal pollution (Roy et al., 2010). The zooplankton community composition was often suggested as a measure of the water quality in coastal environments (Thirunavukkarasu et al., 2020). Heavy metals are found in the sediments of aquatic ecosystems due to a variety of causes, including as the land application of fertilisers, animal manures, sewage sludge, pesticides, wastewater irrigation, and the dumping of high-metal wastes (Zhang et al., 2014). In the aquatic environment, metals and metalloids are dispersed throughout many media, including sediment, water, and biota. Metals and metalloids exist in dissolved form in the aqueous phase and are absorbed by suspended matter. However, because of their adsorption capacity, they mostly accumulate in sediments. Later, they are retransferred to the food chain and water column through the processes of desorption and remobilization (Hasan et al., 2016). According to Haris & Aris (2015), organic matter and sediment particle size have a significant impact on the ability of sediments in rivers to retain metals. Rapidly deteriorating water quality in Penang's rivers remains a pressing concern for many long-time residents. A majority of Penang's main rivers (6 out of 7) are classified as Class III according to Water Quality Index readings from 2016 to 2022 (Dermawan, 2022).

1.2 Statement of the Problem

Recently, swift economic development in Malaysia may have been responsible for increased environmental elemental concentrations (Looi et al., 2019). Unbelievably, river water quality has been declining, making it more difficult than ever to be usable. The point and non-point causes of pollution are threatening Malaysia's rivers. Sulfur and effluent water from residential buildings, sewage treatment plants, manufacturing and agricultural businesses, and pig farms are examples of point sources of pollution (Daud, 2008).

Toxins and river pollutants consumed by aquatic animals (Zooplankton, fish, shellfish, etc.), accumulate across the food web, posing danger to humans. The Sungai Perai is located in an urban area that has undergone significant development over time. Unfortunately, this has led to several issues with regard to pollution. The river receives pollution from sewage treatment plants and industries located near the riverbank. In addition to that, non-point pollution sources, such as domestic drainage and land run-off, also contribute to the pollution problem. Furthermore, some tributaries and drains of the river are being used as dumping sites for rubbish, which is a major concern. This can be seen from reports where the Department of Environment (DoE) had fines a food factory RM 18000 for illegally discharging effluents into Sungai Perai. Complaints of pollution and the presence of dead fish in Sungai have also been received by the DOE in 2021 (Audey, 2021). In 2020, an NGO stated that rice farmers and oil palm smallholders along Sungai Kreh in Tasek Gelugor, Prai, have endured significant losses for decades due to pig excrement contaminating their key water source (FMT, 2020). The contamination of Sungai Perai was determined to have originated from considerable inland sources. The tributaries are already polluted and transmit their pollutants to the river (Mohamad Faudzi et al., 2023).

Primary consumers, zooplankton, are essential to the biogeochemical cycle of metals in aquatic ecosystems' water column, especially particle-reactive metals. Zooplankton is a crucial biological component of Environmental Impact Assessment (EIA) to ensure that the effects on biodiversity are considered, and negative outcomes are avoided or lessened (Superadmin, 2017). It is essential to understand the potential for metals to accumulate in their body tissues (Stewart et al., 2005). The major sources of heavy metals in river sediments are chemical weathering of bedrock, soil, and runoff from the riverbank. The accumulation of metals in urban river sediments can harm the environment (Haris et al., 2017). Since sediments are sinks for heavy metals, understanding how they build up can reveal information about the general condition of river ecosystems as well as the degrees of contamination (C. Zhang et al., 2014).

Sungai Perai, which spans 45 km and has a surface area of 447.824 km^2, is the longest and biggest river in Penang, Malaysia. Sungai Perai should be protected from pollution as a future resource and used as an alternative water supply for Penang. Heavy metals' impacts on aquatic organisms have been documented such as the works of (Achary et al., 2020; Chevrollier et al., 2022; Chouvelon et al., 2019; El-Metwally et al., 2022; Ju et al., 2019), but more needs to be written about how they may affect sediments and Zooplankton in Malaysian river ecosystems. Hence, this study aims to evaluate the potential sources of pollution and impacts of toxic metals at the tributaries and main drainage on sediments and Zooplankton in the downstream area of Sungai Perai Penang.

1.3 Research Objectives (RO)

RO1: To determine the water quality of Sungai Perai Penang, Malaysia.

RO2: To determine the concentration of heavy metals in zooplankton and sediments from Sungai Perai Penang, Malaysia.

RO3: To assess the potential contamination in sediment and zooplankton as in indicative pollution across the food chain .

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

Waterborne trade has become so prevalent, human settlements and enterprises have historically been clustered along rivers, estuaries, and coastal zones. The usage of water by humans results in the introduction of various pollutants, which degrade the quality of the water. Seas and rivers may naturally purify themselves, but when pollution levels in water bodies rise above this threshold, issues arise (Radojević & Baškin, 2006). The main factors affecting river ecosystems are pollution, habitat degradation, changes in hydromorphology, impermeable coverage (such as metropolitan areas), loss of riparian zones, and urbanised floodplains (Sabater et al., 2019). A close relationship exists between the surface water quality and the nature of its catchment area (Oszczapińska et al., 2018). The conditions of catchment and transport processes could affect the delivery of contaminants to rivers. Runoff formation depends on its topographic setting and the intensity of rainfall, which could introduce these pollutants to water bodies (Anh et al., 2023).

Even at very low concentrations, heavy metals can pose a major hazard to both plant and wildlife since they are poisonous and nonbiodegradable in the natural world. Heavy metals sources in the rivers may be natural and anthropogenic (Rani et al., 2022). However, most of the heavy metal pollution in river ecosystems is caused by human activity, particularly the discharge of untreated industrial effluents. Through bioaccumulation and/or biomagnification, they may have an impact on the aquatic organisms after they enter the river ecosystem. Furthermore, these harmful metals can enter the bodies of people and other animals through the food chain and result in major health issues (Rani et al., 2022). The industries that usually attribute heavy metal contamination to river water are the mining, metalworking, paint, pigment, varnish, pulp and paper, tanneries, distilleries, rayon, cotton textiles, rubber, thermal power plants, steel plants, galvanization of iron products, and the careless application of fertilisers and pesticides in agricultural fields (Paul, 2017). Heavy metals are natural elements known for their high atomic mass and density. Despite usually being present in low concentrations, they are distributed across the Earth's crust. A density of 5 g cm^{-3} is typically used to categorize a metal as heavy and distinguish it from lighter metals (Duffus, 2002).

Heavy metals such as copper, selenium, and zinc are vital trace elements that play crucial roles in numerous biological processes and are needed for human metabolism (Mertz, 1981). Conversely, elements like mercury, cadmium, arsenic, chromium, thallium, lead, and others are known for their poisonous properties even at low concentrations, making them the "dark side of chemistry" (Duruibe et al., 2007). Heavy metal ion pollution in rivers is a major concern because of the ions' toxicity, persistence, and capacity to accumulate biologically. Heavy metal pollution can pose a major risks to public health through the food web, especially from drinking water, which can deteriorate the entire biological environment (Singh & Kumar, 2017). Since the river provides around 98% of Malaysia's water needs, the country's riverine environment is particularly significant. Therefore, pollution of river water presents a real risk to public health. The Department of Environment (DOE) in Malaysia is responsible for monitoring the quality of river water in Malaysia (Harguinteguy et al., 2016).

Given how often we consume water in our daily lives, determining the quality of the water is crucial. The purpose of the water quality measurement is to ensure that the water supply's contents are safe for human consumption (Zanuri et al., 2020). Testing for water quality, which involves examining the physical, chemical, and biological properties of water, can provide insight into how healthy rivers are and if they meet the needs of biotic species to survive or to be utilised by humans for a variety of uses (Azizan et al., 2020). It has become clear that toxic metal contamination in sediment is an issue that is becoming more widespread (Kucuksezgin et al., 2008) and it is thought to be a major hazard to the aquatic ecosystem because of their toxicity, persistent nature, inability to biodegrade, and capacity to enrich the food chain (Nobi et al., 2010). It is commonly recognised that zooplankton can also transmit metals to consumers at the upper trophic level (Calbet et al., 2016). Thus, in order to shed light on the potential impact of metal on aquatic environments and ecosystems, it is necessary to comprehend the accumulation of metal in zooplankton (Battuello et al., 2016).

2.2 Heavy metal accumulation in sediment

Sediment is comprised of different organic and inorganic components transported by rivers; the sediment deposition is influenced by the energy of the flow. (Kumar et al., 2016). Rivers accumulate sediment when their velocity decreases. Heavier and larger particles like pebbles and sand settle before lighter silt and clay, which only settle when the water is almost still (*Geological Society - Rivers & Deltas*, n.d.). River sediment is widely considered a suitable indicator of a river's pollution level (Haris et al., 2017).

Heavy metals released into aquatic systems during transportation are partitioned between the water phase and sediments. Due to adsorption, hydrolysis, and co-precipitation of metal ions, a significant amount of them accumulate in the sediment, whereas only a small fraction of free metal ions remain dissolved in the water column. Various parameters such as sediment particle nature, adsorbed compound qualities, metal features, redox processes, and biodegradation of sorptive substances under certain conditions govern the accumulation and movement of heavy metals in sediments (Bastami et al., 2014). Sediment serves as a sink for metals and offers a reliable indication of the river's baseline pollution. Sediment can retain toxins and could discharge them back into the river system (Shafie et al., 2013). Heavy metals in river sediments mostly originate from the chemical weathering of bedrocks, soil, and runoff along the riverbank (Haris et al., 2017). Heavy metals exhibit a low water solubility and can accumulate in sediments (K. P. Singh et al., 2005). Human activities and natural processes like rock weathering and volcanic eruptions are significant factors in determining the levels of heavy metals in sediments. Anthropogenic heavy metals in coastal areas primarily come from local sources including agriculture, industries, urban growth, and other human activities near rivers and estuaries (Liza Evianti, 2013). Research on sediment analysis of dated and/or undisturbed varved sediments has revealed three key conclusions.

Sediments:

(i) Offer a thorough perspective on both known and unknown pollution from the beginning of industrialization,

(ii) Systematically record the occurrence and disappearance times of pollutants.

(iii) Examine how they evolve in reaction to various management strategies, regulations, or environmental shifts. Spatial examination of sediments can provide information about the movement and settling of pollutants (Chiaia-Hernández et al., 2022).

Heavy metal levels in river sediments rise as you move downstream (Tang et al., 2014). The heavy metal concentration in the river sediments is influenced by the combined effects of pollution sources and natural processes associated with geological structure and climatic conditions (Emenike et al., 2020), and the characteristics of the point and non-point sources of pollution (Maanan et al., 2014). Each catchment has a distinct pattern for transporting heavy metals to water, influenced by the nature, and spread of pollution sources and emission rates (Sojka et al., 2018). Identifying the sources of heavy metals is crucial in environmental research. Sediments are becoming reliable markers of pollution in aquatic environments (Liber et al., 2019).

2.2.1 Heavy metal pollution in Malaysian rivers sediments

The heavy metals found in certain stations along the Perai river in Penang, Malaysia are believed to originate from urban runoff, industrial activity near the river, fishing and shipping operations, and land transportation (Foo et al., 2021). Haris and Aris (2015) reported that metal distribution in the sediment was concentrated in the estuaries of the Klang River (Cd, Cr, Cu, Ni, Pb, and Zn), Langat River (Co, Cr, and Ni), and Lumut Strait (Cd, Co, Cr, Mn, Ni, Pb, and Zn).

Arsenic is a toxic metal that is released into the surface ground water through geological formation that happens in sedimentary rocks, geothermal water, and from weathered volcanic rocks. Anthropogenic inputs from mining, manufacturing, metal works, wood preservation, and use of pesticides also introduce arsenic into the water bodies (Freije, 2015). The accumulation of arsenic in sediments affects bioaccumulation in fish (Kamaruzzaman et al., 2020).

Aluminium is another commonly introduced metal into rivers through natural weathering of minerals and anthropogenic sources such as industrial effluents, alum sludge from water treatment, and waste dumping (Shaari et al., 2015). As for Mn, the use of manganese-containing fertilizers (e.g., $MnSO_4$ and MnO) in agriculture can introduce manganese into soils and sediments via runoff, especially in paddy and non-paddy cultivated areas. This input can alter manganese speciation and bioavailability in sediments (Shaari et al., 2018). High levels of Mn in sediments can negatively impact aquatic ecosystems, including the bioaccumulation and biomagnification of Mn in aquatic organisms (Mazilamani et al., 2024).

The natural weathering of lead-bearing minerals in granite and other rocks also contributes to background lead levels in river sediments (Ahmed et al., 2017). The anthropogenic activities that release Pb into river sediments could have been from the spillage of leaded petrol from the boats and leaching from marine paint and undercoating (Haris et al., 2017).

Increased levels of heavy metals were typically found at sample locations near human-made sources such as domestic, industrial, shipping, or other sources of pollution in some rivers around the west coast of the Malaysian peninsula (Buhari & Ismail, 2020). Clay, silt, and organic debris in the sediment of Langat River, Malaysia, facilitated the retention of metals (Haris & Aris, 2015). The research on the horizontal spatial distribution of heavy metals in water and sediment of Kelantan river tributaries in Gua Musang indicates contamination with manganese, iron, aluminum, and zinc (Yusoff et al., 2020). Various human activities along the Perak river, like chemical manufacture and municipal effluents, have led to the presence of heavy metals, causing a decline in the water quality of the river (Muyibi et al., 2008). The metal concentrations in Setiu River sediments were mostly affected by human activities in the nearby locations. The activities of humans around the river led to contamination by arsenic, lead, copper, and zinc. Pollution from metals can also originate from fish loading and offloading, cleaning, maintenance tasks like painting and antifouling coatings, fueling operations, and urban runoff. Furthermore, the use of leaded gasoline by fishermen resulted in elevated Pb levels in the area (Madzlan et al., 2023).

2.2.2 Heavy metal accumulation in Zooplankton

Zooplankton are free-floating organisms found in oceans, especially in the pelagic and littoral zones, as well as in rivers, lakes, and ponds. Most of them are tiny, single-celled or multi-celled organisms that vary in size from a few microns to a millimetre or larger. Zooplankton are a heterotrophic species of plankton that consume phytoplankton. Zooplankton can be categorised into two groups: holoplankton, which complete their full existence as plankton, and meroplankton, which spend only portion of their lifecycle as plankton. Meroplankton comprises larval and juvenile stages of organisms that undergo a lifestyle change upon reaching maturity, such as fish, prawns and crab larvae (Superadmin, 2017).

Zooplankton are crucial in aquatic ecosystems as they act as a primary link in food webs, facilitating energy transfer from phytoplankton (primary producers) to higher trophic levels. They also play a role in nutrient cycling and regulating phytoplankton populations. Changes in zooplankton communities can significantly impact water quality and the overall balance of the ecosystem (Pereira, 2025). Heavy metal pollution can severely impact the biological equilibrium of the surrounding ecosystem and a variety of aquatic life (Farombi et al., 2007). Fishes and zooplankton are the animal species most affected by pollutants and are unable to avoid their harmful effects (Amoatey & Baawain, 2019). Algae that have absorbed heavy metals transmit these metals to zooplankton when they are ingested, leading to decreased survival and reproductive rates in the zooplankton (Barata et al., 2002). Zooplankton populations can serve as a valuable indicator for assessing the ecological condition of a river environment (Ismail et al., 2022). Heavy metals can accumulate in the food chain in a hierarchical manner, posing a risk to human health when consumed (El-Metwally et al., 2021; Pandey et al., 2019). Zooplankton are microscopic organisms with a high surface area to volume ratio, allowing them to absorb or collect heavy metals in their bodies (Achary et al., 2020). Zooplankton, as primary consumers, significantly influence the biogeochemical cycling of metals, particularly particle-reactive metals, in the water column of aquatic environments. Therefore, understanding metal concentrations and accumulation in zooplankton is crucial for effective biomonitoring (Chevrollier et al., 2022). These primary consumers are often used as biomonitors to assess the bioavailability of contaminants in marine environments (Achary et al., 2020).

Copepods, the most notable zooplankton, are the most abundant multicellular animals on Earth. One crucial role they play is as the primary grazers in aquatic food webs, serving as the main conduit for energy transfer from primary producers to higher trophic levels of consumers (Richardson, 2008). Zooplankton is an important food source for pelagic and planktivorous fishes. Zooplankton's ecological responsibilities contribute significantly to the biomagnification of trace metals in the food chain, leading to harmful amounts in higher trophic levels (Ikhuoriah et al., 2015). Zooplankton may accumulate trace metals, leading to the poisoning of aquatic food chains through bioaccumulation in higher trophic levels. During the dry season, Cladocera and Rotifera are highly susceptible to trace metals, leading to declines in their biodiversity. Specifically, Cladocera (like *Daphnia magna* and *Ceriodaphnia dubia*) and Rotifers (like *Brachionus calyciflorus* and *Lecane inermis*) are sensitive to trace metals.

Exposure to these metals can result in reduced population sizes and a decrease in the overall diversity of these groups (Isibor et al., 2020). Zooplankton play a crucial role in aquatic ecosystems by transforming phytoplankton, benthic plants, bacteria, fungi, and decaying organic materials into animal tissue, which then becomes part of the food chain for higher organisms (Nneka & Ekata, 2017). High levels of heavy metals are seen in mixed zooplankton species along the shore because of the untreated discharge of waste products from sewage treatment plants located near the coast or rivers (Robin et al., 2012).

Calanoida is a zooplankton order under the Copepoda subclass. There are 43 families including approximately 2000 species of copepods, found in both marine and freshwater environments. Calanoid copepods play a crucial role in several food chains by obtaining energy from phytoplankton and algae and transferring it to higher trophic level predators such as birds, fish, and mammals. Several commercial fish rely on *Calanoida Copepoda* as a primary food source throughout their larval or adult phases (Omoregie, 2017). In view of this, it is important to comprehend how zooplankton accumulate heavy metals in various environments to consider using them as bioindicators for heavy metal presence in the lower levels of the food chain (Achary et al., 2020; Ju et al., 2019).

The community composition of zooplankton is often suggested as an indicator of water quality in coastal settings (Thirunavukkarasu et al., 2020). Plankton communities are responsive to specific dissolved heavy metals (Ju et al., 2019), and their populations can quickly alter according to variations in physical (such as temperature, dissolved oxygen, and salinity), chemical (such nutrients and heavy metals), and biological factors in the water (Thirunavukkarasu et al., 2020). It is known that zooplankton are widely used as a bioindicator in biomonitoring research. Metal levels in zooplankton can provide valuable information about spatial and temporal changes of toxic and essential elements (Uluturhan et al., 2023).

2.3 Water quality of rivers

Water quality is the assessment of water suitability for various uses (drinking, industrial, agricultural, recreational, and habitat) based on physical, chemical, and biological factors. Water quality is crucial for all living things on Earth and is of interest to a wide array of scientists, researchers, and water resource managers. Water quality fluctuates based on geographical location, time, meteorological conditions, and pollution sources (Giri & Qiu, 2016). Monitoring water quality is crucial for assessing the condition and makeup of streams, rivers, and lakes at various time intervals, ranging from a single moment to weeks, months, and years.

Rivers and streams are the main channels via which freshwater travels on Earth. These waters have diverse roles including irrigation, providing habitat for aquatic life, supplying drinking water, facilitating transportation, generating power via hydroelectric dams, and supporting recreational activities such as swimming and boating. Maintaining the health of these waterways is crucial for preserving the equilibrium between nature and human society (Azura, 2021). River water supplies may be impacted by both natural elements and human actions (Ameen & Mourshed, 2017).

Land use patterns may influence physicochemical water quality measures positively or negatively. Land clearance, livestock manure, and farming activities can result in the release of sediment, nutrients, organic matter, heavy metals, and diseases through runoff or irrigation (Camara et al., 2019). Contaminants from wastewater or emissions may lead to a decline in river water quality. Climate variables such as temperature and precipitation fluctuate based on location and time of year, causing spatial and temporal changes in river water quality (Chen et al., 2018).

Rivers in Malaysia have consistently raised concerns regarding water quality among local authorities, government entities, and the general population. Malaysia's rivers, such as Sungai Klang in Selangor, Sungai Juru in Penang, and Sungai Segget in Johor, are commonly known to be polluted. Based on visual inspection alone, it is evident that there are issues with the existing water quality of these rivers (Zainudin, n.d.). The quality of river water in Malaysia has deteriorated, posing challenges in making it suitable for use. Malaysian rivers face pollution from both point and non-point causes. Main sources of pollution include sewage treatment plants, agro-industry, manufacturing, sulfur or greywater from commercial and residential establishments, and pig farms (Daud, 2008).

2.3.1 Water quality index (WQI)

The water quality index (WQI), which is based on several water quality criteria, gives a single number that represents the total water quality at a specific place and time. Using some of the often used water metrics (BOD, temperature, turbidity, conductivity, etc.), the index shows the degree of quality of a body of water, such as a lake, river, or stream (Adelagun et al., 2021). Since WQI is a useful technique in Malaysia, the Department of Environment (DOE) advised using it for ranking and indication (good, moderate, or poor). The basic steps of the WQI method include: (i) selection of parameters, (ii) determination of values of sub-index, (iii) assignment of appropriate parameter weights, (iv) aggregation of the weighted sub-index to compute the final WQI. At present, there is no single standard accepted for the WQI calculation method. Therefore, various kinds of WQI models such as Canadian Council of Ministers of Environment Water Quality Index (CCMEWQI), National Sanitation Foundation Water

Quality Index (NSFWQI), Integrated Water Quality Index (IWQI), and WQI Malaysia are introduced with different and unique calculations (Liang et al., 2023).

2.4 Factors affecting river water quality in urban and rural areas

Urban and industrial land use expansion is associated with less infiltration, higher runoff, and the movement of pollutants from the catchment area to aquatic bodies. This may result in the degradation of water quality in rivers. Human activities related to urban land, such as discharging domestic and industrial sewage, applying fertilizers and pesticides, surface runoff, and other non-point pollution sources, affected the concentration of pollutants in river water (Chen et al., 2020).

Seasonal fluctuations in precipitation, runoff, and underground flow can impact river flow, leading to alterations in pollution levels in river water. Under specific climate conditions, temperatures may be elevated during the dry season compared to the rainy season, resulting in less water flow and decreased disturbance and dilution. Seasonal changes in river water quality can be elucidated by analyzing the findings obtained over different seasons (Cruz et al., 2019).

The topographic setting plays a crucial role in influencing how contaminants are transported to water bodies, dictating whether runoff enters surface water or seeps into subsurface or groundwater (Anh et al., 2023). Urban areas are seeing a widespread trend of expanding residential and industrial land, which has significant effects on the environment. Research on water pollution in three Malaysian rivers revealed that the Sungai Galing in urban regions had the poorest water quality, attributed to inadequate wastewater treatment, resulting in the highest average concentrations of chemical oxygen demand COD, total phosphorus TP, and total inorganic nitrogen TIN (Kozaki et al., 2016). Urbanization characteristics like impermeable areas and high population density or expansion are frequently studied to determine their correlation with river water quality metrics. Population growth was identified as the primary factor contributing to the decline in river water quality in the Atoyac River in Puebla, Mexico (Estrada-Rivera et al., 2022).

Rural areas typically have predominant land use types such agricultural land, forest land, and vegetation cover, which have been shown to influence the fluctuations in river water quality in prior studies (Li et al., 2022; Mainali & Chang, 2021). The case study conducted in the Maotiao River Basin, China, demonstrated a steady decline in river water quality from the upper to the lower reaches. The inverse correlation between water quality and forested areas suggests that the quality of river water is greatly affected by topography, landscapes, and soil depth. Heavy metals, total nitrogen (TN), total phosphorus (TP), and biochemical oxygen demand (BOD5) were crucial factors influencing the fluctuations in river water quality (Li et al., 2022). Land use considerations were the primary determinants of river water quality in Kuantan, Malaysia. Nitrate NO_3^- levels in the Kuantan River in rural regions exceeded those in the Galing River in urban areas due to residential wastewater (Kozaki et al., 2016).

Identifying the fundamental elements and pollution sources that contribute to the decline in river water quality is crucial for effectively managing river basins to achieve sustainable water management. River water quality is being impacted by both natural and human-induced processes, leading to high pollution levels in numerous river basins globally. This could adversely affect the health of animals, humans, and ecosystems. Land use and urbanization significantly impact river water quality in urban areas, while agriculture land, forest land, climate conditions, and catchment conditions are key factors affecting river water quality in rural rivers (Anh et al., 2023).

2.5 Implications of heavy metals accumulation in sediments and zooplankton on aquatic ecosystem health

Sediments are identified as primary origins of heavy metals in aquatic environments and are crucial in the distribution and settling of these metals. Organisms can chemically transform accumulated heavy metals in sediment into organic compounds, which can become increasingly dangerous to animal and human life through the food chain (Bastami et al., 2014).

Heavy metals are non-biodegradable and have the potential to accumulate in sediments, posing a threat to aquatic organisms (Tang et al., 2008). Trace metals can accumulate in suspended particles and bottom sediments in the water system, leading to long-term contamination (Li et al., 2015). Heavy metal buildup in sediment poses a persistent danger to the components of the natural ecosystem. Heavy metals leached from sediment at the bottom of bodies of water can endanger plant and animal life. If water contaminated with heavy metals is used for irrigation, it can also harm agricultural crops. Research has shown that certain metals that gather in sediment at the bottom of bodies of water can be dangerous, even in little amounts.

It is crucial to study the indicators that describe the quality of soil sediments. These indicators allow for assessing the pollution risk and the toxicity level of heavy metals in the aquatic ecosystem (Ahamad et al., 2020; Jaskuła et al., 2021). Heavy metal accumulation in sediment presents an ongoing risk to the quality of water and other components of the natural environment. Heavy metals, once liberated from silt at the river bottom, can accumulate in plants through irrigation water, posing a threat to river flora and agricultural crops, as well as producing detrimental effects on the fauna (Lee et al., 2019).

Heavy metal water contamination is a significant form of pollution that can impact the biotic community in aquatic ecosystems. Heavy metals are important in ecotoxicology due to their persistence and potential toxicity to living organisms, encompassing both essential and non-essential elements. If the concentration of certain elements exceeds a particular threshold, they become poisonous at bio-available quantities (Udechukwu et al., 2015). Heavy metals are discharged from sediments into water bodies and then transferred to live creatures, depending on the metal's speciation and parameters like sediment pH and organic matter. Overall, they exhibit distinct physical and chemical characteristics regarding chemical reactions, movement, biological accessibility, and possible toxicity (Li et al., 2000).

Evaluating metal concentrations and bioaccumulation in plankton aids in assessing the impact of pollutants on basic trophic levels and the food web (Ho, 2006). Even at low metal concentrations in water, phytoplankton absorbs metals and transfer them to zooplankton and other aquatic organisms in the food web such as fish. Metal bioaccumulation in plankton has a deleterious influence on both the environment and food safety (Chouvelon et al., 2019; Vilhena et al., 2021). Zooplankton play an important role in the transference of energy between primary producers and organisms at the next higher trophic level (Hsiao et al., 2011). Zooplankton are a vital component of aquatic food webs, serving as a primary food source for many fish species. The accumulation of heavy metals in zooplankton can have cascading effects on higher trophic levels, including commercially important fish species, and ultimately, human health. Zooplankton are recognized for their ability to transmit metals to higher trophic level consumers (Calbet et al., 2016). Zooplankton play a crucial role in studying how pollution affects aquatic ecosystems and how disturbances can spread via food chains (Shah et al., 2018). Zooplankton species can transfer metals to higher trophic levels, making them a preferred choice for baseline research in marine environments (Khudhair et al., 2014).

CHAPTER 3
METHODOLOGY

3.1 Study Area

The Malaysian state of Penang is the focus of this study. Penang state has two parts: Penang island, where the capital city, George Town, is located, and the mainland on the Malay Peninsula. These two halves are physically connected by the Penang Bridge and the Second Penang Bridge (Sultan Abdul Halim Muadzam Shah Bridge). A length of land on the west coast of the Malaysian Peninsula and an island (Penang Island) makes up the state of Pulau Pinang or Penang (Seberang Perai). Penang, located on Peninsular Malaysia's northwest coast, is located "between latitudes 5.59° and 5.12°N and longitudes 100.17° and 100.56°E (Facts on Penang, 2022)". The river for this study is Sungai Perai in the state of Penang, Malaysia. The river is at Latitude: 5° 22' 59.99 N and Longitude: 100° 21' 59.99 E (Figure 3.1).

Sungai Perai, is a major river located in Seberang Perai, Penang, Malaysia. It splits Butterworth from Prai to the north and meanders across Seberang Perai's mangrove flatlands before emptying into the channel. Throughout its entire course, the river is not known by that name; it is only 21 kilometres upriver from its estuary at the meeting point of the Sungai Air Hitam, Sungai Kulim, and Sungai Jarak that it is referred to as the Prai River (*Prai River (Sungai Perai)*, 2019). The Muda River provides more than 80% of the state's water (Tan et al., 2019). Water from downstream of the Muda River is abstracted by Penang's sole water delivery business, Perbadanan Bekalan Air Pulau Pinang (PBAPP).

The Perai River has a total basin area of 64.89 km2 and a length of 18.42 km, making it a significant regional water resource. The river is also home to various aquatic species, including fish, crustaceans, and molluscs, which are essential for the local ecosystem and economy. However, the Perai River's aquatic biodiversity and water quality have declined as a result of human activities including industrialization, urbanisation, and agriculture (Feisal et al., 2023). Eight sampling sites were chosen from tributaries that drain downstream to mid-stream of the river around the industrial estate, where the river is exposed to industrial effluents and dumps (Figure 3.1) (Table 3.1).

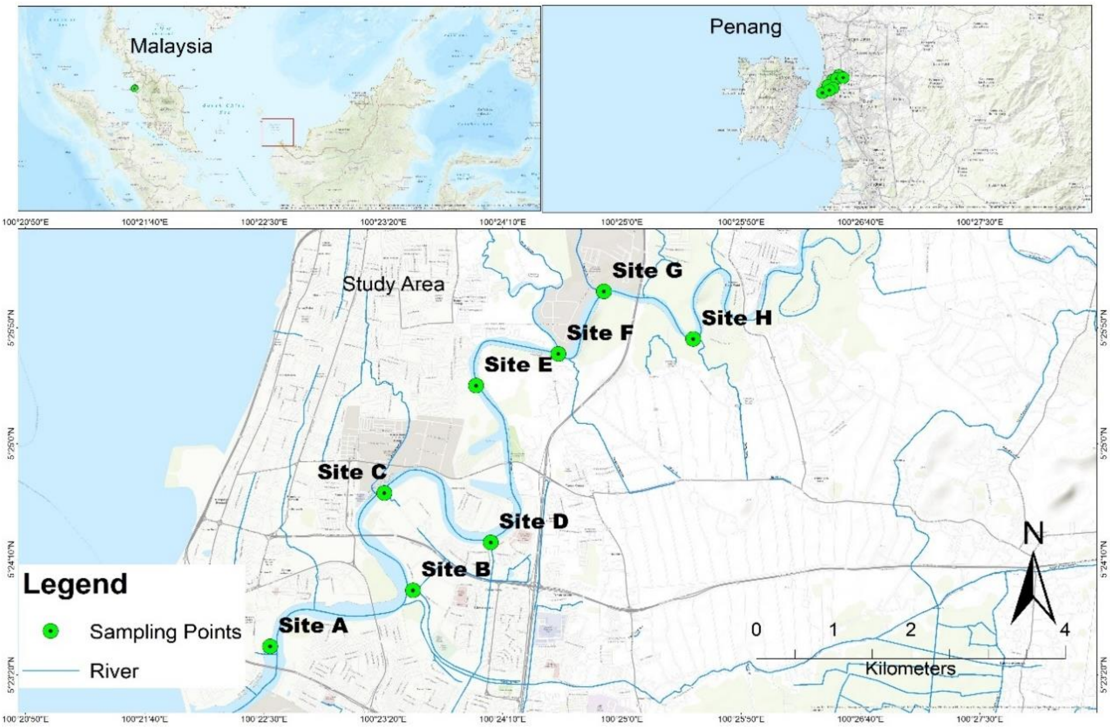

Figure 3.1 Map of Sungai Perai showing sampling sites