HEAVY METAL CONTAMINATION AND OTOLITH SR:CA SIGNATURES AMONG ANGUILLIDS FROM THE ATHI-GALANA-SABAKI AND RAMISI RIVERS, KENYA

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DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been presented in this university or any other for the award of a degree.

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DEDICATION

I dedicate this work to my family for their love and support that have made this journey possible. Your belief in me has been a great motivation, and this thesis is a testament to the values you have instilled in me. This achievement is as much yours as it is mine.

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ABSTRACT

The utilisation of different habitats by catadromous anguillids increasingly exposes them to threats such as pollution in freshwater and marine environments. The yellow eel is especially vulnerable to pollutant bioaccumulation as a result of its benthic predatory behaviour and high-fat reserves. However, further investigation is needed to understand whether contamination levels depend on the species of anguillids and stage-specific habitat use. This study examined heavy metal contamination and habitat use using otolith Sr:Ca signatures among anguillids from Rivers Athi-Galana-Sabaki and Ramisi, Kenya, with varying contaminant sources and anthropogenic disturbance levels. Heavy metal (As, Cd, and Pb) and trace metal (Cr, Cu, Zn, Fe, Co, Ni, and Mn) concentrations in eel tissues, sediment, and water, along with otolith strontium:calcium (Sr:Ca) ratios, were measured using an Inductively Coupled Plasma Optical Emission Spectroscopy. Results showed that arsenic (4.45±2.88 mg/kg) and cadmium (0.88±0.61 mg/kg) concentrations were significantly higher in silver eel tissues ($p \leq 0.05$) than in yellow eels, elvers, and glass eels. This suggests accumulation of heavy metals during the feeding stage (yellow eel), which involves long residency in rivers that are often polluted. Additionally, arsenic (As), lead (Pb), and cadmium (Cd) concentrations in all eel life stages exceeded the FAO/WHO maximum permissible limits in freshwater fishes. Significant differences were observed in muscle and liver heavy and trace metal concentrations among the four anguillid species ($p \leq 0.05$). Significant differences were also observed in the mean concentrations of heavy and trace metals in water and sediment between the Athi-Galana-Sabaki and Ramisi rivers ($p \leq 0.05$). Results revealed the presence of As (0.184±0.006 mg/L) and Cd (0.035±0.002 mg/L) in water, as well as Pb (14.032±5.662 mg/kg), As (4.342±0.241 mg/kg), and Cd (0.687±0.039 mg/kg) in sediment from River Athi-Galana-Sabaki. As (0.192±0.009 mg/L), Cd (0.033±0.002 mg/L), and Pb (0.003±0.003 mg/L) were present in water from River Ramisi. The mean As, Pb, and Cd concentrations in sediment from River Ramisi were 4.64±0.173, 3.04±2.095, and 0.809±0.045 mg/kg, respectively. Notably, As, Cd, Ni, and Mn levels in water from both rivers were above the WHO-recommended limits for drinking water. Comparison among species indicated that otolith Sr:Ca signatures ranged from 0.023 in *A. marmorata* to 0.182±0.044 in *A. bicolor*, with no significant difference ($p \geq 0.05$). There was no significant association between heavy metal loading in muscle tissues and otolith Sr:Ca signatures among silver and yellow eels ($p \geq 0.05$). Results indicate the presence of As, Cd, and Pb in eels above FAO/WHO permissible limits in fish, which raise concerns for public health and aquatic ecosystems.

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
CTR	Copper Transporter
DMT	Divalent metal transporter
DNA	Deoxyribonucleic Acid
GSH	Glutathione
HNO_3	Nitric Acid
ICES	International Credential Evaluation Service
ICP-OES	Inductively Coupled Plasma-Optical Emission Spectrometry
IRE	Iron Regulatory Proteins
IRP	Iron Responsive Elements
mRNA	Messenger Ribonucleic Acid
NEMA	National Environment Management Authority
ROS	Reactive Oxygen Species
SOD	Superoxide Dismutase
Tf	Transferrin
TGN	Trans-Golgi Network
UNEP	United Nations Environment Programme
WHO	World Health Organisation
WIO	Western Indian Ocean
WIOMSA	Western Indian Ocean Marine Science Association

CHAPTER ONE
INTRODUCTION

1.1 Background

Catadromous anguillids are migratory fish that grow and mature in freshwater and estuarine habitats, before the return migration to tropical oceans as adults to reproduce (Miller *et al.,* 2019). Globally, nineteen anguillid species and subspecies are distributed across landmasses bordering major tropical ocean gyres, with the exception of the South Atlantic and Eastern Pacific (Righton *et al.,* 2012). In the Western Indian Ocean (WIO), four species: *Anguilla bengalensis* (Gray, 1831), *A. bicolor* (McClelland, 1844), *A. marmorata* (Quoy & Gaimard, 1824), and *A. mossambica* (Peters, 1852) occur in virtually all eastward-flowing continental rivers and WIO island rivers, but remain poorly studied. The coast of East Africa and the Mascarene Islands host *A. mossambica* and *A. bengalensis*, whereas *A. bicolor* ranges widely across the Indian Ocean, from East Africa to western Indonesia (Arai *et al.,* 2001). *A. marmorata* is a broadly distributed eel species, occurring from the South African coast to the Japanese archipelago and Polynesia (Arai *et al.,* 2001).

These four WIO anguillid species represent one of the greatest eel diversities in the world (Schabetsberger *et al.,* 2016). By utilising marine and freshwater habitats throughout their life cycle, anguillids are exposed to multiple threats in both ecosystems, acting as the ecological indicators of their well-being (Schabetsberger *et al.,* 2016). Additionally, anguillids contribute to subsistence fishery, and thus support the livelihoods of local communities throughout the WIO region (Shanmughan *et al.,* 2022).

Dekker (2016) reported a decline in Europe, North America, Japan, and New Zealand eel populations associated with multiple anthropogenic stressors, including water pollution, hydropower generation, and land reclamation. Decreasing quality of freshwater, estuarine, and coastal habitats as a result of pollution from agricultural run-off, wastewater, and disposal of raw sewage, as well as the loss of biodiversity, has raised global concern (Davidson, 2014).

Agriculture, manufacturing, mining, and transportation (Sardar *et al.,* 2013), as well as geochemical processes such as weathering, are responsible for heavy metal pollution in water bodies (Li *et al.,* 2007), with detrimental consequences to organisms, including eels (Langston *et al.,* 2002). Govind and Madhuri (2014) reported a decrease in fitness and interference with fish reproduction, resulting from heavy metal exposure, leading to carcinoma and death. In addition, reduced androgenic and estrogenic secretion as well as pathological changes in fish have been associated with exposure to heavy metals (Ebrahimi & Taherianfard, 2011). Geeraerts and Belpaire (2010) reported disruption of the endocrine system, reproduction, and immune system in eels as a result of heavy metal exposure.

Pollution impairs the ability of eels to build up energy reserves, swim, produce viable oocytes, and reproduce (Belpaire *et al.,* 2019). Similar threats impact WIO anguillids, as population decline has been reported in Réunion Island (Valade *et al.,* 2018). Pike *et al.* (2020) reported that *Anguilla bengalensis, A. bicolor,* and *A. mossambica*, which inhabit the Indian and Pacific Oceans, are classified as Near Threatened, particularly due to the constriction of their distribution ranges.

In rivers, anguillids are important top predators and feed on crabs, shrimps, and smaller fish (James & Suzumoto, 2006). They are vulnerable to heavy metal pollution because of their long residence (extended exposure), especially during their yellow eel phase, ranging from three to eighteen years (Tabouret *et al.,* 2011), and bioaccumulation (being apex predators), and are thus suitable indicators of aquatic ecosystem well-being (Hanzen *et al.,* 2020). The yellow eel, a sedentary feeding stage, (Tesch, 2003), is regarded as prone to pollutant bioaccumulation as a result of its benthic lifestyle and rich lipid content (Roche *et al.,* 2003).

Subsequently, metabolization of fat reserves among non-feeding, migrating silver and glass eels (McKeown, 1984) results in increased bioavailability of sequestered pollutants that impact migration and ensuing breeding, spawning and recruitment success. Le *et al.* (2010) reported twofold higher concentrations of copper, zinc and manganese in silver eels than yellow eels, while Celino *et al.* (2009) associated high arsenic concentrations (100 mM) with *in vitro* testis degeneration and germ cell mortality in *Anguilla japonica*.

Maes *et al.* (2005) observed that exposure of adult and juvenile eels to varying cadmium and lead loads resulted in a significant decline of eel condition indices and lipid content, suggesting an impact on fitness. The Nairobi River, draining into the Athi River basin, was reported as the most polluted river in Kenya with heavy metal concentrations exceeding the recommended limits by the World Health Organisation (WHO) for drinking water (UNEP/Nairobi and WIOMSA, 2009). Njuguna *et al.* (2017) reported sewage, industrial and household waste, and natural occurrences as sources of heavy metal pollution in the Nairobi River. A study of tributaries of the Athi-Galana-Sabaki River revealed the presence of cadmium (0.004 mg/L), with levels surpassing the recommended limit (0.003 mg/L) by the WHO for drinking water (Muiruri *et al.,* 2013). River Ramisi, which flows adjacent to the Kwale International Sugar Company Limited (KISCOL), formerly known as the Ramisi Sugar Factory (Oteko, 1986), is known to be saline and highly mineralised (Chalala *et al.,* 2017). Ochieng *et al.* (2009) reported cadmium (0.008 mg/L) and lead (0.034 mg/L) concentrations from River Ramisi that also exceeded WHO guidelines for drinking water.

Sediment in rivers acts as both a carrier and a reservoir for pollutants such as heavy metals and is crucial for monitoring pollution (Asefa & Beranu, 2016). According to Suresh *et al.* (2012), the substate and suspended sediment composition, as well as water chemical makeup, determine metal presence and characteristics in the water column. In addition, physicochemical characteristics of a river affect heavy metal adsorption onto suspended particulate matter, which may subsequently settle into the sediments through gravitational deposition (Lafabrie *et al.,* 2007). Disturbances in physicochemical balance or hydrodynamic conditions can remobilise metals from sediments back into the water column, leading to secondary contamination (Hill *et al.,* 2013).

Fish species show different contamination profiles as a result of variations in feeding behaviour, diet, preference of habitat, genome, physiology, duration of exposure, and the quality of neighbouring water (Velusamy *et al.,* 2014). Tolerance to physicochemical stress (Tesch, 1977) and limited depuration capacity (Larsson *et al.,* 1991) have been reported in eels, allowing them to accumulate pollutants and act as indicators of environmental quality.

Consequently, Le *et al.* (2010) suggested that when using eels as bioindicators of aquatic pollution, life history analysis through otolith Sr:Ca ratios is essential for accurately distinguishing pollutant loads across life stages. Many anguillid eel species exhibit facultative catadromy, where leptocephali, after transforming into glass eels, leave ocean currents and enter freshwater habitats to develop into elvers (Arai *et al.,* 2001). The elvers grow into yellow eels prior to journeying back to the ocean to reproduce and die (Arai & Kadir, 2017). In freshwater, the European eel (*Anguilla anguilla)* occupies diverse river and lake habitats (Benchetrit *et al.,* 2017). However, information on eel habitat utilisation in tropical regions, particularly the Western Indian Ocean (WIO), remains limited.

Otolith microchemical analysis compares the concentration of selected elements such as calcium, barium, and strontium, as environmental signatures (for instance, in water) and is a useful tool to reveal fish habitat use (Halden & Friedrich, 2008). Tracing fish movements across freshwater, estuarine, and marine environments has relied on the otolith strontium-to-calcium (Sr:Ca) ratio, which rises with increasing salinity (Tabouret *et al.,* 2010). Arai and Chino (2017) demonstrated this relationship in Vietnam, where Sr:Ca ratios exceeding 6×10^{-3} were recorded in oceanic leptocephali and early glass eels; however, comparable studies on eels from the Western Indian Ocean (WIO) are still lacking.

This study, therefore, aimed to assess anguillid heavy metal contamination and its associated impact on habitat use (otolith Sr:Ca signatures) along two east-flowing Kenyan rivers with differing anthropogenic stressors, namely River Athi-Galana-Sabaki (relatively disturbed) and River Ramisi (relatively pristine). The findings reveal alarming levels of heavy metals in eels, highlighting risks to public health and aquatic environments. To curb pollution, extensive monitoring and management strategies are needed.

Further study on the long-term effects of heavy metal contamination on eels as well as related habitats is central to informed decision-making and effective conservation. To conserve aquatic biodiversity and ensure the sustainability of natural resources, scientists, policymakers, industries, and local communities must work together to address pollution.

1.2 Statement of the Problem

Deterioration and loss of freshwater habitats as a result of pollution, among other factors, have been associated with the decline in temperate and subtropical eel populations. Similar decline is reported in Western Indian Ocean (WIO) eels amidst rising heavy metal loading into rivers; however, the association with heavy metal contamination in eels remains unknown. Furthermore, the prospect of human contamination through the consumption of WIO eels by fishers and their families remains a looming danger. Variations in contamination levels have been shown to differ among eel life stages, which are linked to ontogenic shifts in feeding habits and habitat use; however, such information is lacking for Kenyan and WIO eels. In addition to being apex predators, utilisation of marine, brackish, and freshwater environments at different life stages exposes eels to varying levels of pollutants, including heavy metals, that impact fitness and health due to their carcinogenic, mutagenic, and teratogenic potential. Otolith Sr:Ca signatures have been employed to explore habitat use strategies of *Anguilla* species, for instance, *A. anguilla* and *A. rostrata*, revealing that some individuals bypass freshwater residence and instead mature in estuarine and marine environments. However, similar information on WIO eels (*A. bengalensis*, *A. marmorata*, *A. bicolor*, and *A. mossambica)* is lacking and thus needs to be determined. Ideally, unpolluted freshwater habitats in the Western Indian Ocean (WIO) region support healthy eel populations with well-understood life histories and habitat use patterns. Sustainable fisheries management and conservation strategies are informed by routine research, employing otolith Sr:Ca ratio analysis. In addition, comprehensive monitoring guarantees minimal heavy metal contamination, thus protecting ecosystem health and food safety.

1.3 Objectives of the Study

1.3.1 General Objective

To determine the impact of heavy metal contamination on habitat use (otolith Sr:Ca signatures) among eel species and life stages along Kenyan east-flowing rivers.

1.3.2 Specific Objectives

i. To evaluate the contamination by selected heavy metals among anguillid tissues from rivers with differing pollution sources: River Athi-Galana-Sabaki (human impact) and River Ramisi (environmental impact).

ii. To assess habitat use patterns of anguillid species and their life stages along the River Athi-Galana-Sabaki and River Ramisi using otolith Sr:Ca signatures.
iii. To determine the association between heavy metal loading and otolith Sr:Ca signatures (habitat use) among anguillids from two rivers, River Athi-Galana-Sabaki and River Ramisi, with differing pollution sources.

1.4 Research Hypotheses

H_o1: There is no significant difference in heavy metal loads among anguillid species and rivers.

H_o2: There is no significant difference in otolith Sr:Ca signatures among anguillid species.

H_o3: There is no significant association between heavy metal loading and otolith Sr:Ca signatures among anguillid species and rivers.

1.5 Justification

Anguillids are a vital part of the human diet, offering a valued source of protein and essential nutrients such as omega-3s, calcium, and vitamins. To build up the energy reserves needed for downstream migration as silver eels to spawning grounds, yellow eels inhabit freshwater habitats, a stage during which access to suitable habitats and sufficient food is essential. However, they are prone to the accumulation of heavy metals, which impacts their health and fitness, as well as poses health risks to human consumers. Otolith Sr:Ca ratios have been used to study the life history of migratory fish species, including anguillid eels. Understanding the association between the life history, survival, and growth of anguillid eels is key to planning the rehabilitation of their vulnerable habitats.

This study aims to contribute to scientific knowledge on heavy metal contamination and otolith Sr:Ca signatures among anguillid eels in Kenya, and identify eel life stages as well as species most at risk from pollution. This information is crucial to developing effective conservation strategies in order to safeguard key eel habitats and mitigate anthropogenic activities that contribute to pollution. To ensure safe consumption, it is important to monitor and control heavy metal levels in fish.

The study also provides information on the quality of water from the Athi-Galana-Sabaki and Ramisi rivers and the suitability of the water for domestic use. This information is vital to establishing comprehensive measures aimed at mitigating heavy metal contamination and conserving the anguillid eel resource in Kenya. In addition, determining the relationship between heavy metal loading and otolith Sr:Ca signatures (habitat use) in anguillid eels is essential in identifying connections between eel habitat preferences and heavy metal bioaccumulation patterns, in an effort to predict bioaccumulation trends.

CHAPTER TWO
LITERATURE REVIEW

2.1 Bioecology of WIO Anguillids

Anguillid eels undergo six life stages, namely egg, leptocephalus, glass eel, elver, yellow, and silver, as shown in Figure 1 (Cresci, 2020). Subtropical and tropical oceanic gyres carry the larvae to continental slopes, where they mature into glass eels. The glass eels leave the oceanic currents and enter estuaries (Righton & Metcalfe, 2011) where they grow into elvers and dwell in rivers, lakes, and estuaries as yellow eels (Cresci, 2020).

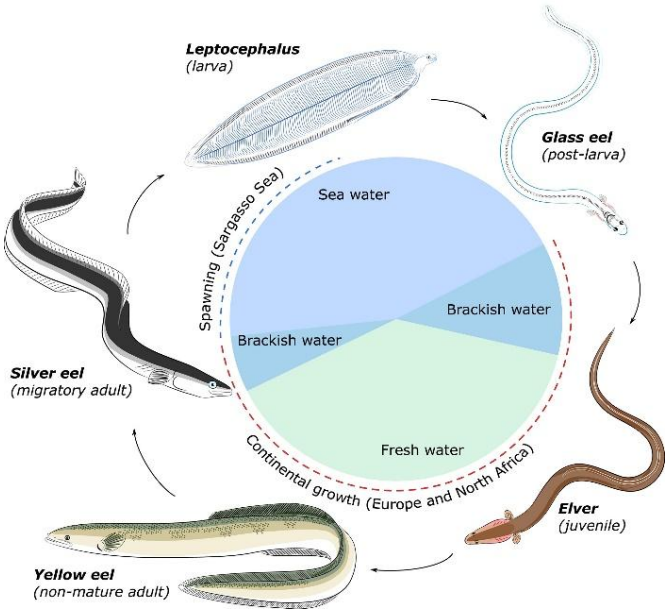


Figure 1: Life history of the European eel *(Anguilla anguilla)*

Source: Cresci (2020)

Upon maturation, following three to fifteen (3-15) years of residence in rivers, yellow eels cease feeding, transform into silver eels, and undergo the maturation of gonads. By metabolising the accumulated fat reserves, they start downstream migration to offshore spawning areas, where they reproduce before dying (Cresci, 2020).

The European eel (*A. anguilla*) and the American eel (*A. rostrata*) spawn in the Sargasso Sea, whereas *A. marmorata* and *A. mossambica* are believed to spawn in the southwestern Indian Ocean, northeast of the Mascarene Ridge between Madagascar and the Mascarene Archipelago (Réveillac *et al.,* 2009; Miller *et al.,* 2019).

On the other hand, a spawning ground for *A. bicolor* has been proposed near the Mentawai Trench off the coast of Sumatra, Indonesia (Jespersen, 1942). The Western Indian Ocean is home to four anguillid species, namely *Anguilla bengalensis, Anguilla bicolor, Anguilla mossambica,* and *Anguilla marmorata*. Van Someren and Whitehead (1959) and later Okeyo (1998) described the presence of eels from the Athi and Tana Rivers. Okeyo (1998) reported the occurrence of *A. bicolor, A. bengalensis,* and *A. mossambica* in the River Athi-Galana-Sabaki.

The catadromous life cycle of anguillid eels exposes them to pollutants at multiple life stages, from early development to adulthood (Bruslé, 1991), making them particularly vulnerable compared to many other fish species. According to Martínez-Gómez *et al*. (2023), eels tend to accumulate larger amounts of environmental chemical contaminants than other fish species due to their physiological and ecological characteristics, namely long life cycle, rich lipid content, benthic feeding, and semelparous reproductive strategy (Steendam *et al.,* 2020). Silver eels cease feeding throughout their migration during which lipid reserves are metabolised, leading to mobilisation and translocation of toxic bioaccumulated contaminants (Sühring *et al.,* 2015).

Heavy metal pollution poses a serious risk to aquatic ecosystems and organisms, including fish (Biswas *et al.,* 2021). Muiruri *et al.* (2013) reported lead (1.42-4.48 mg/kg) and cadmium (0.71-1.77 mg/kg) presence in gills of *Oreochromis niloticus* from Mbagathi and Ruiru rivers, tributaries of the Athi-Galana-Sabaki River. A study conducted in two Spaniard coastal rivers detected cadmium (0.013 mg/kg) and lead (0.023 mg/kg) in *Anguilla anguilla* muscle tissue (Linde *et al.,* 2004). However, studies on heavy metal contamination in top predatory fish such as eels are lacking in Kenya.

Anguillids are a well-appreciated fish in Europe (Drouineau *et al.,* 2018), Asia (Yadav *et al.,* 2020), America (MacGregor *et al.,* 2008), and New Zealand (Jellyman, 2007). They provide highly valued meat, rope, bandage, and eel oil for medicinal (colds) and spiritual (ward off evil spirits) purposes (Macgregor *et al.,* 2008). In Europe, America, and Japan, all eel stages are exploited, with a preference for silver eels due to their higher fat content, whereas glass eels are consumed as appetisers and used to stock aquaculture ponds (Shiraishi and Crook, 2015).

In addition to being consumed and used in several traditional healing rites, eels hold significant social importance for the local populations in the transboundary Incomati River catchment (WIOMSA, 2021). Moreover, they are an important fishery resource to local river fishing communities such as the Kamba (44%), Giriama (21.5%), and Digo (13.5%) in Kenya (Kihia, 2022).

2.2 Otoliths and their Use in Habitat Use Evaluation

Otoliths are calcified, metabolically inert structures consisting of successive aragonite crystal layers deposited on a protein matrix, with each layer representing a daily growth increment in fish (Campana & Thorrold, 2001). The amount of organic matter in each zone determines otolith appearance, which varies from opaque to hyaline (Van Neer *et al.,* 2002). Opaque zones form during rapid growth, while hyaline zones appear during slower growth stages (Rodríguez, 2006).

Furthermore, even when eels are starved, otoliths do not resorb (Campana & Thorrold, 2001). These characteristics make otoliths crucial for studies on fish larval ecology, identification of species, and reconstruction of fish habitat utilisation (Campana, 2005). The distinct daily increments allow accurate and reliable estimation of age for larval and juvenile fish (Tanabe *et al.,* 2003). Otolith annuli provide macroscopical patterns used to determine age and increment periodicity by counting growth bands formed during the life of a fish (Massutí *et al.,* 2000).

The chemistry of the surrounding water influences the composition of elements in otoliths, with temperature having a secondary effect (Ranaldi & Gagnon, 2008). Strontium (Sr) is a suitable tracer for fish movement and habitat use studies due to its sensitivity to environmental conditions (Avigliano & Volpedo, 2013). Secor and Rooker (2000) reported changes in otolith Sr:Ca ratios depending on duration spent in freshwater versus seawater, a pattern also seen in anguillid eels (Campana, 1999; Jessop *et al.,* 2002).

Tsukamoto *et al.* (1998) revealed that *A. anguilla* and *A. japonica* exhibit marine residence with the use of otolith microchemistry. Similarly, *A. anguilla*, *A. japonica*, and *A. rostrata* have been found to reside in estuaries, displaying intermediate otolith signatures between those of marine and freshwater residents (Tzeng *et al.,* 2000; Jessop *et al.,* 2002; Arai *et al.,* 2003). This suggests that eels frequently shift between environments as they grow (Jessop *et al.,* 2002). However, some tropical anguillid species exhibit facultative catadromy, as individuals have been seen to remain in estuarine or marine environments (Tsukamoto & Arai, 2001).

Elsdon and Gillanders (2003) showed a strong positive correlation between Sr:Ca ratios of otolith and those of ambient water, while Zimmerman (2005) showed a positive relationship between water salinity and otolith Sr:Ca ratio. However, Kraus and Secor (2004) observed that freshwater Sr:Ca values may exceed marine levels under conditions of elevated salinity, indicating that salinity does not always show a positive correlation with Sr:Ca levels in water or otoliths, particularly in areas characterised by moderate to high salinity (Dorval *et al.,* 2007). Furthermore, Sr:Ca ratios in both water and otoliths have been shown to vary with temperature (Martin *et al.,* 2004) and through interactions between temperature and salinity (Martin & Wuenschel, 2006). Tzeng *et al.* (2002) reported the highest ratio, ranging between 4.0 and 6.2 (10^{-3}) among leptocephali and glass eels, but it was below values among river-dwelling life stages such as elvers and yellow eels.

Sr:Ca ratios in otoliths have been examined in three northern temperate species; however, comparable research on tropical species from the Western Indian Ocean (WIO) is lacking. Van Someren and Whitehead (1959) noted differences in habitat use among anguillid species found in the Athi and Tana rivers, where the *A. bicolor* was restricted to lower reaches and *A. bengalensis* to >1000m above sea level.

2.3 Heavy Metals and Pollution in Kenyan Rivers

Heavy metals are elements with an atomic number above 20 and a density greater than 5 g/cm³, such as cadmium, mercury, and silver (Raychaudhuri *et al.,* 2021). This group also includes toxic metalloids like cadmium, arsenic, and lead, which are harmful even at low concentrations, as well as trace metals such as copper, zinc, manganese, cobalt, nickel, and magnesium that are essential in small amounts for metabolic functions but become toxic beyond certain thresholds (Jaishankar *et al.,* 2014). Metalloids such as arsenic display metal-like characteristics (Raychaudhuri *et al.,* 2021) and occur naturally in the Earth's crust (He *et al.,* 2005).

Aquatic environments face degradation from domestic waste, industrial effluent, and agricultural run-off (Ndimele, 2008). Liquid waste from industries and farms discharged into water bodies often contains cadmium and lead salts (Qiao *et al.,* 2007). This raises concerns over heavy metal bioaccumulation in food chains and the risks to human health (Agah *et al.,* 2009). Fish absorb heavy metals through their skin, gills, and food since they are heterotrophs in the aquatic food chain (Zhao *et al.,* 2012).

Fish play a critical role in nutrition and food security and can thus be used to monitor heavy metal contamination in water and resident biota (Senarathne & Pathiratne, 2007). In addition to water monitoring, studying heavy metal pollution in sediment can provide information on pollution in an aquatic ecosystem and its impact within the catchment (Akele *et al.,* 2016). Heavy metal availability in water is influenced by the mineral content of the sediment, low solubility of metals in water, adsorption, and association of metals with the sediment, in turn making sediment a source or sink (Akele *et al.,* 2016).

Upon entering rivers, heavy metals settle into sediments, where their concentrations are higher than in the water column (Liu *et al.,* 2018). The accumulation of these metals in sediments impacts benthic fauna and spreads effects throughout the food web (Fu *et al.,* 2014). The Athi-Galana-Sabaki River is the second-largest basin in Kenya after the Tana River. It collects water from the eastern slopes of the Rift Valley, the Aberdare ranges, and the Ngong Hills, and flows into the Indian Ocean through semi-arid regions such as Machakos County. The river drains urban areas, including Nairobi and Machakos in the upper catchment and Malindi near its mouth along its course.

The Athi basin flows through areas of urban settlement of 202 persons.km^{-2}, industrial, and agricultural activities that contribute enormous amounts of pollutants, such as heavy metals, resulting in decreased water quality (Muiruri *et al.,* 2013). Kinyua and Pacini (1991) reported increasing levels of heavy metals resulting from industrial effluents. The concentrations of lead and cadmium in the River Athi-Galana-Sabaki exceeded the World Health Organisation's recommended limits for drinking water (Muiruri *et al.,* 2013).

River Ramisi is a semi-perennial river that originates from the Shimba Hills, flows through the Kiwamba mangroves at Shimoni and pours into the Indian Ocean (Wara *et al.,* 2019). It is saline, highly mineralised, and unsuitable for agricultural and industrial use (Earthview Geoconsultants, 2006), and is affected by the intrusion of seawater during high tides. River Ramisi is a semi-perennial river that originates from the Shimba Hills, flows through the Kiwamba mangroves at Shimoni and pours into the Indian Ocean (Wara *et al.,* 2019). It is saline, highly mineralised, and unsuitable for agricultural and industrial use (Earthview Geoconsultants, 2006), and is affected by the intrusion of seawater during high tides.

Mkanda stream is the only freshwater tributary of the Ramisi River, while the other streams, such as Lovu, Maji Moto, Mkundi, and Chorochoro, originate from areas of active volcanic activity (Earthview Geoconsultants, 2006). The Ramisi catchment is occupied by a sparse human population of not more than 80 persons.km^{-2} dominated by smallholder subsistence farmers (KNBS, 2019).

The Water Quality Regulations - Part II, Water Act (2016) - Section 144, and Environmental Management and Co-ordination Act (2012) - Section 72 describe regulations on water for industrial use and effluent discharge; discharge into aquatic environment, provisions relating to water resources conservation and protection and water pollution control, and prohibition of water pollution define existing laws established to help mitigate pollution of water bodies. Additionally, they outline punishment(s) that a person would be liable to if found guilty of discharging pollutants into the aquatic environment, which include a term not exceeding two years and/or a fine not exceeding one million Kenyan shillings.

2.4 Mechanism of Heavy Metal and Trace Metal Cellular Uptake

Approximately one-third of all proteins require metal ions, and nearly 41% of enzymes depend on metals at their catalytic centers (Waldron *et al.,* 2009). Metalloenzymes constitute about 44% of oxidoreductases, 40% of transferases, and 39% of hydrolases; to acquire these essential nutrients, cells have evolved specific mechanisms for metal uptake from the extracellular environment (Waldron *et al.,* 2009). According to Martinez-Finley *et al.* (2012), several transporters for essential trace metals are hijacked by toxic heavy metals such as arsenic (As), cadmium (Cd), and lead (Pb). Several metal ions can pass through the cell membrane alone or in complex with other proteins (Martinez-Finley *et al.,* 2012).

Heavy metals exploit several pathways to enter cells, including ion pumps, calcium channels, anion transporters, and amino acid or organic anion transporters, particularly when metals are bound to amino acids or organic ligands (Martinez-Finley *et al.,* 2012). An increased body burden occurs when metal uptake and accumulation rate surpass detoxification capacity, disrupting the uptake-efflux balance (Martinez & Aschner, 2011).

2.4.1 Heavy Metal Cellular Uptake

2.4.1.1 Arsenic (As)

The uptake of inorganic arsenite [As(III)] is aided by aquaglyceroporins (AQPs), which transport small uncharged molecules such as glycerol, urea, and water (Liu, 2010). Yoshino *et al.* (2011) showed that AQP9 transports As(III) and monomethylarsonous acid in the liver and astrocytes. AQP7 facilitates the uptake of As(III) in the kidney, testis, and adipose tissue (Kageyama *et al.,* 2001).

Phosphate transporters are associated with the uptake of inorganic arsenate [As(V)], a structural analogue of phosphate (Persson *et al.,* 1999). Additionally, glucose transporter isoform 1 (GLUT1), expressed in erythrocytes and epithelial cells of the blood-brain barrier, plays a role in the uptake of As(III) and monomethylarsonous acid (Liu *et al.,* 2006).

2.4.1.2 Cadmium (Cd)

Iron levels influence cadmium uptake (Martinez-Finley *et al.,* 2012), and divalent metal transporter 1 (DMT1) is key in the transportation of free Cd ions (Bannon *et al.,* 2003). Cadmium bound to protein in hepatocytes can be absorbed by endocytosis (Bressler *et al.,* 2004). In addition, cadmium and lead can displace metals such as copper and zinc from metallothioneins, proteins that serve as intracellular reservoirs for these elements, by cadmium and lead (Martinez-Finley *et al.,* 2012). Cadmium hinders calcium transport through competitive binding and selective inhibition of Ca^{2+}-ATPase transporters (Niyogi *et al.,* 2008). Although voltage-gated L-type calcium channels allow cadmium to enter cells, its absorption is slower than that of calcium (Klinck & Wood, 2011).

Cadmium can replace Zn^{2+} in metallothionein due to its similar oxidation state, reducing its antioxidant functions and increasing oxidative stress within the cell (Jaishankar *et al.,* 2014). ZIP family transporters, particularly ZIP8 and ZIP14 (Zn^{2+}/HCO_3^- symporters), also facilitate cadmium uptake in mammalian cells (Liu *et al.,* 2008). Cadmium often forms conjugates with thiol-containing molecules such as cysteine and glutathione. These complexes can be carried by organic cation/anion or amino acid transporters (Zalups & Ahmad, 2003).

2.4.1.3 Lead (Pb)

Lead is transported across the plasma membrane via calcium and iron transport mechanisms (Martinez-Finley *et al.,* 2012). It is absorbed by red blood cells in the bloodstream through anion exchange and Ca^{2+} uptake channels, where it mainly binds to δ-aminolevulinic acid dehydratase (Bannon *et al.,* 2000). Additionally, lead can enter cells through L-type Ca^{2+} channels (Tomsig and Suszkiw, 1999) and the divalent metal transporter 1 (DMT1) (Gu *et al.,* 2009). Lead is transported intracellularly by calbindin, a calcium-binding protein (Haberman *et al.,* 1983).

The ability of lead to mimic and replace ions such as Mg^{2+}, Ca^{2+}, Fe^{2+}, and Na^{+} influences its toxicity (Generalova *et al.,* 2025). This ionic substitution disrupts processes in the cell and deters normal metabolic functions (Jaishankar *et al.,* 2014).

2.4.2 Trace Metal Cellular Uptake

2.4.2.1 Copper (Cu)

Uptake of copper in the intestine is facilitated by copper transporter 1 (CTR1); however, the divalent metal transporter 1 (DMT1) can transport copper under certain conditions (Espinoza *et al.,* 2011). CTR1, a ubiquitously expressed high-affinity transporter, is localised to the plasma membrane and functions as a homotrimer to facilitate copper entry into the cytosol, including transport from endosomes (Martinez-Finley *et al.,* 2012). Copper binding in CTR1 is facilitated by an extracellular N-terminal region rich in methionine and histidine (De Feo *et al.,* 2007).

Besides CTR1, a related low-affinity transporter, CTR2, shares homology with CTR1 and may also facilitate iron uptake (Martinez-Finley *et al.,* 2012). Unlike CTR1, CTR2 is found mainly in intracellular compartments, with only limited expression at the plasma membrane, and is thought to mobilise copper from lysosomes or other vesicular stores (Bertinato *et al.,* 2008; Martinez-Finley *et al.,* 2012).

Within cells, copper is tightly regulated by binding proteins and chaperones that prevent toxic accumulation. Unbound copper can catalyse the production of reactive oxygen species (ROS) through Fenton chemistry and displace other metals from proteins (Martinez-Finley *et al.,* 2012). To avoid this, copper trafficking occurs along affinity gradients established by copper-binding molecules (Banci *et al.,* 2010).

Metallothioneins and glutathione (GSH) buffer cytosolic copper and act as storage pools, although metallothioneins cannot directly deliver copper to cuproproteins. Instead, specific chaperones mediate targeted delivery: Ccs1 provides copper to superoxide dismutase 1 (SOD1) (Schmidt *et al.,* 2000); Cox17, Cox11, and Sco1/2 deliver copper to cytochrome c oxidase (Robinson & Winge, 2010); and Atox1 functions as a multifunctional chaperone, transferring copper to the exporters ATP7A and ATP7B, while also regulating copper protein expression and localization (Lutsenko *et al.,* 2010).

Copper efflux and distribution are mediated by the P-type ATPases ATP7A and ATP7B. Under low copper conditions, both transporters localise to the trans-Golgi network (TGN) to supply copper for cuproprotein biosynthesis. On the other hand, too much copper triggers the relocation of the transporters to vesicles near the plasma membrane, allowing excretion of copper via exocytosis (Martinez-Finley *et al.,* 2012). In enterocytes, hepatocytes, among other polarised cells, ATP7A is located at the basolateral membrane, while ATP7B is found on the apical membrane, showing their specific roles in directional copper transport (Linz & Lutsenko, 2007).

Besides CTR1, CTR2 is homologous to CTR1 and facilitates the uptake of iron (Martinez-Finley *et al.,* 2012). CTR2 is located in intracellular compartments, with partial expression at the plasma membrane, and mobilises copper from lysosomes or other vesicular stores (Bertinato *et al.,* 2008; Martinez-Finley *et al.,* 2012).

Within cells, copper is tightly regulated by binding proteins and chaperones that prevent toxic accumulation. Unbound copper can catalyse the production of reactive oxygen species (ROS) through Fenton chemistry and displace other metals from proteins (Martinez-Finley *et al.,* 2012). To avoid this, copper trafficking occurs along affinity gradients established by copper-binding molecules (Banci *et al.,* 2010). Metallothioneins and glutathione (GSH) buffer cytosolic copper and act as storage pools, although metallothioneins cannot directly transport copper to cuproproteins. Instead, specific chaperones mediate targeted delivery: Ccs1 provides copper to superoxide dismutase 1 (SOD1) (Schmidt *et al.,* 2000); Cox17, Cox11, and Sco1/2 deliver copper to cytochrome c oxidase (Robinson & Winge, 2010); and Atox1 functions as a multifunctional chaperone, transferring copper to the exporters ATP7A and ATP7B, while also regulating copper protein expression and localization (Lutsenko *et al.,* 2010).

Copper efflux and distribution are mediated by the P-type ATPases ATP7A and ATP7B. Under low copper conditions, both transporters localise to the trans-Golgi network (TGN) to supply copper for cuproprotein biosynthesis. In contrast, excess copper triggers their relocation to vesicles near the plasma membrane, enabling copper excretion via exocytosis (Martinez-Finley *et al.,* 2012). In polarised cells, such as enterocytes and hepatocytes, ATP7A is positioned at the basolateral membrane, while ATP7B is localised at the apical membrane, reflecting their specialised roles in directional copper transport (Linz & Lutsenko, 2007).

2.4.2.2 Iron (Fe)

Iron is a component of haemoglobin, which transports oxygen in the blood, and once absorbed, homeostasis is controlled at uptake, storage, and intracellular distribution stages (Martinez-Finley *et al.,* 2012). This regulation depends on its oxidation state, as iron readily cycles between ferric (Fe^{3+}) and ferrous (Fe^{2+}) forms (Martinez-Finley *et al.,* 2012). In plasma, extracellular iron is predominantly bound to transferrin (Tf) in its ferric form and subsequently recognised by the widely expressed transferrin receptor 1 (TfR1) (Cheng *et al.,* 2004).

The Fe-Tf complex is internalised into endocytic vesicles, where acidification promotes Fe^{3+} release, followed by its reduction to Fe^{2+} by ferroreductases. DMT1 then mediates Fe^{2+} transport into the cytoplasm (Sendamarai *et al.,* 2008; Touret *et al.,* 2003). A related receptor, TfR2, exhibits lower affinity for Fe-Tf and is restricted to hepatocytes, erythroid precursors, and duodenal crypt cells (Kawabata *et al.,* 1999). Iron that is not bound to transferrin can be absorbed directly via DMT1 on intestinal cells (Gunshin *et al.,* 1997).

Alternative uptake pathways also exist. Under iron overload, Fe entry may occur through L-type Ca^{2+} channels (Oudit *et al.,* 2003). Ferritin is a key cellular storage, sequestering surplus iron to avert toxicity and releasing it when needed (Harrison & Arosio, 1996). In addition, iron derived from haemoglobin in plasma can be internalised via a haemoglobin scavenger receptor known as CD163, located on monocytes and macrophages (Kristiansen *et al.,* 2001).

Iron equilibrium is also controlled by iron regulatory proteins (IRPs), which interact with iron-responsive elements (IREs) in the untranslated regions of iron-related mRNAs, modulating translation according to the status of cellular iron (Martinez-Finley *et al.,* 2012). Under iron-deficient conditions or oxidative stress, IRP1 and IRP2 stabilise transcripts, including ferritin,

DMT1, and TfR; this in turn enhances the uptake of iron and ensures compensation when the availability of iron reduces or oxidative demand increases (Haile, 1999).

2.4.2.3 Manganese (Mn)

Manganese enters the gastrointestinal tract via passive diffusion (Bell *et al.,* 1989) or active transport facilitated by the divalent metal transporter 1 (DMT1) (Garcia-Aranda *et al.,* 1983). This takes place through a proton gradient dependent on the potential of the cell's membrane (Gunshin *et al.,* 1997). In the bloodstream, about 80% of manganese binds to β-globulin and albumin, with the remaining transported by transferrin in its trivalent state (Dobson *et al.,* 2004).

Expressed on the surface of intestinal enterocytes and the epithelium of the olfactory system, DMT1 plays a vital role in the transport of Mn^{2+} (Thompson *et al.,* 2007). In the brain, DMT1 is abundant in the basal ganglia (Huang *et al.,* 2004), cerebellum and thalamus (Burdo *et al.,* 2001), and the hippocampus and cortex (Wang *et al.,* 2013). Transferrin (Tf), which interacts with the transferrin receptor (TfR) to gain entry into endosomes, offers an alternative pathway by binding Mn^{3+}. To transport Mn into the cytosol by endosomal DMT1, v-ATPases acidify the endosome, promoting the release of Mn and its reduction to Mn^{2+} (Gruenheid *et al.,* 1995; Touret *et al.,* 2003). Furthermore, at the blood-brain barrier, the choline transporter mediates Mn uptake with predominantly high affinity (Martinez-Finley *et al.,* 2012).

Other mechanisms, including voltage-gated and store-operated calcium channels, also contribute to Mn^{2+} uptake across the blood-brain barrier (Lucaciu *et al.,* 1997), ionotropic glutamate receptor Ca^{2+} channels (Kannurpatti *et al.,* 2000), and citrate transporters (Crossgrove *et al.,* 2003). Additionally, overlap exists with zinc transport, as Mn can be transported via ZIP8 and ZIP14 (Fujishiro *et al.,* 2011). Though less common, Mn^{3+} can form complexes with transferrin and cross the blood-brain barrier through iron- and pH-dependent binding to transferrin (Aschner & Gannon, 1994).

Manganese is a key cofactor for several enzymes (pyruvate carboxylase, glutamine synthetase, and arginase) key to digestion, immunity, reproduction, growth, energy metabolism, and antioxidant defense (Aschner & Aschner, 2005). Mn^{3+} is a catalytic cofactor for manganese superoxide dismutase (MnSOD), which detoxifies superoxide radicals in mitochondria and protects cells from death induced by oxidative stress (Li *et al.,* 1995). Other Mn^{2}-dependent

metalloproteins, such as lectins and integrins, highlight the vital role manganese plays in the organisation and structural integrity of the cytoskeleton (Weatherburn, 2001).

2.4.2.4 Zinc (Zn)

Cellular zinc uptake is regulated by two transporter families: the ZIP (Zrt- and Irt-like protein) family and the ZnT/CDF (cation diffusion facilitator) family (Martinez-Finley *et al.,* 2012). ZIP transporters mediate the movement of zinc from the extracellular environment or intestinal lumen into the cytoplasm (Martinez-Finley *et al.,* 2012). Some ZIP transporters are localised on intracellular organelles; for example, Zip7, which facilitates the release of zinc from the Golgi into the cytoplasm (Huang *et al.,* 2004).

In contrast, the ZnT/CDF transporters (SLC30 family) reduce zinc levels in the cytoplasm by exporting it either to the extracellular space or into organelles (Martinez-Finley *et al.,* 2012). These transporters depend on active transport mechanisms driven by ion gradients to expel zinc (Liuzzi & Cousins, 2004). While several ZnT proteins are expressed on the plasma membrane to support zinc efflux, others are localised to vesicles, directing zinc into intracellular compartments.

Furthermore, DMT1 facilitates the transportation of zinc (Espinoza *et al.,* 2011); however, its transport capacity for zinc differs from that of divalent metals such as copper and iron. This variation reflects the dominant role of ZnT and ZIP transporters in maintaining zinc homeostasis (Martinez-Finley *et al.,* 2012).

2.4.2.5 Cobalt (Co)

Cobalt is readily absorbed in the small intestine; however, retained cobalt has no direct physiological function since human tissues cannot synthesise vitamin B12 in the gut via *E. coli* (Valko *et al.,* 2005). Its only known biological role is as a constituent of vitamin B12 (Saad *et al.,* 2014). In human erythrocytes, the cobalt (Co^{2+}) uptake pathway is shared with calcium (Ca^{2+}) (Simonsen *et al.,* 2011b). However, its uptake is essentially irreversible since cobalt is bound in the cytosol and is not extruded by the Ca-pump (Simonsen *et al.,* 2011a).

2.4.2.6 Nickel (Ni)

Soluble nickel compounds enter the cell through ionomycin, a calcium ionophore channel, which increases the uptake of nickel (Valko *et al.,* 2005). Nickel also functions as a calcium channel blocker, reducing Ca^{2+} entry into the intracellular space; this reduction is offset by the release of free Ca^{2+} from intracellular stores (Valko *et al.,* 2005). Valko *et al.* (2005) also reported that insoluble nickel particles enter animal cells via phagocytosis. Albumin is the key transport protein in the blood; however, nickeloplasmin, a nickel-containing α2-macroglobulin, also transports nickel (Saad *et al.,* 2014).

2.4.2.7 Chromium (Cr)

Chromium is crucial to enhancing the action of insulin and for normal glucose metabolism (ATSDR, 2000). After absorption, Cr(III) binds to transferrin, an iron-transporting protein in the plasma (Saad *et al.,* 2014). Chromates can actively enter cells via anion transport channels used for isoelectric and isostructural ions such as SO_4^{2-} and HPO_4^{2-} (ATSDR, 2000), while insoluble chromates are internalised through phagocytosis (Saad *et al.,* 2014).

Metals such as Fe (II), Cu, Cr (III), (V) and (IV), Co (II), and Ni (II) generate free radicals via the Fenton-type reaction, with differing capacities (Jeong-Chae *et al.,* 2012). The Haber-Weiss reaction generates metal-induced ROS where O^{2-} mediates the generation of OH^- from H_2O_2 and participates in the reduction of Fe (III), leading to the Fenton reaction (Jeong-Chae *et al.,* 2012). Jeong-Chae *et al.* (2012) further suggested that the Haber-Weiss mechanism is more dominant *in vivo*, particularly during ROS production in macrophages undergoing phagocytosis.

2.5 Mechanism of Heavy Metal Toxicity

Heavy metal toxicity in biological systems is induced via the bonding of heavy metals to sulfhydryl groups and reactive oxygen species (ROS) processes that inactivate essential macromolecules, trigger oxidative stress, and deplete glutathione (Balali-Mood *et al.,* 2021). After being absorbed into the body, heavy metals bind to thiol (-SH) groups and alter cysteine residues within protein structures to interact with enzymes and proteins (Balali-Mood *et al.,* 2021). Amino acids containing thiol groups act as ligands during the formation of metal-protein complexes, which facilitate metal coordination (Burford *et al.,* 2005).

Heavy metals lead to the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), inducing oxidative stress (Balali-Mood *et al.,* 2021). Arsenic produces superoxide ($O_2^{\bullet -}$), oxygen ($O_2\bullet$), nitric oxide (NO•), hydrogen peroxide (H_2O_2), and peroxyl (ROO•) radicals (Balali-Mood *et al.,* 2021). In addition, lead toxicity arises from ROS formation and weakening of cellular antioxidant defenses. Exposure to lead diminishes levels of antioxidant enzymes such as glutathione peroxidase (GPx), catalase (CAT), superoxide dismutase (SOD), glutathione S-transferase (GST), and glutathione (GSH), and increases oxidative markers like malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) (Wang *et al.,* 2013).

Similarly, cadmium generates $O_2^{\bullet -}$, hydroxyl (OH•), and NO• radicals, overwhelming antioxidant defenses (Rani *et al.,* 2014; Balali-Mood *et al.,* 2021). This induces oxidative stress by displacing iron and copper ions from cellular proteins, leading to the accumulation of free Fe and Cu capable of catalysing the formation of reactive oxygen species (Balali-Mood *et al.,* 2021). Additionally, cadmium depletes glutathione, weakening the antioxidant defense system and increasing oxidative stress (Wu *et al.,* 2016).

Carcinogenic effects of heavy metals result from their interactions with regulatory proteins that control DNA synthesis and repair, the progression of the cell cycle, and cell death (Kim *et al.,* 2015). Cadmium and arsenic disrupt the regulation of protective genes and promote uncontrolled cellular proliferation by interfering with transcription factors such as Nuclear Factor kappa B (NF-κB), Activator Protein 1 (AP-1), and p53 (Valko *et al.,* 2005). Ngalame *et al.* (2014) reported that exposure to arsenic results from the overexpression of Ras in human prostate epithelial cells. Moreover, exposure to arsenic hinders DNA repair by suppressing poly(ADP-ribose) polymerase-1 (PARP-1), a key enzyme in DNA repair processes (Ding *et al.,* 2009).

Exposure to arsenic and cadmium has been associated with changes in DNA methylation and specific histone modifications (Ma *et al.,* 2016), mainly driven by overexpression of proto-oncogenes mediated by ROS and silencing of tumour suppressor genes (Cheng *et al.,* 2012). Abnormal DNA methylation suppresses the transcription of tumour suppressor genes, leading to dysregulated cell division and malignant transformation (Li & Chen, 2016).

Lead toxicity comprises the inhibition of ferrochelatase and δ-aminolevulinic acid dehydratase (ALAD), resulting in reduced heme production and consequent anaemia (Mense & Zhang, 2006). Lead also induces oxidative damage through lipid peroxidation and depletion of antioxidant defenses in various organs (Kasperczyk *et al.,* 2008). Similar to zinc, cadmium binds to plasma

albumin and disrupts the homeostasis of calcium, zinc, and iron (Schaefer *et al.,* 2020). Cadmium-induced liver injury has been linked to disruption of the regulation of calcium, triggering mitochondrial fragmentation via elevated levels of dynamin-related protein-1 (Xu *et al.,* 2013).

According to Yu *et al.* (2006), arsenic toxicity leads to carcinogenesis through oxidative stress, altered expression of the growth factor, and chromosomal anomalies. Furthermore, lipid peroxidation resulting from arsenic-induced oxidative stress causes DNA damage and death of neuronal cells (Mochizuki, 2019).

2.6 Impact of Heavy Metal Contamination on Eels

Eel diet comprises mussels, insect larvae, crustaceans, snails, and bottom-dwelling fish species (Belpaire & Goemans, 2007b). Eels bioaccumulate heavy metals through the food chain, often reaching levels beyond their metabolic tolerance and causing toxic effects (Le *et al.,* 2009). The accumulation of high amounts of body fat during their continental phase makes eels a perfect candidate for bioaccumulation of persistent contaminants such as heavy metals (Belpaire & Goemans, 2007a, b). Eels are long-lived and semelparous, unlike other sympatric fish species, resulting in high loads of contaminants (Bodin *et al.,* 2014).

Pierron *et al.* (2007a) revealed that cadmium toxicity impacts liver lipolysis and lipogenesis in yellow eels. Cadmium contamination increases the consumption of fat, which compromises fat storage for successful spawning migration and egg yolk reserve for leptocephali (Pierron *et al.,* 2007a).

In addition, cadmium (0.1-10µM) impairs the regulation of glucose metabolism in eels as well as other functions mediated by hormones (Fabbri *et al.,* 2003). Similarly, Pierron *et al.* (2008) reported accumulation of cadmium (5 µg/L) in the liver, kidney, gills, and digestive tract of eels following 30 days of exposure to 15 µg/L of cadmium. Celino *et al.* (2009) revealed that arsenic (0.1-100µM) inhibits *in vitro* spermatogenesis and induces apoptossis of germ cells in *Anguilla japonica*.

Heavy metals are highly toxic, and their accumulation in tissues can cause intoxication, cellular damage, apoptosis, reduced fertility, and organ dysfunction in humans (Damek-Proprawa & Sawicka-Kapusta, 2003). Specifically, lead, cadmium, and arsenic are associated with kidney impairment, neurological disorders, and various cancers (Cobbina *et al.,* 2015), while arsenic exposure is linked to stillbirths, abortion, and cardiovascular abnormalities in man (WHO, 2010).

CHAPTER THREE
RESEARCH METHODOLOGY

3.1 Study Area

The study was conducted along River Athi-Galana-Sabaki and River Ramisi, two Kenyan east-flowing rivers that drain into the Indian Ocean, between March and August 2023 (Figure 2). Two sampling locations were selected at each river: Kiaoni (upstream) and Sabaki Bridge (downstream) along River Athi-Galana-Sabaki, and Eshu (upstream) and Taliani (downstream) along River Ramisi. These river catchments receive long rains from March to May and short rains from October to December, interspersed with dry seasons from January to February and June to September. Both rivers are natural habitats for anguillid eels, and their exploitation is a source of food and income for local fishermen. The rivers also provide water for drinking and agricultural purposes for riparian communities along the rivers. Heavy metal contamination in rivers impacts not only the growth and survival of fish but also the fishing industry, a major source of food and livelihood.

3.1.1 Athi-Galana-Sabaki River Catchment

The Athi-Galana-Sabaki (3° 10' S, 40° 09' E) is the second longest, east-flowing river in Kenya that rises near Aberdare and Ngong hills, with a catchment of over 70,000 km^2, and drains into the Indian Ocean (Leauthaud *et al.,* 2013), see Figure 2. It is known as the Athi River in its upper reach, the Galana River after its confluence with the Tsavo, and the Sabaki River in its lower course. The upper catchment of the river is dominated by urban and peri-urban settlements, industries, and transport infrastructure, particularly within Nairobi and Machakos counties (Kosgey *et al.,* 2015). Additionally, there is an ongoing construction of the Thwake multipurpose dam designed to harness the fluctuating flows of the Athi River and seasonal water from the Thwake River.

The middle and lower reaches of the river flow through Arid and Semi-Arid Lands (ASAL), dominated by agropastoralism (Ocheng'o *et al.,* 2018), with several irrigation schemes such as Galana-Kulalu. The Galana-Kulalu irrigation scheme is a food security project established in 2014 to boost maize production and covers an area of one million acres (Mulupi, 2018).

The lower reach of the river, Sabaki, empties into the WIO at the Sabaki estuary, north of Malindi (KWTA, 2020). The average catchment population density is estimated at 249 persons.km^{-2} but varies from higher density in the upstream reach, through low density in the middle, and moderate population at the lower reaches (KNBS, 2019).

3.1.2 Ramisi River Catchment

Ramisi River (4° 27' 58" S, 39° 17' 44" E) is situated in Kwale County on the southern coast of Kenya near the Tanzanian border. It is a semi-perennial river, with a catchment of 1800 km^2 that rises from the Shimba hills and flows through the Kiwamba mangrove forest at Shimoni before pouring into the Indian Ocean (Wara *et al.,* 2019), see Figure 2. Surface runoff from the Ramisi River is slightly saline due to the draining of Duruma sandstone outcrops (KWTA, 2020). Kwale International Sugar Company Limited (KISCOL), together with its 15,000-acre farm, lies downstream of the Ramisi River (KCIDP, 2018). A sparse human population of less than 80 persons.km^{-2} dominated by smallholder subsistence farmers inhabits the catchment (KNBS, 2019).

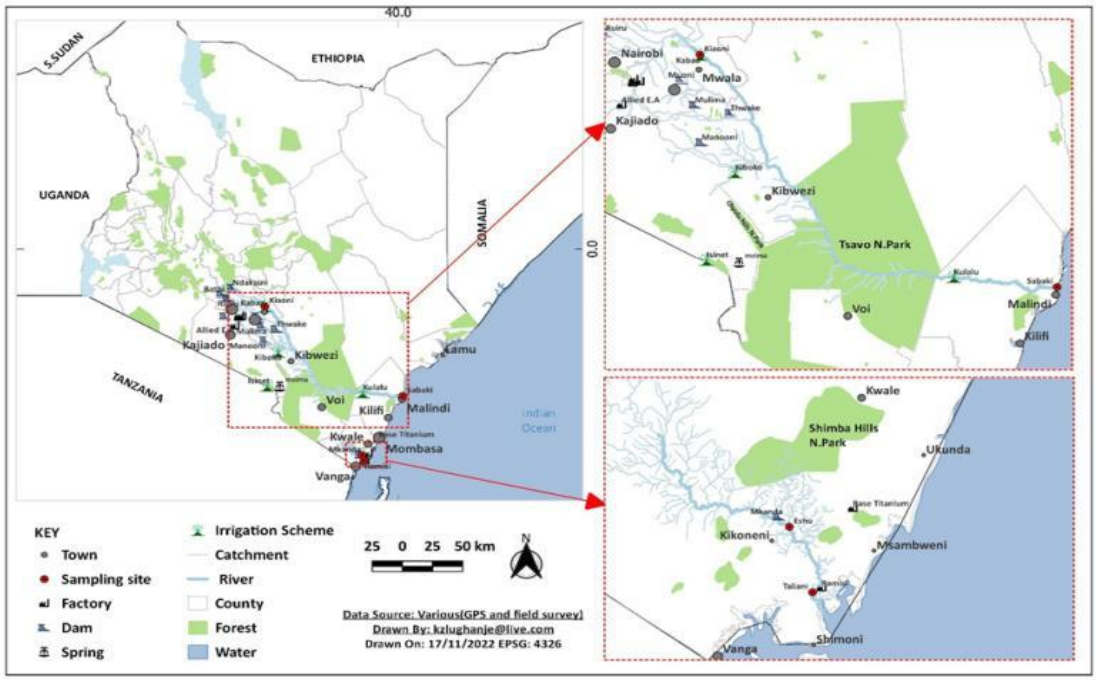


Figure 2: Map of Kenya showing River Athi-Galana-Sabaki and River Ramisi, highlighting some of the villages sampled along each river catchment

3.2 Sampling

3.2.1 River Water and Sediment Sampling

River water (500 ml) was collected in triplicate using precleaned plastic sample bottles at the respective upstream and downstream locations of rivers Athi-Galana-Sabaki and Ramisi. During water sample collection, the bottle was immersed about 30 cm below the water surface with the mouth of the bottle facing upstream and the neck tilted slightly upwards. 2 mL of concentrated HNO_3 (65% Suprapur®) was subsequently added to reduce adsorption of metals onto the walls of the plastic bottles. At each sampling occasion, physicochemical parameters of the river water, including pH, dissolved oxygen (DO), salinity, and temperature, were measured using a YSI Model 85/50 multi-meter probe and recorded. All samples were stored in a cool box with crushed ice and transported to the Chemistry Laboratory at the Kenya Marine and Fisheries Research Institute (KMFRI), Mombasa, where they were refrigerated at 4 °C until analysis.

Simultaneously, 100 g to 200 g of the top surface sediment samples were collected from each river at the same sites as water samples were collected. The samples were placed in sterile centrifuge tubes, properly labelled, stored in a cool box containing crushed ice to limit chemical and biological activities, and transported to the Chemistry Laboratory at the Kenya Marine and Fisheries Research Institute (KMFRI), Mombasa, for analysis.

3.2.2 Eel Sampling

Juvenile and adult anguillids (Plate 1) were captured using glass eel and double fyke nets at upstream and downstream locations of the Athi-Galana-Sabaki and Ramisi Rivers. Captured eels were immediately anaesthetised using clove oil diluted with river water before morphological characterisation and identification. Morphological characteristics, namely body coloration, maxillary tooth bands, and eye index (Equation 1), were determined and used to delimit eel life stages and species (Pankhurst, 1982; Réveillac *et al.,* 2009; Silfvergrip, 2009).

$$\text{Eye Index} = \left[\left((A+B)/4\right)^2 \times \pi / L\right] \times 100 \qquad 1$$

where:

A is the horizontal eye diameter

B is the vertical eye diameter

L is the total body length

The total length (to the nearest millimeter) and total weight (to the nearest gram) were recorded, and their mean values calculated. Specimens from thirty-seven anguillid eels (4 silver eels, 14 yellow eels, 2 elvers, and 17 glass eels) were collected for this study. A small sample size was used based on ethical and practical considerations, making sure that the research objectives were met without compromising conservation priorities surrounding anguillid eel species in the Western Indian Ocean. This was consistent with the 3Rs principle (Reduction, Replacement, Refinement) in an effort to minimise impact on wild populations, particularly as recruitment levels and local stock stability are poorly understood in Kenya.

Plate 1: An eel (*Anguilla bengalensis*) captured at the upstream location (Kiaoni) of River Athi-Galana-Sabaki

The subsamples of silver and yellow eels were sacrificed, and their otoliths (for microchemistry analysis), together with muscle and liver tissues (for heavy metal analysis) obtained. Sagittal otoliths were extracted by making a longitudinal incision along the dorsal surface of the eel's head using a sharp, heavy-bladed knife. The otoliths were then removed from the otic capsules with forceps, cleaned, measured (length and weight), and stored dry in labeled plastic vials. Otoliths were subsequently stored in a cool, dry cabinet pending Sr:Ca ratio analysis.

Dorsal muscle and liver tissue samples were obtained by snipping approximately 5g of the respective organs of the dissected silver and yellow eels. Muscle samples were wrapped in aluminium foil and stored in sealed containers, whereas liver samples were put in precleaned 50 ml centrifuge tubes.

The samples were properly labelled and kept in a cool box with crushed ice during transport to the Chemistry Laboratory at the Kenya Marine and Fisheries Research Institute (KMFRI), Mombasa. The samples were kept in a freezer at -20°C, pending analysis. Eel muscle was chosen to examine the risk posed to consumers. Additionally, the liver was analysed as a reliable marker of long-term exposure to heavy metals.

3.3 Determination of Heavy and Trace Metal Load in Specimen

3.3.1 Digestion of Muscle and Liver Samples

Muscle and liver samples were thawed, oven-dried at 80 °C to constant weight, and ground to a fine powder using a mortar grinder (Retsch RM 200). Triplicate subsamples (0.2 g) were placed in Teflon digestion vessels, treated with 5 ml of concentrated nitric acid (65% Suprapur®), and pre-digested for 1 h at room temperature. Thereafter, 2ml of hydrogen peroxide was added, and the samples were digested in an advanced microwave digestion system (ETHOS EASY, Milestone) programmed to run at 1200 W for 30 minutes as described by Yang *et al.* (2004). The samples were allowed to cool for five (5) minutes and filtered using a 0.45 µm Whatman filter paper into labelled 50ml Eppendorf tubes. Deionised distilled water was used to dilute the filtrate to the 50 ml mark. A blank digest using nitric acid and hydrogen peroxide was similarly prepared. A certified reference material was prepared using scallop (*Pecten maximus*) (IAEA-452), similar to the samples, for each digestion batch.

3.3.2 Digestion of Sediment Samples

Sediment samples were oven dried at 80°C for 24 hours to a constant weight and crushed with a motor grinder (Retsch RM 200) into fine powder. 0.2 g of dry sample was obtained and placed in a labelled Teflon reactor (CEM). A mixture of 5 ml concentrated nitric acid (HNO_3) and 2 ml concentrated hydrofluoric acid (HF) was added to the reactor, and the samples were left to stand at room temperature (25 °C) for 1 h. Subsequently, 2 ml of hydrogen peroxide (H_2O_2) was introduced, and digestion was carried out in an advanced microwave system (ETHOS EASY,

Milestone) for 30 min, see Plate 2. After cooling to room temperature, the reactor valve was carefully opened to release pressure before opening. A 5 ml aliquot of 4 g/l boric acid solution was then added, and the reactor was resealed and subjected to an additional 45 min of microwave digestion. Finally, the samples were cooled, transferred into pre-labelled sterile 50 ml polyethylene centrifuge tubes, and diluted to volume with double-distilled water. Double-distilled water was used to dilute the samples to the 50 ml mark. A blank solution was similarly prepared for each batch of analysis, except that no sample was added to the digestion vessels. A certified reference material using a marine sediment sample (IAEA-57) was prepared as the sample for each digestion batch.

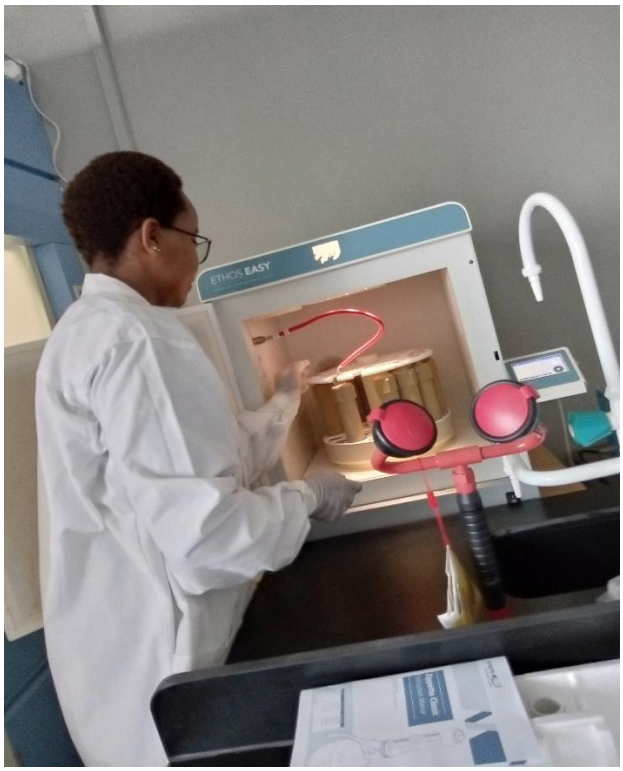


Plate 2: Samples placed in an advanced microwave digestion system (ETHOS EASY, Milestone)

3.3.3 Heavy and Trace Metal Analysis

Heavy (As, Cd, and Pb) and trace metals (Co, Cr, Cu, Fe, Mn, Ni, and Zn) in the filtrates of digested muscle, liver, sediment, and water samples were determined using the Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES; Agilent Technologies 5110). 2 ml of the sample was introduced into the ICP-OES, nebulized, and the aerosol was transported to a high-frequency plasma in which the constituents of the solution were atomized and partially ionized.

The characteristic emission lines of the atoms and ions were dispersed by a monochromator/polychromator, and the intensity of the lines was recorded with a charge-coupled device detector (Rüdel *et al.,* 2007). Element concentrations were quantified through calibration with appropriate standard solutions, based on the linear relationship between emission signal intensity and elemental concentration. Data were statistically analysed using the fitting of a straight line ($r > 0.999$). Blank readings were applied to correct concentration values, and method accuracy was verified using certified reference material (CRM) IAEA-452 scallop (*Pecten maximus*).

The concentrations of the heavy and trace metals in the muscle, liver, sediment, and water samples were determined by the ICP-OES instrument software. Further calculations of the element content in the muscle, liver, glass eel, and sediment samples were done using the following equation (Equation 2).

$$\omega_{E} = {}^{V}/_{M} \, X \, \rho_{\epsilon} \, X \, F \qquad 2$$

where:

ω_E is the proportion by mass of the element in the solid matter, stated, for example, as μg/g;

M is the mass of the sample used, stated in mg (for instance, 200 mg);

V is the volume to which replenished, stated in mL (for example, 50 mL);

ρ_E is the concentration of the element under consideration in the digestion solution, stated, for instance, in μg/L;

F is the conversion factor (1 L/mL × mg/g).

3.3.4 Bioaccumulation Factor among Anguillid Species

The bioaccumulation factor (BAF) was used to standardise the variability contributed by the sampling location and thus compare the levels of heavy and trace metal accumulation among the four species collected.

The BAF values were calculated as follows (Equation 3):

$$BAF = \frac{[X]organism}{[X]water} \qquad 3$$

where:

[X]organism is the concentration of the element X in the examined organism in mg/kg.

[X]water is the concentration of X in the water in mg/L.

3.4 Determination of Otolith Sr:Ca Signatures

3.4.1 Preparation of Otoliths

Otoliths were weighed and subsequently washed with deionised distilled water in acid-washed vials.

3.4.2 Sr:Ca Analysis

Otolith samples were placed in labelled Teflon reactors and digested with 5 ml of concentrated nitric acid (65% Suprapur®) for one hour at room temperature (~25°C). Afterwards, 2 ml of hydrogen peroxide was added, and the reactors were subjected to microwave digestion using an ETHOS EASY system (Milestone) at 1200 W for 30 minutes. The digested samples were cooled for 5 minutes, filtered through 0.45 μm Whatman filter paper, and the filtrates were transferred into labelled 50 ml Eppendorf tubes. Each was then diluted to the 50 ml mark with deionised distilled water, following Mohammed *et al.* (2017). A blank digest containing concentrated nitric acid and hydrogen peroxide was similarly prepared, and the samples were analysed in triplicate. Strontium and calcium concentrations were determined against aqueous standards using an Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES; Agilent Technologies 5110). The following equation was used to calculate the otolith Sr:Ca signatures/ratio (Equation 4).

$$\text{Sr:Ca ratio}_i = \frac{\text{Sr}_i}{\text{Ca}_i} \qquad 4$$

where:

Sr_i is the concentration of strontium (Sr) in sample i.

Ca_i is the concentration of calcium (Ca) in sample i.

$Sr:Ca_i$ is the ratio in sample i.

3.5 Determination of Association between Metal Load and Otolith Sr:Ca Signatures

Pearson correlation coefficient was used to determine the statistical relationship between the concentrations of heavy and trace metals from silver and yellow eel muscle tissues and otolith Sr:Ca signatures.

3.6 Data Analysis

The data were tested for homogeneity, and $\log_{10}$ transformation was applied where variances were non-homogeneous. Descriptive statistics were used to analyse metal concentrations in muscle and liver tissues, glass eels, sediment, and water. The results were expressed as mean±SE. A one-way analysis of variance (ANOVA) was performed, and significant differences were accepted at $p \leq 0.05$. Tukey's honest significant difference (HSD) test was used to separate the mean values where significant differences were found. To determine relationships between different variables, a correlation analysis was done. R version 4.1 (R Core Team, 2021) and Microsoft Excel were used for all statistical analyses.

3.7 Ethical Approval

Approvals to conduct this research were sought from the National Commission for Science, Technology and Innovation (NACOSTI) and the Egerton University Institutional Scientific and Ethics Review Committee (EUISERC).

CHAPTER FOUR

RESULTS

4.1 River Water Physicochemical Parameters

The physicochemical parameters of water from the Athi-Galana-Sabaki and Ramisi rivers measured in this study are shown in Table 1. The water pH values recorded (6.7-8.1) in both rivers were comparable; however, significant differences in the water temperature, dissolved oxygen, and salinity among the rivers ($p \leq 0.05$). Additionally, temperature and salinity values along the River Ramisi were comparable in both upstream and downstream sampling locations.

However, there was a significant difference ($p \leq 0.05$) in dissolved oxygen values of both sampling locations along the River Ramisi. Significant differences ($p \leq 0.05$) were observed in temperature, dissolved oxygen, and salinity values recorded at upstream and downstream sampling locations along the River Athi-Galana-Sabaki, with values from the downstream location being significantly higher than those from the upstream location.

Table 1: Physicochemical properties of water samples from Athi-Galana-Sabaki and Ramisi rivers (values are presented as mean ±SE)

River	Sampling Location	pH	Temperature (°C)	Dissolved Oxygen (mg/L)	Salinity (ppt)
Athi-Galana-	Upstream	$8.10 \pm 0.34_a$	$25.43 \pm 0.16_b$	$5.24 \pm 0.40_c$	$0.28 \pm 0.02_c$
Sabaki	Downstream	$7.10 \pm 0.08_a$	$27.17 \pm 0.45_a$	$6.10 \pm 0.20_b$	$0.43 \pm 0.03_b$
Ramisi	Upstream	$7.61 \pm 0.22_a$	$29.64 \pm 0.66_a$	$6.79 \pm 0.17_a$	$1.37 \pm 0.14_a$
	Downstream	$6.69 \pm 0.11_a$	$26.22 \pm 0.42_a$	$4.87 \pm 0.42_c$	$1.62 \pm 0.34_a$

Means are ranked using the Tukey HSD test; column means followed by different subscript letters a, b, or c are significantly different ($p \leq 0.05$)

4.2 Eel Morphometrics

A total of 37 anguillids, eighteen (18) from River Athi-Galana-Sabaki and nineteen (19) from River Ramisi, were selected for this study (Tables 2 and 3). The specimens were classified into four life stages and four species using keys developed by Réveillac *et al.* (2009) and Silfvergrip (2009).

They were categorised into four life stages, namely silver eels (n=4), yellow eels (n=14), elvers (n=2), and glass eels (n=17). Yellow and glass eels were the most common along the Athi-Galana-Sabaki (44.44%) and Ramisi rivers (63.16%), respectively. Four anguillid species were recorded, namely *Anguilla bengalensis* (n=14), *A. bicolor* (n=16), *A. marmorata* (5), and *A. mossambica* (n=2); accounting for 37.84%, 43.24%, 13.51%, and 5.41% of the specimens, respectively.

The body weight values of glass eels collected from both rivers were comparable (Table 2). However, there was a significant difference ($p \leq 0.05$) in glass eel body length with glass eels from River Ramisi recording a higher mean value than those from River Athi-Galana-Sabaki. This was likely due to the warmer temperatures recorded in River Ramisi required for maximum eel growth. Among glass eels, only *Anguilla bicolor* (5) were captured in River Athi-Galana-Sabaki, whereas all four species, namely *Anguilla bengalensis* (1), *A. marmorata* (1), *A. mossambica* (1), and *A. bicolor* (9) were collected along River Ramisi. There was no significant difference ($p \geq 0.05$) in body weight, eye index, and body length values among elvers collected from the two rivers.

Table 2: Selected morphometrics among glass eel and elvers collected from the rivers Athi-Galana-Sabaki and Ramisi, Kenya (values are presented as mean±SE)

River	Life stage	Species	N	Body weight (g)	Body length (mm)	Eye index
Athi-Galana-Sabaki	Glass	*A. bicolor*	5	$0.20\pm0.18_a$	$53.20\pm2.28_b$	-
	Elver	*A. bengalensis*	1	4.00	140.00	0.65
Ramisi	Glass	*A. bengalensis*	1	0.16	55.00	-
		A. marmorata	1	0.12	53.00	-
		A. mossambica	1	0.19	59.00	-
		A. bicolor	9	$0.26\pm0.02_a$	$65.89\pm0.98_a$	-
	Elver	*A. marmorata*	1	3.50	137.00	0.79

Column means are ranked using the Tukey HSD test; means followed by similar letters are not significantly different ($p \geq 0.05$)

Among silver eels, the mean eye index of *A. bengalensis* was twofold higher than that of *A. bicolor* (Table 3). Yellow eels were the most common along the Athi-Galana-Sabaki (44.44%). Among the yellow eels from River Athi-Galana-Sabaki and River Ramisi, the body weight, body

length, and eye index values of *Anguilla bengalensis* and *A. marmorata* were tenfold, twofold, and threefold higher compared to those of *A. mossambica*, respectively. However, there were no significant differences ($p \geq 0.05$) in the body weight, body length, and eye index values of yellow eels, *Anguilla bengalensis* and *A. marmorata,* collected from River Athi-Galana-Sabaki and River Ramisi.

Table 3: Selected morphometrics among yellow and silver eel species collected from the rivers Athi-Galana-Sabaki and Ramisi, Kenya (values are presented as mean±SE)

River	Life stage	Species	N	Body weight (g)	Body length (mm)	Eye index
Athi-Galana-Sabaki	Yellow	*A. bengalensis*	4	$433.75 \pm 99.59_a$	$596.75 \pm 33.70_a$	$7.07 \pm 2.28_a$
		A. marmorata	3	$393.33 \pm 143.58_a$	$558.33 \pm 57.28_a$	$5.00 \pm 0.43_a$
		A. mossambica	1	35.00_b	290.00_b	1.83_b
	Silver	*A. bengalensis*	2	$640.00 \pm 130.00_a$	$698.50 \pm 11.50_a$	$12.04 \pm 0.08_a$
		A. bicolor	2	$650.00 \pm 50.00_a$	$689.00 \pm 11.00_a$	$7.68 \pm 1.29_b$
Ramisi	Yellow	*A. bengalensis*	6	$363.67 \pm 167.95_a$	$496.67 \pm 74.71_a$	$5.85 \pm 1.21_a$

Means are ranked using the Tukey HSD test; means followed by different letters a, b, or c are significantly different ($p \leq 0.05$)

4.3 Heavy and Trace Metal Contamination in River Water

A total of thirty-four (34) water samples, sixteen (16) from the Athi-Galana-Sabaki River and eighteen (18) from the Ramisi River were analysed for heavy (As, Cd, and Pb) and trace metals (Co, Cr, Cu, Fe, Ni, Mn, V, and Zn) (Table 4 and Table 5). Among the heavy metals, water samples from River Ramisi recorded lead (0.003 mg/L), cadmium (0.033±0.002 mg/L), and arsenic (0.192±0.009 mg/L). Lead was only present at the upstream location of the river; however, its concentration was below the ICP-OES detectable limit at the downstream sampling location of the river. Arsenic (0.184±0.006 mg/L) and cadmium (0.035±0.002 mg/L) were recorded in water samples from River Athi-Galana-Sabaki; however, lead was below the ICP-OES detectable limit. The concentrations of arsenic and cadmium in both rivers exceeded the WHO maximum permissible limit for drinking water by more than tenfold.

Table 4: Concentrations of selected heavy metals in water (mg/L) along River Athi-Galana-Sabaki and River Ramisi (values are presented as mean±SE)

Element	Athi-Galana-Sabaki (mg/L)	Ramisi (mg/L)	WHO Permissible Limit (mg/L)
Arsenic	$0.18\pm0.01_a^*$	$0.19\pm0.01_a^*$	0.01
Cadmium	$0.04\pm0.002_a^*$	$0.03\pm0.002_a^*$	0.003
Lead	BDL	0.003±0.003	0.01

BDL- Below Detectable Limit; row means followed by similar letters are not significantly different ($p\geq0.05$); means with superscript * attached are higher than WHO maximum permissible limits for drinking water (mg/L)

All trace elements were present in River Ramisi, with relatively high iron, manganese, and nickel levels recorded. This was likely a result of the weathering of highly mineralised rocks over which River Ramisi and its tributaries flow, making the water unsuitable for household and irrigation use. Notably, chromium, iron, and nickel concentrations were below the ICP-OES detectable limits. The concentrations of trace metals were higher at River Ramisi than at River Athi-Galana-Sabaki; however, the magnitude varied, likely due to differences in the rates of release into the water column following the weathering of the rock minerals. There was a significant difference in trace metal content between River Athi-Galana-Sabaki and River Ramisi ($p\leq0.05$), possibly due to the influence of different point sources of pollution along the two basins.

The average cobalt concentration in River Ramisi was twofold higher than that of River Athi-Galana-Sabaki. Additionally, the mean concentrations of copper, manganese, and zinc in River Ramisi were sevenfold, twentyfold, and thirtyfold higher than those in River Athi-Galana-Sabaki, respectively, possibly as a result of the River Maji Moto, a tributary of River Ramisi, which discharges highly mineralised water into the Ramisi River. This indicates the unsuitability of water from River Ramisi for domestic/industrial/agricultural use. However, there was no significant difference ($p\geq0.05$) in heavy and trace metal concentration between the upstream and downstream locations of the River Athi-Galana-Sabaki and the River Ramisi.

Table 5: Concentration levels of trace metals in water (mg/L) along River Athi-Galana-Sabaki and River Ramisi (values are presented as mean±SE)

Element	Athi-Galana-Sabaki (mg/L)	Ramisi (mg/L)	WHO Permissible Limit (mg/L)
Cobalt	$0.23\pm0.03_b$	$0.66\pm0.12_a$	-
Chromium	BDL	0.009±0.008	0.05
Copper	$0.004\pm0.001_b$	$0.03\pm0.02_a$	2
Iron	BDL	$18.97\pm10.59^*$	2
Manganese	$0.30\pm0.15_b$	$6.78\pm4.57_a^*$	0.4
Nickel	BDL	$1.09\pm0.75^*$	0.07
Zinc	$0.003\pm0.003_b$	$0.10\pm0.07_a$	3

BDL - Below Detectable Limit; - no set guideline; column means followed by different letters (a, b) are significantly different ($p\leq0.05$); means with superscript * attached are higher than WHO maximum permissible limits for drinking water (mg/L)

4.4 Heavy and Trace Metal Load in River Sediment

Fourteen (14) sediment samples from River Athi-Galana-Sabaki and twenty (20) from River Ramisi were analysed. The samples recorded varying heavy metal and trace metal mean concentrations (Tables 6 and 7). Cadmium (0.687±0.0386 mg/kg), arsenic (4.342±0.241 mg/kg), and lead (14.032±5.662 mg/kg) were present in sediment samples from the Athi-Galana-Sabaki River. In River Ramisi, arsenic (4.64±0.173 mg/kg), cadmium (0.809±0.045 mg/kg), and lead (3.04±2.095 mg/kg) were detected in sediment samples collected from the river bed. Heavy metals were distributed in the following sequence As>Pb>Cd and Pb>As>Cd in River Ramisi and River Athi-Galana-Sabaki, respectively.

The arsenic concentration in River Athi-Galana-Sabaki was comparable to that of River Ramisi. However, the concentration of lead was fivefold higher in River Athi-Galana-Sabaki (14.032±5.662 mg/kg) than in River Ramisi (3.040±2.095 mg/kg). This is likely a result of the reported pollution in tributaries such as the Nairobi River that is attributed to industrial effluents, agricultural activities, and sewerage discharge (Njuguna *et al.,* 2017). Arsenic, cadmium, and lead concentrations in both rivers were below the EPA freshwater sediment benchmark (EPA, 2006).

Table 6: Mean heavy metal concentration (mg/kg) in sediment samples from River Athi-Galana-Sabaki and River Ramisi (values are presented as mean±SE)

Element	Athi-Galana-Sabaki (mg/kg)	Ramisi (mg/kg)	EPA Region III BTAG Benchmark (mg/kg)
Arsenic	$4.34\pm0.24_a$	$4.64\pm0.17_a$	9.8
Cadmium	$0.69\pm0.04_b$	$0.81\pm0.05_a$	0.99
Lead	$14.03\pm5.66_a$	$3.040\pm2.10_b$	35.8

BDL - Below Detectable Limit; means followed by similar letters are not significantly different ($p\geq0.05$)

All trace metals were found in sediment samples from both rivers, with River Athi-Galana-Sabaki recording higher trace metal concentrations than River Ramisi. Trace metals arising from industrial effluent discharge, solid waste dumps and landfills, and surface runoff from agricultural lands are adsorbed by river sediment. The mean concentrations of cobalt, chromium, copper, and iron were threefold higher in River Athi-Galana-Sabaki than in River Ramisi. Furthermore, the average concentrations of manganese and nickel in River Athi-Galana-Sabaki were twofold and fourfold higher than in River Ramisi, respectively. Cobalt and nickel mean concentrations from both rivers were above the Environmental Protection Agency (EPA) Region III BTAG freshwater sediment screening benchmark (EPA, 2006).

At River Athi-Galana-Sabaki, cobalt and nickel concentrations were fourfold and thirteenfold higher than the EPA freshwater sediment benchmark, respectively. River Ramisi recorded cobalt (1.4-fold) and nickel (threefold) values higher than the EPA freshwater sediment benchmark. Trace metals such as cobalt and nickel are naturally present in the Earth's crust; however, they are also released into the environment through urban waste, wastewater irrigation, and industrial effluent and emissions. The presence of high concentrations of trace metals in the environment is of major concern because of their toxicity, bioaccumulation, and threat to not only human beings but also other living organisms.

Table 7: Mean trace metal concentration (mg/kg) in sediment samples from River Athi-Galana-Sabaki and River Ramisi (values are presented as mean±SE)

Element	Athi-Galana-Sabaki (mg/kg)	Ramisi (mg/kg)	EPA Region III BTAG Benchmark (mg/kg)
Cobalt	$179.38\pm34.18_a^*$	$68.12\pm11.90_b^*$	50
Chromium	$18.05\pm7.20_a$	$6.05\pm0.89_b$	43.4
Copper	$3.41\pm0.82_a$	$1.24\pm0.39_b$	31.6
Iron	$3387.74\pm710.68_a$	$1194.34\pm351.14_b$	20000
Manganese	$113.93\pm15.61_a$	$74.84\pm33.42_b$	460
Nickel	$296.36\pm166.52_a^*$	$70.24\pm23.58_b^*$	22.7
Zinc	$8.87\pm1.54_a$	$6.39\pm2.99_b$	121

BDL - Below Detectable Limit; - no set guideline; means followed by similar letters are not significantly different ($p\geq0.05$); means with superscript * attached are higher than EPA freshwater sediment benchmark values (mg/kg)

4.5 Heavy and Trace Metal Concentration among Anguillid Life Stages

The mean concentrations of heavy and trace metals in muscle tissues from silver eels, yellow eels, elvers, and entire glass eel specimens are presented in Table 8 and Table 9, respectively. Arsenic, cadmium, and lead were present in glass eels; however, lead was absent in elvers, yellow, and silver eels. Lead was only detected in glass eels collected from the upstream area of River Ramisi.

The mean values of heavy metals were found to be significantly higher in silver eel muscle tissues ($p\leq0.05$), as compared to the other three life stages, possibly as a result of the long residency and feeding habit of yellow eels in the rivers. Among heavy metals, arsenic recorded the highest mean concentrations. Additionally, the average concentrations of arsenic, cadmium, and lead exceeded the FAO/WHO maximum permissible limits in freshwater fishes. The order of heavy metal mean concentrations was As>Cd>Pb.

Table 8: The mean concentration of heavy metals (mg/kg dry weight) in silver, yellow, and elver muscle tissues, and entire glass eels (values are presented as mean±SE)

Element	Silver (n=4)	Yellow (n=14)	Elver (n=2)	Glass (n=12)	FAO/WHO Limit (mg/kg)
Arsenic	$4.45\pm2.88_a^*$	$2.31\pm1.87_a^*$	$0.48\pm0.19_b^*$	$0.39\pm0.04_b^*$	0.12
Cadmium	$0.88\pm0.61_a^*$	$0.43\pm0.29_a^*$	$0.11\pm0.06_b^*$	$0.09\pm0.05_b^*$	0.05
Lead	BDL	BDL	BDL	$0.59\pm0.42^*$	0.2

BDL- Below Detectable Limit; means followed by similar letters are not significantly different ($p\geq0.05$); means with superscript * attached are higher than FAO/WHO maximum permissible limits in freshwater fishes (mg/kg)

The mean values of trace metals were found to be significantly higher in silver eel muscle tissues ($p\leq0.05$), as compared to the other three life stages. Cobalt was below the detectable limit in elvers and glass eels. The mean concentrations of nickel and cobalt in silver eels, chromium in all life stages, and manganese in silver and yellow eels were above the FAO/WHO maximum permissible limits in freshwater fishes.

Glass eels and elvers contain heavy and trace metal levels below the FAO/WHO maximum permissible limits for freshwater fishes. However, with prolonged residency in rivers, these levels increase, and yellow and silver eels accumulate metal concentrations several times higher than the FAO/WHO limits. The distribution pattern of mean trace metal concentrations was as follows: Fe>Zn>Cu>Ni>Mn>Cr>V>Co.

Table 9: The average concentration of trace metals (mg/kg dry weight) in silver, yellow, and elver muscle tissues, and entire glass eels (values are presented as mean±SE)

Element	Silver (n=4)	Yellow (n=14)	Elver	Glass (n=17)	FAO/WHO Limit (mg/kg)
Cobalt	$0.53\pm0.20_{a}^{*}$	$0.33\pm0.11_{b}$	BDL	BDL	0.5
Chromium	$0.97\pm0.45_{a}^{*}$	$0.43\pm0.06_{a}^{*}$	$0.18\pm0.08_{b}^{*}$	$0.14\pm0.03_{c}^{*}$	0.05
Copper	$2.23\pm1.70_{a}$	$1.42\pm1.21_{a}$	$1.13\pm1.05_{a}$	$0.42\pm0.31_{b}$	30
Iron	$14.33\pm12.42_{a}$	$6.14\pm3.89_{a}$	$3.24\pm1.32_{a}$	$2.56\pm1.05_{a}$	30
Manganese	$1.13\pm0.052_{a}^{*}$	$1.1\pm0.11_{a}^{*}$	$0.08\pm0.05_{b}$	$0.05\pm0.03_{b}$	1.0
Nickel	$1.51\pm1.30_{a}^{*}$	$0.18\pm0.26_{a}$	$0.10\pm0.04_{b}$	$0.10\pm0.02_{b}$	0.5
Zinc	$4.95\pm2.39_{a}$	$2.57\pm2.35_{a}$	$1.33\pm0.54_{b}$	$1.03\pm0.68_{b}$	30

BDL- Below Detectable Limit; - no set guideline; means followed by similar letters are not significantly different ($p\geq0.05$); means with superscript * attached are higher than FAO/WHO maximum permissible limits in freshwater fishes (mg/kg)

4.6 Heavy and Trace Metal Concentration in Eel Liver

The average heavy and trace metal concentrations in liver tissues from silver and yellow eels are presented in Tables 10 and 11, respectively. Liver tissue samples from silver and yellow eels recorded varying trace and heavy metal mean concentrations. Lead was below the detectable limit in both silver and yellow eels. The mean concentration of arsenic in yellow eels was 1.989 mg/kg, while silver eels recorded an average arsenic concentration of 1.713 mg/kg. In silver eels, cadmium had a mean concentration of 0.657 mg/kg, whereas the average concentration of cadmium in yellow eels was 0.234 mg/kg. There was no significant difference in mean heavy metal concentrations ($p\geq0.05$) between silver and yellow eel liver tissues.

The mean arsenic concentrations of both silver and yellow eels were over tenfold higher than the FAO/WHO maximum permissible limit (0.12 mg/kg) in freshwater fishes, raising concerns of bioaccumulation higher up the food chain. Compared to the FAO/WHO maximum permissible limit (0.05 mg/kg) in freshwater fishes, the average concentration of cadmium among silver eels and yellow eels was thirteenfold and fivefold higher, respectively. Similarly, chromium, iron, and nickel mean concentrations in both silver and yellow eels were above the FAO/WHO maximum permissible limit in freshwater fishes.

Table 10: Average heavy metal concentrations (mg/kg dry weight) in liver tissues from silver and yellow eels (values are presented as mean±SE)

Element	Silver (n=4)	Yellow (n=14)	FAO/WHO Limit (mg/kg)
Arsenic	$1.71\pm0.81_a^*$	$1.99\pm1.16_a^*$	0.12
Cadmium	$0.66\pm0.56_a^*$	$0.23\pm0.02_a^*$	0.05
Lead	BDL	BDL	0.2

BDL- Below Detectable Limit; BDL- Below Detectable Limit; means followed by similar letters are not significantly different ($p\geq0.05$); means with superscript * attached are higher than FAO/WHO maximum permissible limits in freshwater fishes (mg/kg)

There was no significant difference in mean trace metal concentrations ($p\geq0.05$) except for zinc, which was significantly higher in silver eels compared to yellow eels ($p\leq0.05$). The distribution of average trace metal concentrations in silver eels was as follows: Fe>Zn>Cu>Ni>V>Mn>Cr>Co. The mean concentrations of trace metals in yellow eels were ordered in the following sequence: Fe>Ni>Zn>Cu>V>Mn>Co>Cu.

Table 11: Trace metal mean concentrations in liver tissues from silver and yellow eels (mg/kg dry weight) (values are presented as mean±SE)

Element	Silver (n=4)	Yellow (n=14)	FAO/WHO Limit (mg/kg)
Cobalt	$0.17\pm0.08_a$	$0.74\pm0.35_a$	0.5
Chromium	$0.24\pm0.09_a^*$	$0.39\pm0.21_a^*$	0.05
Copper	$7.52\pm3.32_a$	$4.95\pm2.22_a$	30
Iron	$98.42\pm37.40_a^*$	$47.75\pm28.34_a^*$	30
Manganese	$1.63\pm1.14_a$	$0.85\pm0.37_a$	1.0
Nickel	$5.84\pm1.85_a^*$	$10.50\pm6.77_a^*$	0.5
Zinc	$23.707\pm5.191_a$	$9.569\pm4.364_b$	30

BDL- Below Detectable Limit; means followed by similar letters are not significantly different ($p\geq0.05$); means with superscript * attached are higher than FAO/WHO maximum permissible limits in freshwater fishes (mg/kg)

4.7 Heavy and Trace Metal Load among Anguillid Species

The average concentrations of trace and heavy metals in the muscle and liver tissues of silver and yellow eels across four anguillid species are shown in Tables 12 and 13, respectively. Lead was below the ICP-OES detectable limit in all four species. Significant differences among the four anguillid species were observed in muscle and liver heavy and trace metal concentrations ($p \leq 0.05$). Arsenic and cadmium mean values in all four species were over tenfold higher FAO/WHO maximum permissible limits in freshwater fishes. Among species, *Anguilla bengalensis* had the highest arsenic and cadmium concentrations, whereas *A. mossambica* had the lowest. However, *Anguilla marmorata* and *A. bicolor* had intermediate levels of arsenic and cadmium.

Furthermore, Arsenic concentrations in liver tissues exceeded the FAO/WHO maximum permissible limits for freshwater fishes by more than tenfold. Similarly, the average concentration of cadmium in *Anguilla bengalensis* (twofold), *A. bicolor* (sixfold), *A. marmorata* (threefold), and *A. mossambica* (eightfold) liver tissues was higher than the FAO/WHO maximum permissible limits in freshwater fishes.

Table 12: Heavy metal concentrations (mg/kg) in yellow and silver eel muscle and liver tissues of four anguillid eel species from Rivers Athi-Galana-Sabaki and Ramisi, Kenya

Tissue	**Element**	***A. bengalensis***	***A. bicolor***	***A. marmorata***	***A. mossambica***	**FAO/WHO Limit (mg/kg)**
Muscle	Arsenic	$4.61 \pm 0.14_a^*$	$2.90 \pm 0.67_b^*$	$3.54 \pm 0.77_b^*$	2.49_c^*	0.12
	Cadmium	$1.10 \pm 0.57_a^*$	$0.50 \pm 0.23_b^*$	$1.21 \pm 0.72_a^*$	0.11_c^*	0.05
	Lead	BDL	BDL	BDL	BDL	0.2
Liver	Arsenic	$1.52 \pm 0.35_b^*$	$2.41 \pm 0.26_a^*$	$2.11 \pm 1.45_a^*$	5.34^*	0.12
	Cadmium	$0.30 \pm 0.27_b^*$	$1.14 \pm 0.05_a^*$	$0.64 \pm 0.74_a^*$	1.53^*	0.05
	Lead	BDL	BDL	BDL	BDL	0.2

BDL- Below Detectable Limit; means followed by similar letters are not significantly different ($p \geq 0.05$); means with superscript * attached are higher than FAO/WHO maximum permissible limits in freshwater fishes (mg/kg)

Cobalt was below the detectable limit in both muscle and liver tissues of *Anguilla bicolor* and *Anguilla mossambica,* whereas copper was below the detectable limit in muscle tissues of *Anguilla mossambica*. Chromium and nickel were similarly below the detectable limit in the liver tissues of *Anguilla mossambica.*

In muscle tissues, the mean chromium concentrations in *A. bengalensis* (fivefold), *A. marmorata* (threefold), and *A. bicolor* (fivefold) were higher than the FAO/WHO maximum permissible limits in freshwater fishes. Notably, *A. mossambica* recorded an average chromium concentration fortyfold higher than the FAO/WHO maximum permissible limits in freshwater fishes; possibly an outlier. Moreover, nickel recorded average concentrations higher than the FAO/WHO maximum permissible limits in freshwater fishes in *A. bengalensis* (twofold) and *A. mossambica* (threefold).

However, in liver tissues, iron recorded the highest mean concentration in liver tissues of all species, while chromium recorded the lowest mean concentration in *Anguilla bengalensis, Anguilla bicolor*, and *Anguilla marmorata*. In *Anguilla mossambica*, zinc recorded the lowest mean concentration. Except for zinc, which was highest in *A. bicolor*, but comparable among the other three species, all other trace metals had similar mean concentrations among all the species. The nickel mean concentrations among all four species were over tenfold higher than the FAO/WHO maximum permissible limits in freshwater fishes.

Table 13: Mean ± standard error values for trace metal concentrations (mg/kg dry weight) in yellow and silver eel muscle and liver tissues of four anguillid eel species from Rivers Athi-Galana-Sabaki and Ramisi, Kenya

Tissue	Element	*A. bengalensis*	*A. bicolor*	*A. marmorata*	*A. mossambica*	FAO/WHO Limit (mg/kg)
Muscle	Cobalt	$0.52\pm0.11_{a}$	BDL	$0.13\pm 0.04_{b}$	BDL	0.5
	Chromium	$0.26\pm0.17_{b}^{*}$	$0.28\pm0.12_{b}^{*}$	$0.19\pm0.08_{b}^{*}$	2.35_{a}^{*}	0.05
	Copper	$1.02\pm0.80_{a}$	$0.22\pm0.01_{a}$	$0.63\pm0.02_{a}$	BDL	30
	Iron	$4.92\pm0.21_{c}$	$17.40\pm0.14_{a}$	$5.31\pm0.04_{b}$	1.29_{d}	30
	Manganese	$0.61\pm0.03_{b}$	$1.44\pm0.10_{a}$	$0.32\pm0.02_{c}$	0.12_{d}	1.0
	Nickel	$0.91\pm0.35_{a}^{*}$	$0.10\pm0.05_{b}$	$0.61\pm0.53_{a}^{*}$	1.66_{a}^{*}	0.5
	Zinc	$4.13\pm0.07_{b}$	$4.75\pm0.08_{a}$	$3.43\pm0.12_{c}$	2.91	30
Liver	Cobalt	$0.62\pm0.44_{a}$	BDL	$0.50\pm0.48_{a}$	BDL	0.5
	Chromium	$0.40\pm0.29_{a}^{*}$	$0.12\pm0.17_{a}^{*}$	$0.18\pm0.11_{a}^{*}$	BDL	0.05
	Copper	$5.46\pm2.71_{a}$	$4.68\pm0.30_{a}$	$5.61\pm3.93_{a}$	7.63	30
	Iron	$55.40\pm34.87_{a}^{*}$	$106.32\pm50.63_{a}^{*}$	$49.10\pm25.39_{a}^{*}$	37.41	30
	Manganese	$1.11\pm0.80_{a}$	$0.80\pm0.13_{a}$	$0.89\pm0.27_{a}$	0.76	1.0
	Nickel	$8.39\pm1.76_{a}^{*}$	$6.33\pm2.025_{a}^{*}$	$10.66\pm8.06_{a}^{**}$	BDL	0.5
	Zinc	$11.01\pm5.84_{b}$	$27.41\pm1.19_{a}$	$12.16\pm5.13_{b}$	5.34	30

BDL- Below Detectable Limit; means followed by similar letters are not significantly different ($p\geq0.05$); means with superscript * attached are higher than FAO/WHO maximum permissible limits in freshwater fishes (mg/kg)

4.8 Bioaccumulation Factors among Anguillid Species

The bioaccumulation factors (BAFs) of muscle and liver tissues from four anguillid species are shown in Figures 3 and 4, respectively. There was no significant difference in the bioaccumulation of heavy metals (As and Cd) and trace metals (Co, Cr, Cu, Fe, Mn, Ni, and Zn) between species and tissues ($p\geq0.05$).

However, muscle chromium BAF values were the highest, followed by zinc, copper, and lastly cadmium and arsenic. All muscle metal BAF values were below the recommended values for BAF in edible parts of freshwater fish, BAF Freshwater NCRP 123 1996 (Karlsson *et al.,* 2002), except chromium in *Anguilla mossambica* (522.667 L/kg), which was 2.6-fold greater than the recommended value for chromium BAF in edible parts of freshwater fish (200 L/kg).

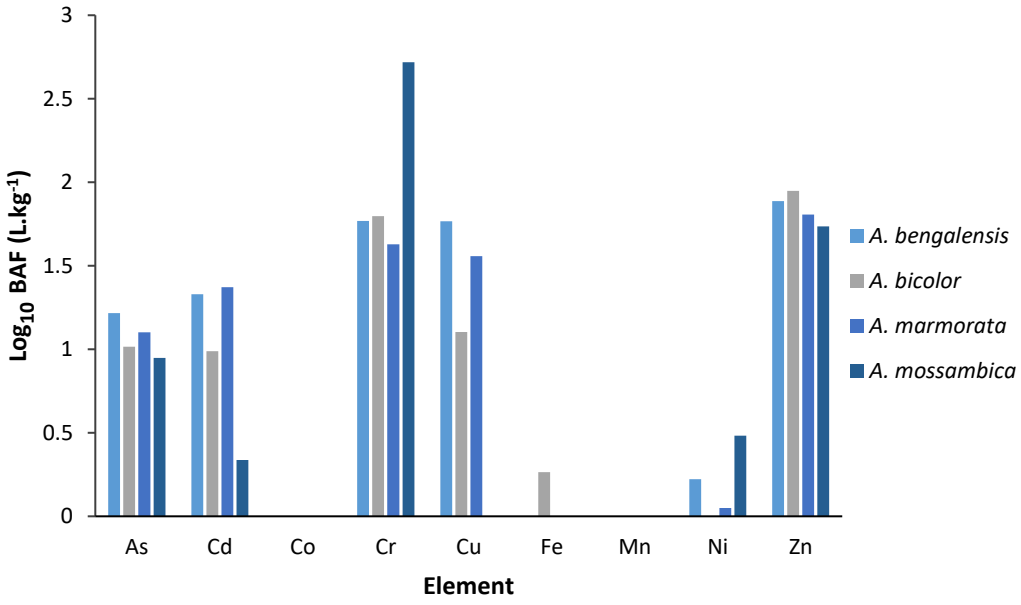


Figure 3: Log_{10}-transformed bioaccumulation factor (BAF; $L.kg^{-1}$) of heavy and trace metals measured in muscle tissues of four anguillid species

Liver copper BAF values were the highest, followed by zinc, chromium, cadmium, and arsenic. *A. mossambica* recorded a copper BAF twofold higher than that of *A. bicolor*. Most liver metal BAF values were below the recommended values for BAF in edible parts of freshwater fish (Karlsson *et al.,* 2002). However, copper BAF values in *Anguilla mossambica* (436.23 L/kg), *A. marmorata* (320.34 L/kg), and *A. bengalensis* (312.06 L/kg) were 2-fold greater than the recommended value for copper BAF in edible parts of freshwater fish (200 L/kg) (Karlsson *et al.,* 2002).

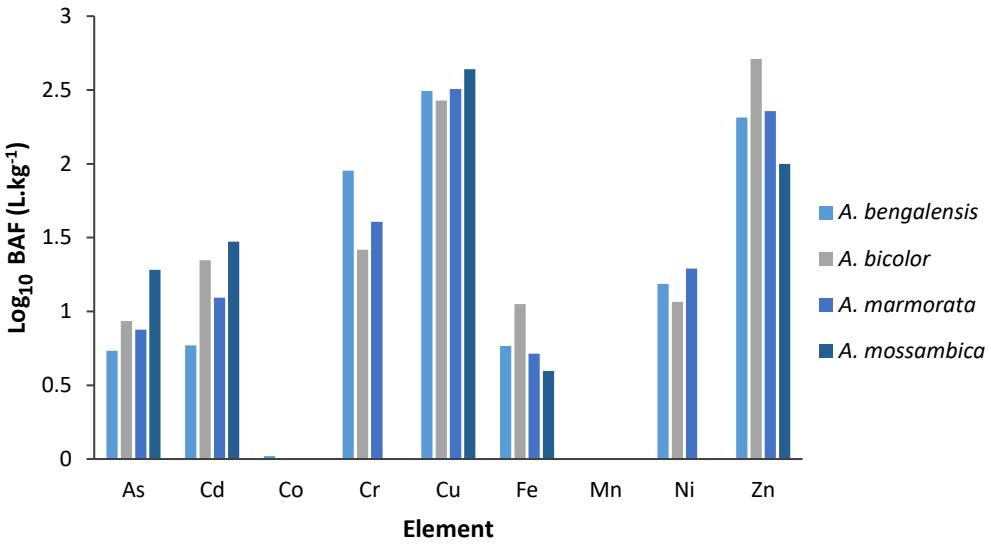


Figure 4: Log_{10}-transformed bioaccumulation factor (BAF; $L.kg^{-1}$) of heavy and trace metals in liver tissues of four anguillid species

4.9 Otolith Sr:Ca Signatures

Otolith elemental signatures were analysed to infer habitat use patterns among subsampled yellow eels (Table 14). Among the species, *A. bengalensis* had the highest number (13) of otoliths analysed, while *A. marmorata* and *A. mossambica* were each represented by a single specimen, limiting statistical comparisons for these two species. Significant differences were observed in the mean length values ($p \leq 0.05$). Eel otolith mean length, width, and weight were 2.42 mm, 1.48 mm, and 0.008 g, respectively. A comparison between *A. bengalensis* and *A. bicolor* revealed that *A. bicolor* otoliths were significantly longer (3.70 ± 0.30 mm) than those of *A. bengalensis* (2.99 ± 0.19 mm) ($p \leq 0.05$), although width and weight were statistically similar (Table 14).

Table 14. Dimensions (length, width) and weight of otoliths of yellow eel species collected from the rivers Athi-Galana-Sabaki and Ramisi, Kenya (values are presented as mean±SE)

Species	N	Length (mm)	Width (mm)	Weight (g)
A. bengalensis	13	$2.99\pm0.19_b$	$1.89\pm0.16_a$	$0.01\pm0.001_a$
A. bicolor	3	$3.70\pm0.30_a$	$2.03\pm0.03_a$	$0.01\pm0.002_a$
A. marmorata	1	2.00	1.00	0.01
A. mossambica	1	1.00	1.00	0.002

Means are ranked using the Tukey HSD test; means followed by similar letters are not significantly different ($p \geq 0.05$)

Sr:Ca ratios were determined for 18 otoliths to assess environmental histories based on strontium (Sr) and calcium (Ca) incorporation (Table 15). The mean Sr:Ca ratio across all samples was 0.169 ± 0.03. Sr:Ca variation was strongly influenced by the river system ($p \leq 0.001$), but not by species ($p \geq 0.05$). Otoliths from the Ramisi River exhibited markedly higher mean Sr:Ca ratios (0.263 ± 0.04) compared to those from the Athi-Galana-Sabaki River (0.042 ± 0.01), suggesting higher salinity exposure or estuarine residency in the Ramisi population.

Table 15: Mean Sr:Ca ratios in anguillid eel otoliths from River Athi-Galana-Sabaki and River Ramisi (values are presented as mean±SE)

Species	N	Strontium (Sr)	Calcium (Ca)	Sr:Ca
A. bengalensis	13			
		51.38±23.62	281.72±57.73	$0.175\pm0.04_a$
A. bicolor	3	62.90±25.55	403.52±173.02	$0.182\pm0.044_a$
A. marmorata	1	10.99	484.09	0.02_b
A. mossambica	1	3.72	17.89	0.21_b

Means are ranked using the Tukey HSD test; means followed by similar letters are not significantly different ($p \geq 0.05$)

Among species, Sr:Ca ratios ranged from a low of 0.023 in *A. marmorata* to a high of 0.182 ± 0.044 in *A. bicolor*. While interspecific differences were not statistically significant, *A. bicolor* and *A. bengalensis* showed marked contrasts in Sr:Ca values at the Athi River.

In contrast, *A. bengalensis* and *A. mossambica* exhibited similar elevated Sr:Ca ratios at Ramisi, implying shared use of more saline or estuarine habitats (Figure 5).

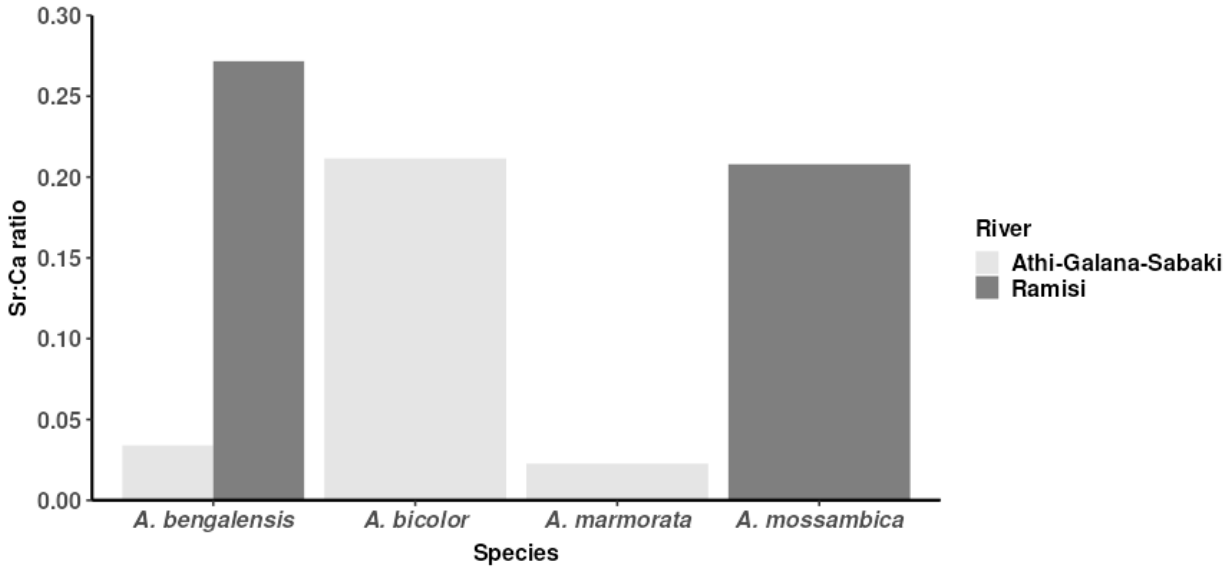


Figure 5: Otolith Sr:Ca signatures of anguillids: *Anguilla bengalensis*, *A. bicolor*, *A. marmorata*, and *A. mossambica* from River Athi-Galana-Sabaki and River Ramisi, Kenya

4.10 Correlation Analysis between Heavy Metal Loading and Otolith Sr:Ca Signatures in Anguillid Eels

4.10.1 Life Stage

Pearson correlation analysis revealed a relationship between heavy and trace metals content in muscle tissues and otolith Sr:Ca ratios among silver and yellow eels. Both muscle arsenic and cadmium concentrations had a negative correlation to the otolith Sr:Ca signatures among silver and yellow eels (Table 16). Notably, there was a significant association between the arsenic level in yellow eels and otolith Sr:Ca ratios ($p \geq 0.05$).

Table 16: Correlation coefficients (r) between muscle heavy metal concentrations and otolith Sr:Ca ratios in yellow and silver eels

Life Stage	N	As	Cd
Silver	4	-0.49	-0.46
Yellow	14	-0.03	-0.16

Iron (0.94) and manganese (0.89) concentrations showed the strongest positive correlation to otolith Sr:Ca ratios among silver eels (Table 17). These values were higher than those observed in silver eel copper (9-fold), cobalt (6-fold), as well as chromium and zinc (2-fold) contents, implying preferential uptake of iron and manganese along with the deposition of strontium and calcium in otoliths. The concentrations of cobalt (-0.22), copper (-0.09), iron (-0.05), nickel (-0.04), and zinc (-0.30) were inversely related to otolith Sr:Ca signatures among yellow eels (Table 17). Chromium (0.12) and manganese (0.07) levels were positively correlated with otolith Sr:Ca signatures in yellow eels. There was a significant association between nickel concentration and otolith Sr:Ca signatures (r = -0.04).

Table 17: Correlation coefficients (r) showing the relationship between muscle trace metal content and otolith Sr:Ca signatures in yellow and silver eels

Life Stage	N	Co	Cr	Cu	Fe	Mn	Ni	Zn
Silver	4	0.14	0.45	0.10	0.94	0.89	0.70	0.45
Yellow	14	-0.22	0.12	-0.09	-0.05	0.07	-0.04	-0.30

4.10.2 Species

There was a negative correlation between arsenic content and otolith Sr:Ca ratios among *A. bengalensis* (-0.17) and *A. bicolor* (-0.98) (Table 18). *A. bengalensis* cadmium concentration was inversely related to otolith Sr:Ca ratios (-0.28). However, there was a complete positive correlation (1.00) between cadmium concentration and otolith Sr:Ca ratios in *A. bicolor*. There was no significant correlation between arsenic and cadmium concentrations and otolith Sr:Ca ratios ($p \geq 0.05$).

Table 18: Correlation coefficients (r) showing the relationship between muscle heavy metal content and otolith Sr:Ca signatures in anguillid species

Species	N	As	Cd
A. bengalensis	13	-0.17	-0.28
A. bicolor	3	-0.98	1.00

Chromium (0.18), manganese (0.06), and nickel (0.04) concentrations were positively correlated to otolith Sr:Ca ratios in *A. bengalensis* (Table 19). Zinc (-0.45), cobalt (-0.21), iron (-0.05), and copper (-0.002) levels were inversely related to otolith Sr:Ca signatures *A. bengalensis*. Among *A. bicolor*, chromium (-1.00) and copper (-0.56) had a negative association with otolith Sr:Ca signatures, while nickel (0.87), manganese (0.69), iron (0.54), cobalt (0.34), and zinc (0.20) were positively correlated with otolith Sr:Ca signatures. However, there was a significant correlation between nickel (0.04) and copper (0.002) concentrations and otolith Sr:Ca ratios in *A. bengalensis* ($p \leq 0.05$).

Table 19: Correlation coefficients (r) showing the relationship between muscle trace metal content and otolith Sr:Ca signatures in anguillid species

Species	N	Co	Cr	Cu	Fe	Mn	Ni	Zn
A. bengalensis	13	-0.21	0.18	-0.002	-0.05	0.06	0.04	-0.45
A. bicolor	3	0.34	-1.00	-0.56	0.54	0.69	0.8	0.20

CHAPTER FIVE
DISCUSSIONS

5.1 Physicochemical Parameters

The water pH values recorded (6.7-8.1) in both rivers were within the recommended WHO range of 6.5-8.5 for drinking water (WHO, 2007). Sila (2019) reported a pH of 7.31±0.14 at Athi River, which is within the range of the results of this study, and the natural pH of water is 7. Higher salinity values were recorded in River Ramisi, likely as a result of seawater intrusion during high tides, as reported by Chalala *et al.* (2017). Water temperature was measured at different time periods to capture temporal variations.

Temperature impacts the biological activity and growth of aquatic flora and fauna, with variations from the preferred range resulting in the death and/or migration of fish due to stress (Monette *et al.,* 2006). Dissolved oxygen ranged from 6.79 mg/L to 4.87 mg/L, with a decrease observed in downstream and upstream locations of River Ramisi and River Athi-Galana-Sabaki, respectively, possibly due to the discharge of waste by riparian communities. This suggests that most of the discharge into the rivers is organic and hence requires oxygen for decomposition (EPA, 2024). Sufficient dissolved oxygen is vital for fish growth and reproduction (Murphy, 2006).

5.2 Heavy and Trace Metals in River Athi-Galana-Sabaki and River Ramisi

5.2.1 Heavy Metals in River Athi-Galana-Sabaki and River Ramisi

Heavy metal (As, Pb, and Cd) contamination in the River Athi-Galana-Sabaki arises from industrial effluent, the disposal of untreated sewage, and intensive agriculture upstream. Among heavy metals, cadmium concentration in River Athi-Galana-Sabaki was notably higher than in River Ramisi, perhaps as a result of the use of fertilisers in farms, particularly those derived from phosphate rock (Muiruri *et al.*, 2013), along the river. Phosphorite and apatite rocks used in the production of phosphate fertilisers contain cadmium, and the presence of cadmium in soil greatly depends on the use of phosphate fertilisers (Suciu *et al.*, 2022). The application of mineral phosphate (P) fertilisers is mainly responsible for the accumulation of cadmium in agricultural soils and river basins (Parkh *et al.,* 2021). In addition, pesticides applied in farms comprise heavy metals either as impurities or active ingredients (Lewis *et al.,* 2016; Alnuwaiser, 2019).

River Ramisi recorded a higher arsenic mean concentration of 0.1925 mg/L as opposed to the mean arsenic concentration of 0.1835 mg/L observed in River Athi-Galana-Sabaki. These values were lower than the mean concentration in the Nairobi River, which ranged from 0.174 mg/L to 0.001 mg/L (Njuguna *et al.,* 2017). Notably, the levels in both rivers were higher than the recommended limit of 0.01 mg/L in drinking water (WHO, 2004). Arsenic pollution results from natural processes and the use of arsenic-containing compounds, such as copper arsenite, applied as an insecticide and wood preservative (ATSDR, 2000; Bencko & Slámová, 2007).

River Athi-Galana-Sabaki recorded a higher mean value of cadmium (0.035 mg/L), contrary to 0.033 mg/L observed in River Ramisi. These values were higher than the value (0.0012 mg/L) recorded in the Nairobi River (Njuguna *et al.,* 2017). Additionally, cadmium levels in both rivers were higher than the recommended limit (0.03 mg/L) in drinking water (WHO, 2004). This is likely as a result of mining activities and the use of commercial phosphate fertilisers in agricultural areas (Ngo *et al.,* 2005; Parkh *et al.,* 2021).

5.2.2 Trace Metals in River Athi-Galana-Sabaki and River Ramisi

River Ramisi recorded higher mean concentrations of chromium, copper, manganese, and nickel at the upstream location, possibly due to the influence of industrial effluents from KISCOL and agricultural waste from sugarcane cultivation. River Ramisi recorded higher cobalt concentration levels (0.6785 mg/L) than River Athi-Galana-Sabaki (0.272 mg/L). Cobalt is an essential element and is required as a key component of Vitamin B12, as the body cannot synthesise it (Valko *et al.,* 2005). It occurs in association with copper, manganese, nickel, and arsenic and is not only found naturally in the environment but is also released to the environment by anthropogenic activities (Saad *et al.,* 2014).

The mean copper concentration observed in River Athi-Galana-Sabaki (0.004 mg/L) and River Ramisi (0.003 mg/L) did not exceed the WHO limits (1.00 mg/L) in drinking water (WHO, 2004). Studies by Njuguna *et al.* (2017) found higher copper values in waters of the Nairobi River that ranged from 0.006 to 0.01 mg/L. Copper is a cofactor of many enzymes involved in redox reactions (Carolyn *et al.,* 2004). It is released into the environment during soil weathering of soil, and as a result of sewage treatment plants and industrial discharge (Hutchinson, 2002). Extensive use of pesticide sprays in farms leads to copper contamination in surface water (Al-Weher, 2008).

Iron concentration in River Ramisi (0.713 mg/L) was below the recommended limit of 2 mg/L in drinking water (WHO, 2004). This was lower than the values recorded in the Nairobi River (2.476-0.008 mg/L) as reported by Njuguna *et al.* (2017). Iron in aquatic ecosystems originates from weathering of rocks, steel production, and industrial effluents (Hossain *et al.,* 2023).

The mean manganese concentration level (0.167 mg/L) in River Athi-Galana-Sabaki did not exceed the recommended limit of 0.4 mg/L for drinking water (WHO, 2008). However, River Ramisi recorded values (1.213 mg/L) higher than the WHO-recommended limit for drinking water (WHO, 2008). Muiruri *et al.* (2013) reported the presence of manganese in River Athi (0.088 - 1.048 mg/L), lower than the mean values observed in both rivers during this study. Manganese occurs in conjunction with iron and serves as an enzyme cofactor (Aschner & Aschner, 2005). It enters rivers from the disposal of waste during glass, dry-cell batteries, and fertiliser manufacturing (Aschner & Aschner, 2005).

The mean concentration of nickel in River Ramisi (0.565 mg/L) was higher than the recommended limit (0.07 mg/L) for drinking water (WHO, 2008) and the values found in River Athi, which ranged from 0.008 mg/L to 0.062 mg/L (Muiruri *et al.,* 2013). Nickel contamination in the environment results from mining, domestic wastewater, and industrial discharges (Begum *et al.,* 2022).

The concentration of zinc in both River Athi-Galana-Sabaki (0.017 mg/L) and River Ramisi (0.198 mg/L) did not exceed the recommended limit (3 mg/L) in drinking water (WHO, 2008). Muiruri *et al.* (2013) reported zinc presence in River Athi (0.010 - 0.695 mg/L), possibly as a result of leaching from fertilisers, runoff, and industrial waste from mining and smelting activities as well as municipal sewage (Damodharan, 2013).

The mean concentration of chromium in River Ramisi (0.009 mg/L) was within the levels observed in the Nairobi River that ranged between 0.033 mg/L and 0.001 mg/L (Njuguna *et al.,* 2017). Furthermore, it did not exceed the recommended limit for chromium (0.05 mg/L) in drinking water (WHO, 2008). Key sources of chromium include waste from municipal sewage sludge and leather manufacturing (Rahman *et al.,* 2012).

Lead was only detected at the upper reaches of the River Ramisi, possibly due to industrial effluent disposal and artisanal sand mining along the river. The concentration recorded, 0.005 mg/L, was below the recommended limit of 0.01 mg/L in drinking water (WHO, 2008). Lead concentration in water increases primarily through human activities; thus, likely sources of Pb in rivers are municipal and industrial wastes, runoff, and soil erosion (Ako *et al.*, 2014). Ako *et al.* (2014) reported high concentrations of copper, nickel, arsenic, cadmium, and lead in soil samples collected in sand mining areas.

5.2.3 Point Sources of Water Pollution

The quality of water across the Athi-Galana-Sabaki basin is heavily affected by point and non-point sources of pollution, with the non-point sources associated with land management and utilisation (Ministry of Water, Sanitation and Irrigation, 2020). Pollution from towns, domestic sewage, municipal and industrial effluents raises concern. Nairobi at the headwaters of the River Athi poses the greatest challenge to the management of water quality due to the disposal of untreated or partially treated domestic sewage and industrial effluent (Ministry of Water, Sanitation and Irrigation, 2020). Further away from the city, the river contains fewer pollutants as a result of the river's natural filtration and assimilative capacity, and dilution effects. However, sediment deposition is greater along the river's lower reaches. In general, the point sources of water pollution in the Athi Basin are classified as:

Upper Athi: Industries discharge their waste into the Athi River. In addition, effluents from Dandora and Kariobangi, as well as Kiambu, Kahawa West, Thika, and Limuru sewage treatment works, are discharged into tributaries of the Athi River, including the Nairobi, Riara, Kiu, and Komo rivers, as well as the Ithanji stream. Agrochemical leachates from farms in Kiambu and Thika also find their way into the river (Ministry of Water, Sanitation and Irrigation, 2020).

Middle Athi: Domestic sewerage and effluents from activities in Machakos, Kitui, Makueni, Tsavo Lodges, Taita Taveta, and Voi towns, as well as septic tank leakage in areas such as Utawala, Rongai, Athi River, Kasarani, Ruiru, and Muthurwa, all contribute to the degradation of water quality. Sediment from soil erosion resulting from overgrazing and runoff from rural roads additionally reaches the river (Ministry of Water, Sanitation and Irrigation, 2020).

Lower Athi: Pollution in the lower Athi basin arises from effluents produced by waste from Kwale, Malindi, Mombasa, and Kilifi towns, which discharge into the Indian Ocean (Ministry of Water, Sanitation and Irrigation, 2020).

Studies have shown that River Ramisi is highly mineralised (Chalala *et al.,* 2017) and flows through the Kwale International Sugar Company Limited (KISCOL), formerly known as the Ramisi Sugar Factory (Oteko, 1986). Kwale International Sugar Company Limited (KISCOL) and its 15,000-acre farm are located downstream of the Ramisi River (KCIDP, 2018). Oteko (1986) reported the discharge of effluents from the Ramisi Sugar Factory into the River Ramisi. Furthermore, sugarcane production has been found to pollute freshwater ecosystems with fertilisers washed from farms and chemical sludge from sugarcane mills (WWF, 2004).

In this study, heavy metals were detected in water; however, higher concentrations were observed in the sediment. In aquatic systems, sediments act as reservoirs for heavy metals due to their low solubility in water and strong tendency to bind to sediment particles (Singh *et al.,* 2005; Sojka & Jaskula, 2022). Badawy *et al.* (2021) reported that sediments exhibit less variation than dissolved metals in overlying water columns, making them a more reliable medium for monitoring.

5.3 Heavy Metal Contamination and Trace Elements in Anguillids

Fish utilise energy not only from food intake but also from muscle tissue reserves, mainly stored as fat, protein, and carbohydrate, for gonad development (Kamler, 1992). Tembo et al. (2023) reported that eels in the Athi-Galana-Sabaki and Ramisi rivers feed on benthic fauna such as prawns, penaeid shrimp, and crabs. Benthic organisms absorb sediment-associated contaminants, such as heavy metals, and their consumption (for instance, by eels) results in biomagnification through the food chain (Oremo *et al.,* 2019). Anguillids use up muscle energy reserves to meet energy requirements during spawning migration (Le *et al.,* 2012). This may account for the higher heavy metal levels in silver eels, raising concern as these toxic metals can accumulate to hazardous concentrations, rendering anguillid eels unsafe for human consumption.

Canli and Atli (2003) reported that heavy metal concentration in the surrounding water and the duration of exposure influence the accumulation of heavy metals in fish tissue. Differences in the heavy metal concentration of muscle tissues among eel life stages may have several causes, for instance, the age and size of the fish, habitat (man-made or natural sources), and seasonal differences (Le *et al.,* 2009).

Le *et al.* (2010) reported significantly higher (approximately 3-fold) copper, zinc, and manganese concentrations in mature eels as opposed to immature eels. Several heavy metal levels in both *Anguilla bicolor* and *Anguilla marmorata* were less than those observed in other countries.

Silver eels had significantly greater concentrations of copper, iron, zinc, and nickel compared to yellow eels. These trace metals play key roles in animal metabolic processes. The high trace metal concentrations detected in the silver eel muscle tissues might be related to the demand for maturation of gonads and migration (Le *et al.,* 2012). Arai *et al.* (2012) reported higher copper, nickel, zinc, and cadmium mean concentration levels in *Anguilla bicolor* as opposed to the eels captured in River Athi-Galana-Sabaki and River Ramisi. The mean concentration of iron in *Anguilla bicolor* in Kenya was within the mean values found in Malaysia (Arai *et al.,* 2012). *Anguilla marmorata* recorded lower concentrations of cadmium, cobalt, copper, manganese, zinc, and chromium as opposed to those observed in Vietnam (Le *et al.,* 2012).

Chromium and nickel concentrations of *A. bengalensis* from this study were 2-fold and 13-fold higher than the levels observed in *A. bengalensis* from Pakistan (Mehmood *et al.,* 2020). Additionally, the concentration of copper in *A. bengalensis* from Kenya was over 100-fold higher than that of Pakistani *A. bengalensis* (Mehmood *et al.,* 2020). However, cadmium was below the detection limit (BDL) in *A. bengalensis* from Pakistan.

Pollutant concentrations in the environment are often below acute toxicity levels for eels; however, as other migratory fish species have shown, sublethal concentrations impact eel physiology (McCormick *et al.,* 2005). Additionally, it is believed that silver eels cease feeding during their transoceanic spawning migration, implying that mobilisation of accumulated pollutants occurs when the fat reserves are broken down (Robinet & Feunteun, 2002). This in turn influences migration and reproduction success, as well as the quality and survival of larvae (van Ginneken *et al.,* 2009).

The bioaccumulation factor (BAF) is defined as the ratio of metal concentration in aquatic organisms ($C_n$biota) to that in the surrounding water ($C_n$water), providing an estimate of potential risks to biota from metal exposure. BAF values observed in this study were higher in liver tissues than in muscle tissues. Copper and zinc were the most frequently bioaccumulated metals in liver tissue, while chromium and zinc predominated in muscle tissue.

However, the ratio of internal to external metal concentrations does not wholly capture uptake, depuration, nutrient essentiality, and detoxification mechanisms via internal sequestration (Karlsson *et al.,* 2002). For instance, high copper and zinc concentrations in the livers of the eel may arise from physiological demands for growth, rather than contamination.

5.4 Otolith Sr:Ca Signatures

Otolith Sr:Ca ratios serve as reliable indicators of habitat utilisation and migratory history in anguillid eels (Shiao *et al.,* 2006). Strontium (Sr) and calcium (Ca) are co-deposited in the otolith matrix, with the Sr:Ca ratio reflecting the salinity of the surrounding water, since seawater comprises Sr levels approximately 4.8 times higher than freshwater (Campana, 1999). Studies have used this ratio to reconstruct the life histories of *A. japonica* and *A. anguilla* (Elsdon & Gillanders, 2003; Shiao *et al.,* 2006).

Otolith Sr:Ca signatures from Ramisi River eels (0.263 ± 0.04) were over fourfold higher than those from Athi-Galana-Sabaki River eels (0.042 ± 0.01). This proposes increased exposure to salinity at upstream and downstream sampling locations along River Ramisi. Sr:Ca ratios varied among species, with *Anguilla mossambica* recording the highest mean Sr:Ca ratio (0.208), which was approximately tenfold higher than that of *A. marmorata* (0.023). *A. bengalensis* (0.175) and *A. bicolor* (0.182) exhibited equally elevated Sr:Ca values, suggesting that different species exploit saline environments to varying degrees.

All four species in this study had Sr:Ca signatures thirtyfold greater than those found in *A. japonica* (Tzeng *et al.,* 2002) and *A. anguilla* (Shiao *et al.,* 2006). Tzeng *et al.* (2002), using Sr:Ca thresholds, classified *A. japonica* habitats into three: freshwater (0.004), seawater (0.0051), and estuarine (0 - 0.01). Similarly, Shiao *et al.* (2003) reported that *A. marmorata* and *A. japonica* exhibited Sr:Ca ratios of 0.0020 and 0.0037, respectively, which were much lower than those observed in this study. These Sr:Ca signatures suggest a distinct regional signature in eels from the Western Indian Ocean or extended exposure to estuarine or saline environments. The four studied species exceeded the critical otolith Sr:Ca ratio (0.00377) used to differentiate freshwater residents from habitat shifters (Lin *et al.,* 2012, 2015; Milton *et al.,* 2008), suggesting non-exclusivity in relation to freshwater residency.

Sr:Ca ratios in the yellow eels are largely governed by the salinity of the surrounding water (Marohn *et al.,* 2009); however, physiological factors impact otolith composition during early development (Lin *et al.,* 2005). This study suggests that habitat use in Kenyan anguillids may reflect facultative catadromy, a migratory flexibility observed in eels across Europe (*A. anguilla*), North America (*A. rostrata*), and Asia (*A. japonica*, *A. bicolor pacifica*, *A. marmorata*) (Chino & Arai, 2010a, 2010b; Jessop *et al.,* 2008; Lin *et al.,* 2012; Tsukamoto *et al.,* 1998). Nonetheless, migratory life histories of WIO anguillids remain poorly understood. The consistently high Sr:Ca ratios reported here underscore the need for further investigations into the facultative nature of catadromy in WIO eel populations.

5.5 Correlation Analysis between Heavy Metal Loading and Otolith Sr:Ca signatures in Anguillid Eels

The negative correlations between the average concentrations of arsenic and cadmium, and otolith Sr:Ca signatures, suggest there is no association between arsenic and cadmium loading and the deposition of strontium and calcium on otoliths. The positive relationship between mean concentrations of manganese, chromium, iron, and nickel, and otolith Sr:Ca signatures suggests concurrent deposition of strontium and calcium alongside these trace elements in otoliths.

CHAPTER SIX
SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary of Findings

This study demonstrated that the Athi-Galana-Sabaki and Ramisi rivers are contaminated with heavy and trace metals, with water concentrations of arsenic, cadmium, and lead exceeding WHO limits by more than tenfold and sediments showing cobalt and nickel levels above EPA freshwater benchmarks. River Ramisi recorded higher trace metal loads, while Athi-Galana-Sabaki reflected urban and industrial inputs. Heavy and trace metal bioaccumulation exceeded FAO/WHO food safety limits, with silver eels carrying the highest burdens; however, all species and life stages were affected. These results show a relationship between heavy metal contamination and habitat utilisation, calling attention to the ecological vulnerability of eels, the role of sediments as metal reservoirs, and the potential health risks to consumers.

6.2 Conclusions

The following conclusions were drawn from this study:

i. Anguillids from River Athi-Galana-Sabaki showed higher levels of cadmium, copper, and zinc, compared to those from River Ramisi. The elevated concentrations, particularly in silver eel tissues, indicate the bioaccumulative nature of heavy and trace metals and suggest more anthropogenic impact in the River Athi-Galana-Sabaki.

ii. Otolith Sr:Ca signatures revealed that anguillid species and life stages utilise the two rivers differently. Lower Sr:Ca ratios in eels from River Athi-Galani-Sabaki indicate longer freshwater residency, while higher ratios in eels from River Ramisi suggest greater exposure to brackish or marine waters. These variations support the use of Sr:Ca ratios as reliable markers of transitions in habitat and salinity profiles in the environment.

iii. The relationship between heavy metal loading and otolith Sr:Ca signatures revealed complex interactions; manganese, chromium, iron, and nickel exhibited positive correlations with Sr:Ca ratios, indicating co-exposure in saline environments. However, negative correlations between Sr:Ca ratios and arsenic as well as cadmium suggest that these metals originate from anthropogenic sources in the rivers. This shows how otolith chemistry can enhance studies on heavy metals to determine exposure pathways and environmental histories in anguillid eels.

6.3 Recommendations

Taking into account the limited sample size used in this study, subsequent research should focus on employing larger datasets to enhance statistical robustness and reliability of inferences. The recommendations include:

i. Enhanced monitoring and mitigation of anthropogenic pollution in River Athi-Galana-Sabaki: To control pollution in River Athi-Galana-Sabaki, intensified monitoring and strengthened regulatory enforcement are recommended, considering the high levels of cadmium and arsenic in River Athi-Galana-Sabaki detected.

ii. Incorporation of otolith microchemistry in long-term fisheries and environmental monitoring: Integrating otolith Sr:Ca signatures with routine ecological monitoring offers information on habitat connectivity and enables effective conservation planning for migratory fishes in freshwater and estuarine environments.

iii. Research on mechanisms of heavy metal uptake and the effect of heavy metals on anguillid eel life stages: Further studies should focus on anguillid heavy metal uptake and detoxification pathways, especially during silvering.

6.4 Suggestions for Further Study

The following suggestions highlight areas for additional research to expand on the current work as well as inform management and policy:

i. Long-term tracking of heavy and trace metal concentrations across seasons and river catchments to identify trends and variability.

ii. Investigation of metal transfer and biomagnification in the aquatic food web from primary producers to trophic levels higher than anguillid eels.

iii. Use of otolith microchemistry, tagging, and telemetry to link migration routes, habitat use, and metal exposure across eel life stages.

REFERENCES

Agah, H., Leermakers, M., Elskens, M., Fatemi, S. M. R., Baeyens, W. (2009). Accumulation of trace metals in the muscles and liver of five fish species from the Persian Gulf. *Environmental Monitoring and Assessment, **157**,* 499-514.

Al-Weher, S. M. (2008). Levels of heavy metal Cd, Cu, and Zn in three fish species collected from the northern Jordan valley. *Jordan Journal of Biological Sciences, **1***(1), 41-46.

Aoyama, J. (2009). Life history and evolution of migration in catadromous eels (genus *Anguilla*). *Aqua-BioScience Monographs, **2***, 1-42.

Arai, T., Limbong, D., Otake, T., Tsukamoto, K. (2001). Recruitment mechanisms of tropical eels, *Anguilla* spp., and implications for the evolution of oceanic migration in the genus Anguilla. *Marine Ecology Progress Series, **216***, 253-264.

Arai, T., Kotake, A., Ohji, M., Miller, M. J., Tsukamoto, K., Miyazaki, N. (2003). Occurrence of sea eels of Anguilla japonica along the Sanriku Coast of Japan. *Ichthyological Research*, ***50***(1), 0078-0081.

Arai, T. & Chino, N. (2017). Influence of water salinity on the strontium: calcium ratios in otoliths of the giant mottled eel, *Anguilla marmorata. Environmental Biology of Fishes, **100***, 281-286.

Arai, T., Chai, I. J., Iizuka, Y., Chang, C. W. (2020). Habitat segregation and migration in tropical anguillid eels, *Anguilla bengalensis* and *A. bicolor*. *Scientific Reports, **10**.*

ATSDR. (2000). Toxicological profile for arsenic TP-92/09. Center for Disease Control, Agency for Toxic Substances and Disease Registry, Atlanta, GA.

Badawy, W. M., Duliu, O. G., El Samman, H., El-Taher, A., Frontasyeva, M. V. (2021). A review of major and trace elements in Nile River and Western Red Sea sediments: An approach of geochemistry, pollution, and associated hazards. *Applied and Radiation Isotopes, **170**,* 109595.

Baker, T. J., Tyler, C. R., Galloway, T. S. (2014). Impacts of metal oxide nanoparticles on marine organisms. *Environmental Pollution, **186**,* 257-271.

Balali-Mood, M., Naseri, K., Tahergorabi, Z., Khazdair, M. R., Sadeghi, M. (2021). Toxic mechanisms of five heavy metals: mercury, lead, chromium, cadmium, and arsenic. *Frontiers in Pharmacology*, ***12***, 643972.

Bannon, D. I., Olivi, L., Bressler, J. (2000). The role of anion exchange in the uptake of Pb by human erythrocytes and Madin-Darby canine kidney cells. *Toxicology*, ***147(2)***, 101-107.

Batty, J. & Pain, D. (1996). Metal concentrations in eels *Anguilla anguilla* from Camargue region of France. *Biological Conservation,* ***76***, 17-23.

Bearhop, S., Waldron, S., Votier, S. C., Furness, R. W. (2002). Factors that influence assimilation rates, and fractionation of nitrogen and carbon isotopes in avian blood and feathers. *Physiological and Biochemical Zoology,* ***75***, 451-458.

Belpaire, C. & Goemans, G. (2007a). The European eel *Anguilla anguilla*, a rapporteur of the chemical status for the Water Framework Directive? *Vie Et Milieu - Life & Environment*, ***57***, 235-252.

Belpaire, C. & Goemans, G. (2007b). Eels: contaminant cocktails pinpointing environmental contamination. *ICES Journal of Marine Science,* ***67***(4), 1423-1436.

Belpaire, C., Pujolar, J. M., Geeraerts, C., Maes, G. E. (2016). Contaminants in Eels and their Role in the Collapse of the Eel Stocks. In: *Biology and Ecology of Anguillid Eels*. (ed T. Arai), CRC Press, 225-250.

Belpaire, C., Hodson, P., Pierron, F., Freese, M. (2019). Impact of chemical pollution on Atlantic eels: Facts, research needs, and implications for management. *Current Opinion in Environmental Science & Health*, ***11***, 26-36.

Benchetrit, J., Beguer-Pon, M., Sirois, P., Castonguay, M., Fitzsi-mons, J., Dodson, J. J. (2017). Using otolith microchemistry to reconstruct habitat use of American eels Anguilla rostrata in the St. Lawrence River-Lake Ontario system. *Ecology of Freshwater Fish,* ***26***(1)*,* 19-33.

Bertinato, J., Swist, E., Plouffe, L. J., Brooks, S. P., L'abbé, M. R. (2008). Ctr2 is partially localized to the plasma membrane and stimulates copper uptake in COS-7 cells. *Biochemical Journal*, ***409***(3), 731-740.

Bervoets, L. & Blast, R. (2003). Metal concentrations in water, sediment and gudgeon (*Gobio gobio*) from a pollution gradient: relationship with fish condition factor. *Environmental Pollution,* ***126****,* 9-19.

Bervoets, L., Knaepkens, G., Eens, M., Blast, R. (2005). Fish community responses to metal pollution. *Environmental Pollution,* ***138****,* 338-349.

Biswas, J. C., Haque, M. M., Maniruzzaman, M., Kalra, N. (2021). Coastal and marine pollution in Bangladesh: Pathways, hotspots and adaptation strategies. *European Journal of Environment and Earth Sciences*, ***2***(4), 26-34.

Bodin, N., Tapie, N., Le Ménach, K., Chassot, E., Elie, P., Rochard, E., Budzinski, H. (2014). PCB contamination in fish community from the Gironde estuary (France): blast from the past. *Chemosphere,* ***98***, 66-72.

Bressler, J. P., Olivi, L., Cheong, J. H., Kim, Y., Bannona, D. (2004). Divalent metal transporter 1 in lead and cadmium transport. *Annals of the New York Academy of Sciences*, ***1012***, 142-152.

Bruslé, J. (1990). Effects of heavy metals on eels, *Anguilla* sp. *Aquatic Living Resources,* ***3***(2), 131-141.

Carlson, E. & Zelikoff, J. T. (2008). The immune system of fish: a target organ of toxicity. In: Di Giulio, R. T., Hinton, D. E. (Eds). *The Toxicology of Fishes.* Taylor and Francis; Washington DC, 489-530.

Campana, S. E. (1999). Chemistry and composition of fish otoliths: pathways, mechanism and applications. *Marine Ecology Progress Series,* ***188***, 263-297.

Campana, S. E. & Thorrold, S. R. (2001). Otoliths, increments, and elements: keys to a comprehensive understanding of fish populations? *Canadian Journal of Fisheries and Aquatic Sciences,* ***58***, 30-38.

Campana, S. E. (2005). Otolith science entering the 21st century. *Marine and Freshwater Research,* ***56,*** 485-495.

Carolyn, S., Bentivegna, J.A., Sean M. B., Katarzyna, C. (2004). Influence of sediment characteristics on heavy metal toxicity in an urban marsh, Department of Biology, Seton Hall University, South Orange, New Jersey, 07079.

Caut, S., Angulo, E., Courchamp, F. (2009). Variation in discrimination factors (Δ15N and Δ13C): the effect of diet isotopic values and applications for diet reconstruction. *Journal of Applied Ecology,* ***46,*** 443-453.

Cazorla, A. L. (2000). Age structure of the population of weakfish *Cynoscion guatucupa* (Cuvier) in the Bahia Blanca waters, Argentina. *Fisheries Research,* ***46***, 279-286.

Celino, F. T., Yamaguchi, S., Miura, C., Miura, T. (2009). Arsenic inhibits in vitro spermatogenesis and induces germ cell apoptosis in Japanese eel (Anguilla japonica). Reproduction, ***138***(2), 279-287.

Costa, M. (1997) Toxicity and carcinogenicity of Cr VI in animal models and humans. *Critical Reviews in Toxicology*, ***27***, 431-442.

Cresci, A., Durif, C., Shema, S., Skiftesvik, A., Browman, H. (2019). Glass eels (Anguilla anguilla) imprint the magnetic direction of tidal currents from their juvenile estuaries. *Communications Biology*, **2**, 366.

Cresci, A. (2020). A comprehensive hypothesis on the migration of European glass eels (*Anguilla anguilla*). *Biological Reviews,* ***95*** (5), 1273-1286.

Crossgrove, J. S., Allen, D. D., Bukaveckas, B. L., Rhineheimer, S. S., Yokel, R. A. (2003). Manganese distribution across the blood–brain barrier: I. Evidence for carrier-mediated influx of manganese citrate as well as manganese and manganese transferrin. *Neurotoxicology*, ***24***(1), 3-13.

Damek-Proprawa, M. & Sawicka-Kapusta, K. (2003). Damage to the liver; kidney and testis with reference to burden of heavy metals in yellow-necked mice from areas around steelworks and zinc smelters in Poland. *Toxicology,* ***186,*** 1-10.

Degens, E. T., Deuser, W. G., Haedrich, R. L. (1969). Molecular structure and composition of fish otoliths. *Marine Biology,* ***2,*** 105-113.

DeNiro, M. & Epstein, S. (1981). Influence of diet on the distribution of nitrogen isotopes in animals. *Geochim Cosmochim Acta,* ***45***, 341-351.

Drouineau, H., Durif, C., Castonguay, M., Mateo, M., Rochard, E., Verreault, G., Yokouchi, K., Lambert, P. (2018). Endangered eels: a symbol of the effects of global change. *Fish and Fisheries,* ***19***, 903-930.

Ebrahimi, M. & Taherianfard, M. (2011). The effects of heavy metals exposure on reproductive systems of cyprinid fish from Kor River. *Iranian Journal of Fisheries Sciences*, ***10***(13).

Ewers, U. & Schlipöter, H. W. (1991) 'Lead', in Merian E. (ed.), Metals and Their Compounds in the Environment - Occurrence: Analysis and Biological Significance, Verlagsgesellschaft, Weinheim, Germany, 971-1014.

Elsdon, T. S. & Gillanders, B.M. (2003). Reconstructing migratory patterns of fish based on environmental influences on otolith chemistry. *Reviews in Fish Biology and Fisheries*, ***13,*** 217–235.

Environmental Protection Agency (EPA). (2006). Region III BTAG Freshwater Sediment Screening Benchmarks 8/2006.

Fabbri, E., Caselli, F., Piano, A., Sartor, G., Capuzzo, A. (2003). Cd and Hg affect glucose release and cAMP-dependent transduction pathway in isolated eel hepatocytes. *Aquatic Toxicology,* ***62****(1),* 55-65.

Fonseca, V. F., Vasconcelos, R. P., Franca, S., Serafim, A., Lopes, B., Company, R., Bebianno, M. J., Costa, M. J., Cabral, H. N. (2014). Modeling fish biological responses to contaminants and natural variability in estuaries. *Marine Environmental Research,* ***96***, 45-55.

Fry, B. (1999). Using stable isotopes to monitor watershed influences on aquatic trophodynamics. *Canadian Journal of Fisheries and Aquatic Sciences,* ***56****,* 2167-2171.

Fujishiro, H., Kubota, K., Inoue, D., Inoue, A., Yanagiya, T., Enomoto, S., Himeno, S. (2011). Cross-resistance of cadmium-resistant cells to manganese is associated with reduced accumulation of both cadmium and manganese. *Toxicology*, ***280***(3), 118-125.

Garcia-Aranda, J. A., Wapnir, R. A., Lifshitz, F. (1983). In vivo intestinal absorption of manganese in the rat. *The Journal of Nutrition*, ***113***(12), 2601-2607.

Generalova, A., Davidova, S., Satchanska, G. (2025). The Mechanisms of Lead Toxicity in Living Organisms. *Journal of Xenobiotics,* ***15(5)***, 146.

Gruenheid, S., Cellier, M., Vidal, S., Gros, P. (1995). Identification and characterization of a second mouse Nramp gene. *Genomics*, ***25***(2), 514-525.

Halldorsson, T. I., Meltzer, H. M., Thorsdottir, I., Knudsen, V., Olsen, S. F. (2007). Is high consumption of fatty fish during pregnancy a risk factor for fetal growth retardation? A study of 44,824 Danish pregnant women. *American Journal of Epidemiology,* ***166***, 687-696.

Hansson, S., Hobbie, J. E., Elmgren, R., Larsson, U., Fry, B., Johansson, S. (1997). The stable nitrogen isotope ratio as a marker of food-web interactions and fish migration. *Ecology,* ***78***, 2249-2257.

Hill, N. A., Simpson, S. L., Johnston, E. L. (2013). Beyond the bed: effects of metal contamination on recruitment to bedded sediments and overlying substrata. *Environmental Pollution*, ***173***, 182-191.

Hobson, K. A., Alisauskas, R. T., Clark, R. G. (1993). Stable-nitrogen isotope enrichment in avian tissues due to fasting and nutritional stress: implications for isotopic analysis of diet. *Condor,* ***95****,* 388-394.

Hogstrand, C. & Haux, C. (2001). Binding and detoxification of heavy metals in lower vertebrates with reference to metallothionein. *Comparative Biochemistry and Physiology Part C,* ***100****,* 137-141.

Hossain, M. A., Inzamul, M. H., Parvin, M. A., Islam, M. N. (2023). Evaluation of iron contamination in groundwater with its associated health risk and potentially suitable depth analysis in Kushtia Sadar Upazila of Bangladesh. *Groundwater for Sustainable Development*, ***21***, 100946.

ICES. (2016). Report of the Workshop of the Working Group on Eel and the Working Group on Biological Effects of Contaminants. *ICES CM 2015/SSGEPD,* ***20***, 98.

Jacoby, D. M. P., Casselman, J. M., Crook, V., De Lucia, M. B., Ahn, H., Kaifu, K., Kurwie, T., Sasal, P., Silfvergrip, A. M. C., Smith, K. G., Uchida, K., Walker, A. M., Gollock, M. J. (2015). Synergistic patterns of threat and the challenges facing global anguillid eel conservation. *Global Ecology and Conservation,* ***4,*** 321-333.

Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B. B., Beeregowda, K. N. (2014). Toxicity, mechanism and health effects of some heavy metals. *Interdisciplinary Toxicology*, ***7***(2), 60-72.

Jeong-Chae, L., Young-Ok, S., Poyil, P., Xianglin, S. (2012). Oxidative stress and metal carcinogenesis. *Free Radical Biology and Medicine*, ***53***(4), 742-757.

Jessop, B. M., Shiao, J. C., Iizuki, Y., Tzeng, W. N. (2002). Migratory behaviour and habitat use by American eels *Anguilla rostrata* as revealed by otolith microchemistry. *Marine Ecology Progress Series,* ***233***, 217-229.

Jessop, B. M., Cairns, D. K., Thibault, I., Tzeng, W. N. (2008). Life history of American eel Anguilla rostrata: new insights from otolith microchemistry. *Aquatic Biology*, ***1***(3), 205-216.

Jespersen, P. (1942). *Indo-Pacific Leptocephalids of the genus Anguilla-Systematic and biological studies*. Copenhagen: Carlsberg Foundation.

Kafemann, R., Alderstein, S., Neukamm, R. (2000). Variation in otolith strontium and calcium ratios as an indicator of life-history strategies of freshwater fish species within a brackish water system. *Fisheries Research,* ***46***, 313-325.

Kannurpatti, S. S., Joshi, P. G., Joshi, N. B. (2000). Calcium sequestering ability of mitochondria modulates influx of calcium through glutamate receptor channel. *Neurochemical Research*, ***25***(12), 1527-1536.

Kasperczyk, A., Kasperczyk, S., Horak, S., Ostałowska, A., Grucka-Mamczar, E., Romuk, E., Birkner, E. (2008). Assessment of semen function and lipid peroxidation among lead exposed men. *Toxicology and Applied Pharmacology*, ***228***(3), 378-384.

KCIDP. (2018). Kwale County Integrated Development Plan (KCIDP), 246.

Kerstan, M. (2000). Estimation of precise ages from the marginal increment widths of differently growing sardine *(Sardinops sagax)* otoliths. *Fisheries Research,* ***46,*** 207-225.

Khumbanyiwa, D. D., Li, M. M., Jiang, T., Liu, H. B., Yang, J. (2018). Unraveling habitat use of *Coilia nasus* from Qiantang River of China by otolith microchemistry. *Regional Studies in Marine Science, **18***, 122-128.

Kim, N. H., Hyun, Y. Y., Lee, K. B., Chang, Y., Rhu, S., Oh, K. H., Ahn, C. (2015). Environmental heavy metal exposure and chronic kidney disease in the general population. *Journal of Korean Medical Science*, ***30***(3), 272.

Kinyua, A. M. & Pacini, N. (1991). The impact of pollution on the ecology of the Nairobi-Athi River system in Kenya. *Journal of Biochemiphysics, **1**(1),* 1-18.

KNBS. (2019). Kenya population and household census, Vol 1 Population by county and sub-county. Kenya National Bureau of Statistics, Nairobi, 49.

Kosgey, J., Koech, J., Bunyasi, S., Bett, K., Muthoka, T. M., Nyabaro, O. M. (2015). Determination of heavy metal pollution in sediments along the banks of Athi river, Machakos County, Kenya. *International Journal of Science & Technology*, ***5**(7),* 1-6.

Kraus, R. T. & Secor, D. H. (2004). Incorporation of strontium in otoliths of an estuarine fish. *Journal of Experimental Marine Biology and Ecology*, ***302***, 85-106.

Krumins, J. A., van Oevelen, D., Bezemer, T. M., De Deyn, G. B., Hol, W. H. G., van Donk, E., de Boer, W., de Ruiter, P. C., Middelburg, J. J., Monroy, F., Soetaert, K., Thebault, E., van de Koppel, J., van Veen, J. A., Viketoft, M., and van der Putten, W. H. (2013). Soil and Freshwater and Marine Sediment Food Webs: Their Structure and Function, *Bioscience, **63**,* 35-42.

Kannurpatti, S. S., Joshi, P. G., Joshi, N. B. (2000). Calcium sequestering ability of mitochondria modulates influx of calcium through glutamate receptor channel. *Neurochemical research*, ***25***(12), 1527-1536.

KWTA. (2020). Kenya Water Towers status report: Shimba Hills. Nairobi, 82.

Larsson, P., Hamrin, S., Okla, L. (1991). Factors determining the uptake of persistent pollutants in an eel population (Anguilla anguilla L.). *Environment Pollution*, ***69***(199), 39-50.

Le Q. D., Shirai, K., Nguyen, D. C., Miyazaki. N., Arai, T. (2009). Heavy metals in tropical eel *Anguilla marmorata* from the central part of Vietnam. *Water, Air and Soil Pollution, **204**,* 405-410.

Leander, N. J., Shen, K. N., Chen, R. T., Tzeng, W. N. (2012). Species composition and seasonal occurrence of recruiting glass eels (*Anguilla* spp.) in the Hsiukuluan River Eastern Taiwan. *Zoological Studies, **51**,* 59-71.

Leauthaud, C., Duvail, S., Hammerlynck, O., Pal, J., Cochet, H., Nyunja, J., Albergel, J., Gruberger, O. (2013). Floods and livelihoods; The importance of changes in water resources on wetland agroecological production system in the Tana River delta, Kenya. *Global Environmental Change*, ***23,*** 252-263.

Li, G., Cao, Z., Lan, D., Xu, J., Wang, S., Yin, W. (2007). Spatial variations in grain size distribution and selected metal contents in the Xiamen Bay, China. *Environmental Geology*, ***52***(8), 1559-1567.

Li, L., Li, C., Mao, H., Du, Z., Chan, W. Y., Murray, P., Tao, Q. (2016). Epigenetic inactivation of the CpG demethylase TET1 as a DNA methylation feedback loop in human cancers. *Scientific Reports*, ***6***(1), 26591.

Lin, S. H., Iizuka, Y., Tzeng, W. N. (2012). Migration behavior and habitat use by juvenile japanese eels Anguilla japonica in continental waters' as indicated by Mark-Recapture Experiments and Otolith Microchemistry. *Zoological Studies*, ***51***(4), 442-452.

López-González, N., Borrego, J., Morales, J. A., Carro, O., Lozano-Soria, O. (2006). Metal Fractionation in Oxic Sediments of an Estuary Affected by Acid Mine Drainage (Southwestern Spain). *Estuarine*, *Coastal and Shelf Science,* ***68,*** 297-304.

Lucaciu, C. M., Dragu, C., Copăescu, L., Morariu, V. V. (1997). Manganese transport through human erythrocyte membranes. An EPR study. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, ***1328***(2), 90-98.

MacGregor, R., Mathers, A., Thompson, P., Casselman, J. M., Dettmers, J. M., LaPan, S., Allen, B. (2008). Declines of American eel in North America: complexities associated with bi-national management. International governance of fisheries ecosystems: learning from the past, finding solutions for the future. *American Fisheries Society,* Bethesda, Maryland, 357-381.

Maes, G. E., Raeymaekers, J. A. M., Pampoulie, C., Seynaeve, A., Goemans, G., Belpaire, C., Volckaert, F. A. M. (2005a). The catadromous European eel *Anguilla anguilla* (L.) as a model for freshwater evolutionary ecotoxicology: Relationship between heavy metal bioaccumulation, condition and genetic variability. *Aquatic Toxicology, **73***, 99-114.

Maes, J., Belpaire, C., Goemans, G. (2008). Spatial variations and temporal trends between 1994 and 2005 in polychlorinated biphenyls, organochlorine pesticides and heavy metals in European eel (*Anguilla anguilla L.*) in Flanders, Belgium. *Environmental Pollution, **153***, 223-237.

Marohn, L., Jakob, E., Hanel, R. (2013). Implications of facultative catadromy in *Anguilla Anguilla.* Does individual migratory behaviour influence eel spawner quality? *Journal of Sea Research, **77,*** 100-106.

Martin, M. H. & Coughtrey, P. J. (1982). Biological Monitoring of heavy metal pollution, 'land and air'. London and New York, 475.

Martin, G. B. & Wuenschel, M. J. (2006). Effect of temperature and salinity on otolith element incorporation in juvenile gray snapper Lutjanus griseus. *Marine Ecology Progress Series*, ***324***, 229-239.

Martin, G. B., Thorrold, S. R., Jones, C. M. (2004). Temperature and salinity effects on strontium incorporation in otoliths of larval spot (Leiostomus xantharus). *Canadian Journal of Fisheries and Aquatic Sciences*, ***61***, 34-42.

Massuti, E., Morales-Nin, B., Moranta, J. (2000). Age and growth of blue-mouth, *Helicolenus dactylopterus* (Osteichthyes: Scorpaenidae), in the western Mediterranean. *Fisheries Research, **46***, 165-176.

McCleave, J. D. & Arnold, G. P. (1999). Movements of yellow- and silver-phase European eels (*Anguilla anguilla L.*) tracked in the western North Sea. *ICES Journal of Marine Science, **56,*** 510-536.

McCormick, B., Popp, C., Andrews, B., Cottrell, E. (2015). Ten years of satellite observations reveal highly variable sulphur dioxide emissions at Anatahan Volcano, Mariana Islands. *Journal of Geophysical Research: Atmospheres*, ***120***(14), 7258-7282.

McHugh, B., Poole, R., Corcoran, J., Anninou, P., Boyle, B., Joyce, E., Foley, B. M., McGovern, E. (2010). The occurrence of persistent chlorinated and brominated organic contaminants in the European eel (*Anguilla anguilla*) in Irish waters. *Chemosphere, **79***, 305-313.

Miller, M. J., Marohn, L., Wysujack, K., Freese, M., Pohlmann, J. D., Westerberg, H., Hanel, R. (2019). Morphology and gut contents of anguillid and marine eel larvae in the Sargasso Sea. *Zoologischer Anzeiger*, ***279***, 138-151.

Milton, D., Halliday, I., Sellin, M., Marsh, R., Staunton-Smith, J., Woodhead, J. (2008). The effect of habitat and environmental history on otolith chemistry of barramundi Lates calcarifer in estuarine populations of a regulated tropical river. *Estuarine, Coastal and Shelf Science*, ***78***(2), 301-315.

Morales-Nin, B. (2000). Review of the growth regulation process of otolith daily increment formation. *Fisheries Research, **46,*** 53-67.

Morrison, W. E. & Secor, D. H. (2003). Demographic attributes of yellow phase American eels (*Anguilla rostrata*) in the Hudson River estuary. *Canadian Journal of Fisheries and Aquatic Sciences, **60**,* 1487-1501.

Mochizuki, C., Adachi, K., Tanaka, Y., Seki, R. (2019). The effect of prenatal moderate aerobic exercise to inhibit the increase of oxidative stress. *The Journal of Japan Academy of Health Sciences*, ***22***(1), 5-15.

Muiruri, J. M., Nyambaka, H. N., Nawiri, M. P. (2013). Heavy metals in water and tilapia fish from Athi-Galana-Sabaki tributaries, Kenya. *International Food Research Journal, **20**(2),* 891-896.

Ndimele, P. E. (2008). Evaluation of phyto-remediative properties of water hyacinth (*Eichhornia crasspies*) and biostimulants in restoration of oil-polluted Wetland in the Niger Delta. PhD. Thesis, University of Ibadan, Nigeria.

Ngalame, N. N., Tokar, E. J., Person, R. J., Waalkes, M. P. (2014). Silencing KRAS overexpression in arsenic-transformed prostate epithelial and stem cells partially mitigates malignant phenotype. *Toxicological Sciences*, ***142***(2), 489-496.

Njinga, R. L., & Tshivhase, V. M. (2019). Major chemical carcinogens in drinking water sources: health implications due to illegal gold mining activities in Zamfara State-Nigeria. *Exposure and Health*, ***11**(1)*, 47-57.

Njuguna, S. M., Yan, X., Gituru, R. W. (2017). Assessment of macrophyte, heavy metal, and nutrient concentrations in the water of the Nairobi River, Kenya. *Environmental Monitoring and Assessment, **189**(9)*, *454*.

Niyogi, S., Kent, R., Wood, C. M. (2008). Effects of water chemistry variables on gill binding and acute toxicity of cadmium in rainbow trout (*Oncorhynchus mykiss*): A biotic ligand model (BLM) approach. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, ***148**(4)*, 305-314.

Ocheng'o, C. N., Ondieki, S. C., Obiero, J. P. (2018). Estimation of Safe Yield of a Multipurpose Reservoir Using Weap: A Case Study of Thwake Reservoir, Makueni and Kitui Counties, Kenya. *Journal of Water Resources and Ocean Science*, *7(4),* 60-69.

Oremo, J., Orata, F., Owino, J., Shivoga, W. (2020). Assessment of Available Phosphates and Nitrates Levels in Water and Sediments of River Isiukhu, Kenya. *Applied Ecology and Environmental Sciences*, ***10***, 12691

Pannella, G. (1971). Fish otolith: daily growth layers and periodical patterns. *Science, **173**,* 1124-1127.

Payan, P., Edeyer, A., De Pontual, H., Borelli, G., Boeuf, G., Mayer-Gostan, N. (1999) Chemical composition of saccular endolymph and otolith in fish inner ear: lack of spatial uniformity. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology,* ***277***, 123-131.

Pierron, F., Baudrimont, M., Bossy, A., Bourdinaud, J. P., Bréthes, D., Elie, P., Massabuau, J. C. (2007a). Impairment of lipid storage by cadmium in the European eel (*Anguilla anguilla*). *Aquatic Toxicology,* ***81***, 304-311.

Pike, C., Crook, V., Jacoby, D., Gollock, M. (2020). Anguilla bicolor (amended version of 2019 assessment). In The IUCN Red List of Threatened Species 2020, *8235*.

Post, D. M. (2002). Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology,* ***83***, 703-718.

Qiao - qiao, H. I., Guang-Wei, Z. H. U., Langdon, A. (2007). Bioaccumulation of heavy metals in fishes from Taihu Lake, China. *Journal of Environmental Sciences,* ***19***, 1500-1504.

Richardson, C. J., DiGiulio, R. T., Tandy, N. E. (1989) Free radical mediated processes as makers of air pollution stress in trees: In Committee on biologic makers of air pollution damage in trees (ed), *Biological Makers of Air Pollution Stress and Damage in Forest*, National Academic Press, Washington, DC, 251-260.

Rietzler, A. C., Fonseca, A. L., Lopes, G. P. (2001). Heavy metals in tributaries of Pampulha reservoir. Minas. Gerais. *Brazillian Journal of Biology,* ***61***, 363-370.

Righton, D., Aarestrup, K., Jellyman, D., Sébert, P., van den Thillart, G., Tsukamoto, K. (2012). The Anguilla spp. migration problem: 40 million years of evolution and two millennia of speculation. *Journal of Fish Biology,* ***81***, 365-386.

Righton, D. A. & Metcalfe, J. D. (2011). Fish migrations| Eel Migrations,1937-1944.

Robinet, T. & Feunteun, E. (2002). Sublethal effects of exposure to chemical compounds: A cause for the decline in Atlantic eels? *Ecotoxicology,* ***11,*** 265-277.

Roche, N., Buet, A., Ramade, F. (2003). Characterization and validation of ecotoxicological biomarkers in an eel population exposed to persistent organic pollutants in the Vaccares lake, French national reserve of Camargue. *Revue D Ecologie-La Terre Et La Vie*, ***58(1)***, 127-141.

Rose, K. A., Allen, J. I., Artioli, Y., Barange, M., Blackford, J., Carlotti, F., Cropp, R., Daewel, U., Edwards, K., Flynn, K., Hill, S. L., HilleRisLambers, R., Huse, G., Mackinson, S., Megrey, B., Moll, A., Rivkin, R., Salihoglu, B. (2010). End-To-End Models for the Analysis of Marine Ecosystems: Challenges, Issues, and Next Steps. *Marine and Coastal Fisheries, **2**,* 115-130.

Rosenblatt, A. E., Nifong, J. C., Heithaus, M. R., Mazzotti, F. J., Cherkiss, M. S., Jeffery, B. M., Elsey, R. M., Decker, R. A., Silliman, B. R., Guillette Jr., L. J., Lowers, R. H., Larson, J. C. (2015). Factors affecting individual foraging specialization and temporal diet stability across the range of a large "generalist" apex predator. *Oecologia, **178**,* 5-16.

Schaefer, H. R., Dennis, S., Fitzpatrick, S. (2020). Cadmium: Mitigation strategies to reduce dietary exposure. *Journal of Food Science*, ***85***(2), 260-267.

Secor D. H. & Rooker J. R. (2000). Is otolith strontium a useful scalar of life cycles in estuarine fishes? *Fisheries Research, **46**,* 359-371.

Senarathne, P. & Pathiratne, K. A. S. (2007). Accumulation of heavy metals in a food fish, *Mystus gulio* inhabiting Bolgoda Lake, Sri Lanka. *Sri Lanka Journal of Aquatic Sciences, **12**,* 61-75.

Sendamarai, A. K., Ohgami, R. S., Fleming, M. D., Lawrence, C. M. (2008). Structure of the membrane proximal oxidoreductase domain of human Steap3, the dominant ferrireductase of the erythroid transferrin cycle. *Proceedings of the National Academy of Sciences*, ***105***(21), 7410-7415.

Shiao, J. C., Ložys, L., Iizuka, Y., Tzeng, W. N. (2006). Migratory patterns and contribution of stocking to the population of European eel in Lithuanian waters as indicated by otolith Sr:Ca ratios. *Journal of Fish Biology, **69***, 749-769.

Shiraishi, H. & Crook, V. (2015). Eel market dynamics: An analysis of Anguilla production, trade and consumption in East Asia. Tokyo, Japan.

Stapp, P., Polis, G. A., Sánchez, P. (1999). Stable isotopes reveal strong marine and El Ninõ effects on island food webs. *Nature, **401***, 467-469.

Sugarman, P. J., McCartan, L., Miller, K. G., Feigenson, M. D., Pekar, S., Kistler, R. W., Robinson, A. G. (1997). Correlation of Miocene sequences and hydrogeologic units, New Jersey Coastal Plain. *In* Proceedings of the Ocean Drilling Program, Scientific Results. *Edited by* K.G. Miller and S.W. Snyder. Ocean Drilling Program, College Station, TX, 147-159.

Tabouret, H., Bareille, G., Claverie, F., Pécheyran, C., Prouzet, P., Donard, O. F. (2010). Simultaneous use of strontium:calcium and barium:calcium ratios in otoliths as markers of habitat: application to the European eel (Anguilla anguilla) in the Adour basin, South West France. *Marine Environmental Research*, ***70****(1)*, 35-45.

Tanabe, T., Kayama, S., Ogura, M., Tanaka, S. (2003): Daily increment formation in otoliths of juvenile skipjack tuna *Katsuwonus pelamis*. *Fisheries Science,* ***69****,* 731-737.

Tesch, F. W. (2003). The Eel. Oxford: Blackwell Science Ltd.

Touret, N., Furuya, W., Forbes, J., Gros, P., Grinstein, S. (2003). Dynamic traffic through the recycling compartment couples the metal transporter Nramp2 (DMT1) with the transferrin receptor. *Journal of Biological Chemistry*, ***278***(28), 25548-25557.

Tsukamoto, K., Aoyama, J. (1998). Evolution of freshwater eels of the genus Anguilla: a probable scenario. *Environmental Biology of Fishes*, ***52***(1), 139-148.

Tsuchiya, K. (1969). Epidemic of mercury poisoning in the Agano River area. An introductory review. *The Keio Journal of Medicine,* ***18****,* 213-227.

Tyagi, O. D. & Mehra, M. (1992). Environmental Chemistry. Anmol Publications, New Delhi, 109-288.

Tzeng, W. N. (1996). Effects of salinity and ontogenetic movements on strontium: calcium ratios in the otoliths of the Japanese eel, Anguilla japonica Temminck & Schlegel. *Journal of Experimental Marine Biology and Ecology,* ***199****,* 111-122.

Tzeng, W. N., Severin, K. P., Wickstrom, H. (1997). Use of otolith microchemistry to investigate the environmental history of European eel *Anguilla anguilla*. *Marine Ecology Progress Series,* ***149****(1-3),* 73-81.

Tzeng, W. N., Lin, H. R., Wang, C. H., Xu, S. N. (2000). Differences in size and growth rates of male and female migrating Japanese eels in Pearl River, China. *Journal of Fish Biology*, ***57***(5), 1245-1253.

Tzeng, W. N., Shiao, J. C., Iizuka, Y. (2002). Use of otolith Sr: Ca ratios to study the riverine migratory behaviors of Japanese eel Anguilla japonica. *Marine Ecology Progress Series*, ***245***, 213-221.

Tzeng, W. N., Iizuka, Y., Shiao, J. C., Yamada, Y., Oka, H. P. (2003). Identification and growth rates comparison of divergent migratory contingents of Japanese eel (Anguilla japonica). *Aquaculture,* ***216***, 77-86.

van den Thillart, G. E. E. J. M., Palstra, A. P., van Ginneken, V. J. T. (2007). Simulated migration of European silver eel: swim capacity and cost of transport. *Journal of Marine Science and Technology,* ***15****,* 1-16.

van Ginneken, V., Palstra, A., Leonards, P., Nieveen, M., Van Den Berg, H., Flik, G., Spannings, T., Niemantsverdriet, P., van den Thillart, G., Murk, A. (2009). PCBs and the energy cost of migration in the European eel (*Anguilla anguilla* L.). *Aquatic Toxicology, **92**,* 213-220.

Valade, P., Hoarau, P., Bonnefoy, A. (2018). Plan Directeur de Conservation en faveur des 675 Anguillidae à l'île de La Réunion 2018-2027. *DEAL Réunion*, 206.

Valko, M. M. H. C. M., Morris, H., Cronin, M. T. D. (2005). Metals, toxicity and oxidative stress. *Current medicinal chemistry*, ***12***(10), 1161-1208.

Vander Zanden, M. J. & Rasmussen, J. B. (1999). Primary consumer δ13C and δ15N and the trophic position of aquatic consumers. *Ecology, **80***, 13951404.

Velusamy, A., Kumar, P. S., Ram, A., Chinnadurai, S. (2014). Bioaccumulation of heavy metals in commercially important marine fishes from Mumbai Harbor, India. *Marine Pollution Bulletin*, ***81***(1), 218-224.

Von Burg, R. & Greenwood, R. M. (1991) 'Mercury', in E. Merian (ed), Metals and Their Compounds in the Environment - Occurrence: Analysis and Biological Significance, Verlagsgesellschaft, Weinheim, Germany, 1045-1053.

Wang, Y., Deng, W., Wang, B., Zhang, Q., Wan, X., Tang, Z., Wan, H. (2013). Chemical synthesis of lactic acid from cellulose catalysed by lead (II) ions in water. *Nature Communications*, ***4***(1), 2141.

Wara, C., Thomas, M., Mwakurya, S., Katuva, J. (2019). Development of River Rating Curves for Simple to Complex Hydraulic Structure Based on Calibrated HEC-RAS Hydraulic Model, in Kwale, Coastal Kenya. *Journal of Water Resource and Protection*, ***11***, 468-490.

Watanabe, S., Aoyama, J., Tsukamoto, K., (2009). A new species of freshwater eel *Anguilla luzonensis* (Teleostei: *Anguillidae*) from Luzon Island of the Philippines. *Fisheries Science, **75***, 387-392.

Willson, J. D., Winn, C. T., Pilgrim, M. A., Romanek, C. S., Gibbons, J. W. (2010). Seasonal variation in terrestrial resource subsidies influences trophic niche width and overlap in two aquatic snake species: a stable isotope approach. *Oikos, **119***, 1161-1171.

World Health Organization. (2009). Evaluation of certain food additives: sixty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives*, **45**,* 65-69.

World Health Organization. (2008). Guidelines for drinking water quality, World Health Organization, Geneva.

Wu, H., Liao, Q., Chillrud, S. N., Yang, Q., Huang, L., Bi, J., Yan, B. (2016). Environmental exposure to cadmium: health risk assessment and its associations with hypertension and impaired kidney function. *Scientific Reports*, ***6***(1), 29989.

Xu, S., Pi, H., Chen, Y., Zhang, N., Guo, P., Lu, Y., Zhou, Z. (2013). Cadmium induced Drp1-dependent mitochondrial fragmentation by disturbing calcium homeostasis in its hepatotoxicity. *Cell Death & Disease*, ***4***(3), e540.

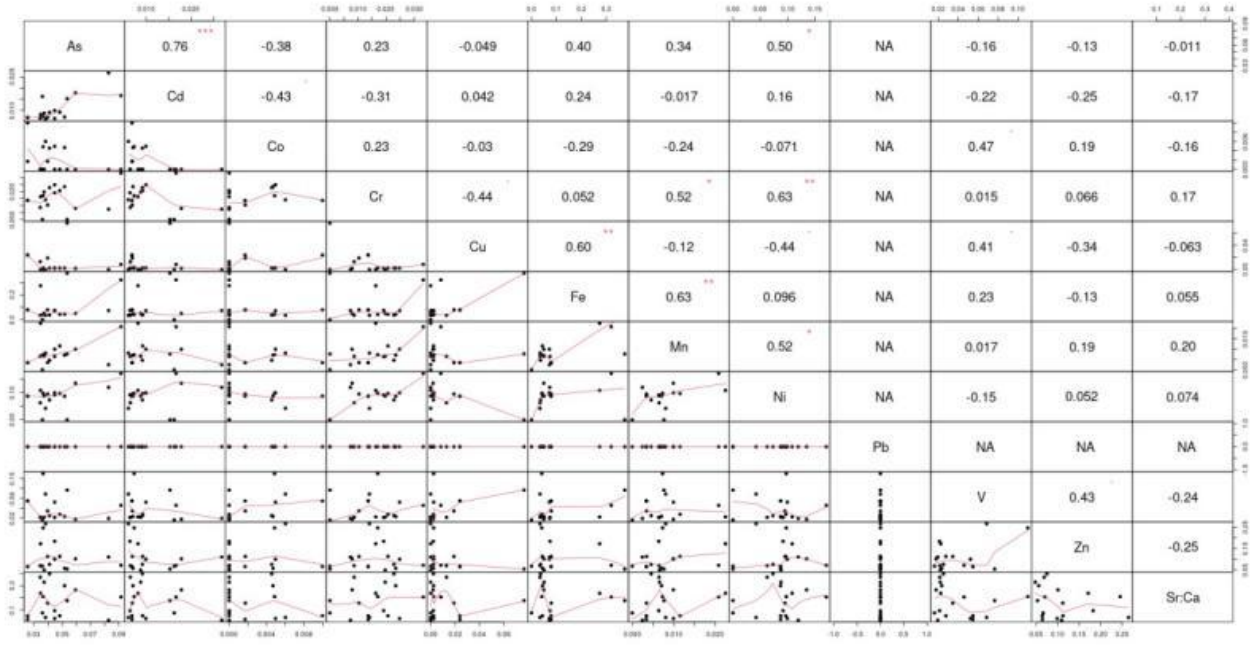
Yadav, V. K., Pandey, S., Kaushik, P. (2020). A brief perspective on Japanese eel (Anguilla japonica Temminck & Schlegel, 1846) an indispensable part of Japanese culture. *Indian Journal of Geo Marine Sciences*, ***49***(06), 933-939.

Yang, J., Kunito, T., Anan, Y., Tanabe, S., Miyazaki, N. (2004). Total and subcellular distribution of trace elements in the liver of a mother–fetus pair of Dall's porpoises (Phocoenoides dalli). *Marine Pollution Bulletin*, ***48***, 1122-1129.

Zimmerman, C. E. (2005). Relationship of otolith strontium-to-calcium ratios and salinity: experimental validation for juvenile salmonids. *Canadian Journal of Fisheries and Aquatic Sciences*, ***62****(1)*, 88-97.

APPENDICES

Appendix A: Correlation matrix showing the association between mean heavy and trace metal concentration and otolith Sr:Ca ratios among yellow and silver eels



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Heavy metal bioaccumulation in anguillids from rivers in Kenya

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Abstract

Heavy metal bioaccumulation was examined in anguillids from the Athi-Galana-Sabaki (AGS) and Ramisi Rivers in Kenya, which have differing contaminant sources and anthropogenic disturbances. Water and eel tissue samples were analysed for conventional water quality parameters and heavy metals, respectively. Arsenic, lead, and cadmium concentrations were determined using ICP-OES. Results showed that pH was comparable among rivers, but significant differences in temperature, dissolved oxygen, and salinity were observed. Arsenic and cadmium concentrations were comparable between the rivers and exceeded the WHO maximum permissible limits for drinking water. Lead was not detected in the AGS River or upstream in the Ramisi River. Arsenic and cadmium concentrations in muscle and liver tissues differed significantly among anguillid species. Arsenic bioaccumulation was comparable amongst the species, whereas cadmium concentrations differed significantly. Metal concentrations in muscle were negatively correlated to the condition factor, suggesting that eel performance was impacted. Although arsenic and cadmium concentrations in tissues were above permissible limits in freshwater fishes, corresponding bioaccumulation factors were below the recommended values suggesting that eels may be safe for consumption. Further assessment of human health risks is recommended to protect public health.

Appendix C: Research permit from the National Commission for Science, Technology & Innovation (NACOSTI)

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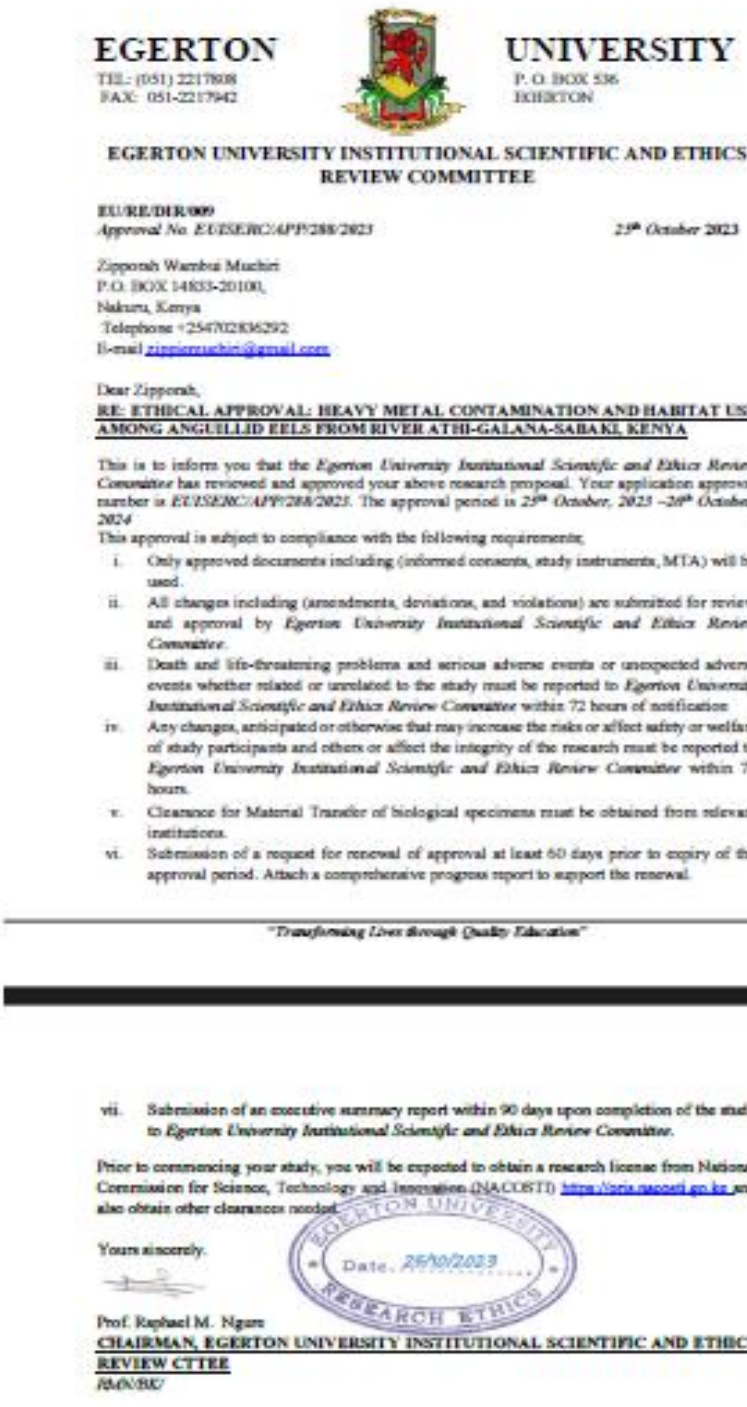

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EGERTON UNIVERSITY INSTITUTIONAL SCIENTIFIC AND ETHICS REVIEW COMMITTEE

EU/RE/DIR/009
Approval No. EUISERC/APP/288/2023 *25th October 2023*

Zipporah Wambui Muchiri
P.O. BOX 14833-20100,
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Dear Zipporah,

RE: ETHICAL APPROVAL: HEAVY METAL CONTAMINATION AND HABITAT USE AMONG ANGUILLID EELS FROM RIVER ATHI-GALANA-SABAKI, KENYA

This is to inform you that the *Egerton University Institutional Scientific and Ethics Review Committee* has reviewed and approved your above research proposal. Your application approval number is *EUISERC/APP/288/2023*. The approval period is *25th October, 2023 –26th October, 2024*

This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, MTA) will be used.
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by *Egerton University Institutional Scientific and Ethics Review Committee*.
iii. Death and life-threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to *Egerton University Institutional Scientific and Ethics Review Committee* within 72 hours of notification
iv. Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to *Egerton University Institutional Scientific and Ethics Review Committee* within 72 hours.
v. Clearance for Material Transfer of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.

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vii. Submission of an executive summary report within 90 days upon completion of the study to *Egerton University Institutional Scientific and Ethics Review Committee*.

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://oris.nacosti.go.ke and also obtain other clearances needed.

Yours sincerely,

EGERTON UNIVERSITY RESEARCH ETHICS
Date 26/10/2023

Prof. Raphael M. Nguro
CHAIRMAN, EGERTON UNIVERSITY INSTITUTIONAL SCIENTIFIC AND ETHICS REVIEW CTTEE
RMN/BC/