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# KENYA *Aquatica*



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Fisheries Research Institute

# KMFRI

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# Editorial

## Editorial: Kenya Aquatica Journal Vol 10(1) – A Showcase of KMFRI's Pioneering Research in Freshwater Ecosystems

The latest edition of Kenya Aquatica Journal, Vol 10(1) showcases pioneering research by KMFRI scientists on Kenya's freshwater ecosystems. This edition, supported by KMFRI and WIOMSA, covers ecological, socio-economic, and environmental challenges, providing valuable insights into sustainable management practices.

One notable study investigates disease surveillance and antimicrobial resistance in fish from lacustrine caged farms, emphasizing responsible antibiotic use to maintain fish health. Another study explores the impact of organochlorine pesticides on macroinvertebrates in Lake ecosystems, advocating for *Rhagovelia* spp. as a bioindicator for pesticide monitoring across food webs.

Research on Lake Baringo's small-scale fishery assesses the catch and effort composition, stressing the need for regulatory enforcement to avoid overfishing and advocating for capacity building among stakeholders for sustainable management. Additionally, a study on wild fish kills in Lake Victoria focuses on eutrophication and pollution, recommending integrated watershed management to protect the lake's fisheries and local livelihoods.

A comprehensive study on Lake Elementaita – one of Kenya's flamingos' sanctuaries, combines water quality, fisheries studies, and community surveys, calling for integrated watershed management, conservation, and sustainable agriculture. Research on fisheries co-management in Lake Baringo highlights the importance of local community involvement and sustained achievements in ecosystem management, despite challenges in law enforcement.

An article on the socio-economic dynamics of Lake Victoria proposes establishing a regulatory framework incorporating citizen science to manage the lake's resources for long-term sustainability. Addressing plastic pollution in Lake Turkana, a study recommends waste management solutions, public awareness, and better enforcement of regulations to tackle the issue.

The journal also features research on antimicrobial resistance (AMR), with a review exploring Kenya's aquatic biodiversity for potential novel antimicrobial agents. A genetic research study evaluates freshwater fish populations, identifying gaps and proposing future directions for conservation and management.

Lastly, the journal presents an evaluation of fish market dynamics in Lake Naivasha, recommending infrastructure development like fish markets and hatcheries to support the region's fishery sector.

This edition of Kenya Aquatica Journal provides crucial insights into Kenya's freshwater ecosystems, covering a wide range of research on sustainable management, environmental challenges, and the socio-economic factors influencing aquatic resources. The research highlights KMFRI's ongoing contributions to understanding and addressing these issues, fostering a deeper understanding of Kenya's aquatic biodiversity.

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Kenya Aquatica is the Scientific Journal of the Kenya Marine and Fisheries Research Institute (KMFRI). The aim of the Journal is to provide an avenue for KMFRI researchers and partners to disseminate knowledge generated from research conducted in the aquatic environment of Kenya and resources therein and adjacent to it. This is in line with KMFRI's mandate to undertake research in "marine and freshwater fisheries, aquaculture, environmental and ecological studies, and marine research including chemical and physical oceanography", in order to provide scientific data and information for sustainable development of the Blue Economy.

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**Featured front cover picture:** Researcher sampling surface plankton in the Kerio River inlet to Lake Turkana. (Photo credit: Mr. John Malala)

**Featured back cover picture:** Chair of KMFRI Board of Management Amb. Dr. Wenwa Akinyi Odinga Oranga (seated middle), on her right, Ag. KMFRI CEO Dr. James Mwaluma, flanked by KMFRI Heads of Sections: Front (L-R) Dr. Victoria Tarus, Ms Caroline Mukira, Dr. Jacob Ochiewo, Dr. Irene Githaiga, Mr. Abraham Kagwima. Back (L-R) Mr. Paul Waluba, Ms Jane Kiguta, Dr. Gladys Okemwa, Dr. Eric Okuku, Dr. Joseph Kamau, Mr. Isaac Koja, Ms Joan Karanja, Mr. Milton Apollo. (Photo credit KMFRI)

Research Vessel MV Mtafiti in the background

# Distribution of organochlorine pesticides in macroinvertebrate functional feeding guild (FFG) of predators, *Rhagovelia* spp. in a tropical estuarine ecosystem

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## Abstract

The current world population stands at approximately 8.5 billion people and this number is likely to shoot up in the coming decades. This increased trend in world population demands for sufficient food, which calls for, improved agricultural production systems. In order to achieve this, a tremendous increase in pesticide application of about 30–40% has been documented and this trend is predicted to increase in the coming years. Due to their negative impacts to the environment, some pesticides mainly those of organochlorines (OCPs) have since been banned, but their residues can still be detected in different media causing deleterious effects on organisms. The aim of this study, therefore, was to assess the distribution of organochlorine pesticides (OCPs) by aquatic macroinvertebrates FFG of *Rhagovelia* spp. in the tropical estuarine ecosystems of South Coast, Kenya. Twelve sampling stations were purposively identified taking into considerations different hydrological and ecological factors. *Rhagovelia* spp. were sampled using established methods and analysis for OCPs detection were performed using a TSQ Vantage Triple-Stage Quadrupole Mass Spectrophotometer (Thermo Electron) equipped with a heated electrospray ionization probe (HESI-II). Separation, detection, identification and quantification of target analyses followed the same established methods. Sixteen OCPs were recorded in *Rhagovelia* spp. samples collected from all the 12 sampling stations.  $\alpha$ -HCH was the lowest (2.74 0.18 ng g<sup>-1</sup> dw) recorded concentration value for OCPs from *Rhagovelia* species samples whereas OCPs Cis\_chlordan, mirex, *p,p'*-DDT, *p,p'*-DDE, *o,p'*-DDE and HCH recorded 10.09 0.35 ng g<sup>-1</sup> dw, being the highest registered value. Analysis of variance (ANOVA) on the mean concentration residues of OCPs in *Rhagovelia* spp. samples yielded a significant variation among the sampled stations ( $F = 77.79$ ,  $df = 11$ ,  $p < 2.2e-16$ ). The statistical analysis revealed that each station played a crucial role in determining the levels of OCPs in *Rhagovelia* spp. due to environmental factors, early life history strategies of the tested bioassay organism, and different sources of OCPs as influenced by anthropogenic activities. The study recommends for the application of macroinvertebrate FFG of *Rhagovelia* spp. in biomonitoring of estuarine ecosystems. The study also recommends the use of different FFGs of macroinvertebrates such as grazers, collector-gatherers, filterers and shredders in order to bring out the general behavior of these pesticides along the food web.

**Keywords:** bioaccumulation, estuarine ecosystems, benthic macroinvertebrates, biomonitoring, persistent organic pollutants (POPs), organochlorine pesticides (OCPs)

## Introduction

Aquatic environmental degradation by emerging pollutants (EPs) including pesticides is of great interest worldwide. Human pressure has led to the rise of anthropogenic activities, which have contributed to high contamination of aquatic ecosystems by EPs (Zhao *et al.*, 2014). EPs have attracted serious scientific attention in the world that has seen increased research in different environmental partitions such as water, sediments, soil, and organisms (Fair *et al.*, 2018). They are persistent in the aquatic ecosystem thus accumulate in the sediments and enter food webs posing public health threats to the living biota (Bervoet *et al.*, 2005; Fraysse *et al.*, 2006; Davis *et al.*, 2007; Combi *et al.*, 2016; Montuori *et al.*, 2016, Kayembe *et al.*, 2018; Nyakeya *et al.*, 2022). Further, EPs have the ability to bioconcentrate, bioaccumulate and biomagnify along the food web causing deleterious biological effects. They are major causes of human maladies such as cancer, damage to the nervous system, poor growth rates among newborns due to their toxic, carcinogenic, and mutagenic effects (IARC, 2014).

Pesticides may enter the aquatic environment via anthropogenic activities mainly agriculture (Nicolau *et al.*, 2006; Reichnberger *et al.*, 2007). It has been argued that their bioavailability in the environment have the tendency to be bioconcentrated in organism tissues directly from the water, bioaccumulate and biomagnify within food chains, causing higher trophic organisms become contaminated with higher concentrations of pollutants than their counterparts in the lower trophic level (Hargrave *et al.*, 2000). It is against the aforesaid backdrop coupled with human health risks upon consumption of sea food that first world countries banned the use of all OCPs in agricultural production (Yuan *et al.*, 2015). In comparison, this is not the case in third world countries who are struggling with the upsurge in population hence feeding their people is a challenge (Suami *et al.*, 2020). OCPs are therefore, widely used to boost their agricultural production by preventing pest attacks (Yuan *et al.*, 2015). In addition, DDTs have been reported to be widely used for sanitation purposes in

third world countries (UNDP, 2009; Verhaert *et al.*, 2013; Zhang *et al.*, 2013; Kilunga *et al.*, 2017;).

OCPs are classified as hydrophobic, take quite a long time to degrade due to their chemical stability, and can easily be adsorbed in the sediments (Montuori *et al.*, 2016). Physicochemical attributes have been shown to affect the concentration and distribution of OCPs in different ecosystems (Poté *et al.*, 2008; Yang *et al.*, 2011; Jiang *et al.*, 2013; Alegria *et al.*, 2016). This, therefore, means that sediments act as sinks for OCPs for an extended period of time (El-Said and Youssef, 2013; Xu *et al.*, 2014) making them to be intimate with functional feeding guilds (FFGs) of macroinvertebrates. It is on this basis that spatial evaluation of OCPs can be of great significance in validating both environmental and ecological risks.

Use of organisms to monitor (biomonitoring) toxicants in aquatic environment has become popular in the recent past (Masese *et al.*, 2013; Nyakeya *et al.*, 2017). Those that have been used widely are fish and benthic macroinvertebrates. Such toxicants as pesticides are absorbed by macroinvertebrates at the base of food webs and biomagnified at higher trophic levels (Bard, 1999). Hargrave *et al.* (2000) averred that some macroinvertebrates depending on the FFGs take up chemicals directly from the water, sediments and/or via predation through bioconcentration. Consequently, they are important prey items for many fish taxa, and create a pathway by which chemical contaminants are bioconcentrated from sediments and bioaccumulated in higher trophic levels (Morrison *et al.*, 1996).

Benthic macroinvertebrates act as the main source of food for fish and other organisms at the top of the food pyramid. They, therefore, provide a clear path of OCPs exposure and other pollutants for fish and other resident biota along the food web (Nyakeya, *et al.*, 2017). They are thus good bioindicators of aquatic environment because they bioconcentrate pollutants such as pesticides, heavy metals and many more contaminants (Lynch *et al.*, 1988; Hare, 1992; Hare and Campbell, 1992; Nyakeya *et al.*, 2009; Masese *et al.*, 2013; Nyakeya *et al.*, 2017; Nyakeya *et al.*, 2018a, b; Nyakeya *et al.*, 2022).

Other reasons for their preference in screening and biomonitoring of the environment include: their ability to live and be intimate to aquatic sediments and their ability to live for a longer period of time (months to years) which make them accumulate contaminants in their bodies; their ability to live in almost all forms of aquatic systems while found in quite diverse groups; many taxa are fairly sedentary and thus representative of local conditions; many are benthic and thus are closely associated with sediments; they may accumulate pollutants and yet tolerate low moderate contaminant concentrations; toxicant concentrations in the animals appear to be related to those in their environment; a life-span of several months to years allows integration of contaminants into their bodies over a reasonable period of time, but not so long that it avoids short-term changes in the environment; since most are the immature stages of the life-cycle, body concentrations are not affected by reproductive cycles or sexual differences; they are near the base of food chains, so may be vital agents of metal entry into food chains (Masese *et al.*, 2013; Aura *et al.*, 2021; Nyakeya *et al.*, 2022).

Although previous studies have reported on how benthic macroinvertebrates and fish respond to pollution, there exists data paucity on the spatial distribution of pesticides in different FFGs of benthic macroinvertebrates in estuarine ecosystems. Second, although there has been increased interest for research in the bioaccumulation of EPs by aquatic organisms (Vicente-Martorell *et al.*, 2009) since 1970s (Kaushik *et al.*, 2009), there is a gap on the biology of pesticides in estuarine and freshwater organisms (Hare, 1992; Zhou *et al.*, 2008), and their effects (Hare & Campbell, 1992; Gower *et al.*, 1994).

Moreover, in Sub-Saharan Africa (SSA) and particularly in Kenya many of the studies have only reported on either the occurrence of pesticides especially in the inland water bodies (Kiyuka, 2022) but have not related them to macroinvertebrates. In the coastal waters, pesticide studies have dwelt only on distribution, fate and occurrence in sediments (Wandiga *et al.*, 2002; Wan-

diga *et al.*, 2005; Okuku *et al.*, 2013, 2019, Wanjeri, *et al.*, 2022) and many studies on emerging pollutants have concentrated on metal pollution (Okuku *et al.*, 2010), which also have not shown their ecological effects on organisms. Little studies on the concentration levels of pesticides in fish have been done in Tana and Sabaki rivers and their respective estuaries (Munga, 1985; Mugachia *et al.*, 1992a, b; Lalah *et al.*, 2003) with little regard to trophic levels. Getting to understand the bioconcentration and biomagnification characteristics of pesticides may not be brought out in order to determine the toxicological risks that likely to impact on organisms in the environment as well as human health.

Going by the above arguments, it is confirmed that the distribution, occurrence and fate of pesticides has not been studied well to report authoritatively on the state of environment in the region. Furthermore, pesticides such as DDT has not been given the attention it deserves given that it is one of the lethal pollutants that is being reported to be ubiquitous in the environment despite its ban in most of the countries globally and is known to cause deleterious impacts to biota including man (Kiyuka, 2022, Wanjeri *et al.*, 2022). In addition, DDT is in most instances investigated comparatively with other pesticides such as organophosphates which were to replace it in the industrial and agricultural applications because of their less harmful effects and may not persist for long in the environment (Okuku *et al.*, 2019). In such a scenario, very little is known in terms of its impacts to the environment in general. There is need, therefore, for comprehensive studies on the distribution, bioconcentration and biomagnification of OCPs and sustain regular bioassessments and biomonitoring for informed policy framework. The present study, therefore, tries to assess the distribution of OCPs by aquatic macroinvertebrates FFG of *Rhagovelia spp.* in the tropical estuarine ecosystems of South Coast, Kenya. In this regard, the null hypothesis which stated that there is no significant difference in the distribution of OCPs by aquatic macroinvertebrates FFGs of *Rhagovelia spp.* between the sampling stations was tested.

## Materials and methods

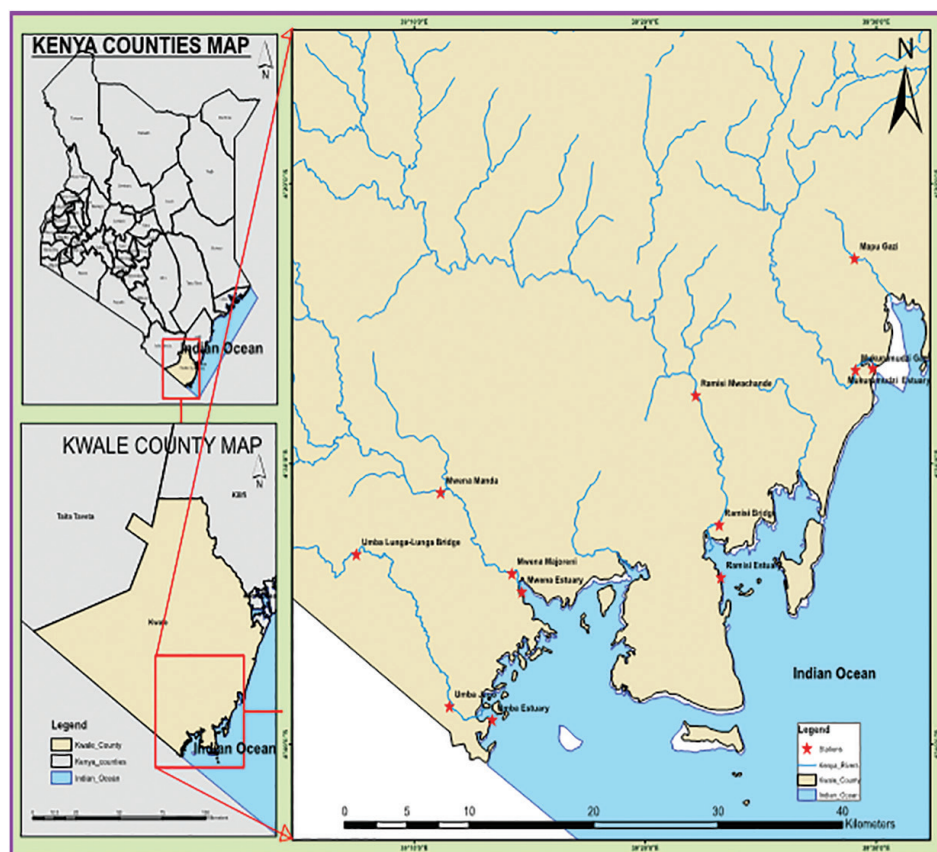
### Study area

The Kenya coast measures about 600 km<sup>2</sup>, bordering Somalia to the North at Kiunga (1°41' S) and Tanzania to the South in a town called Vanga (4°40' S). The region has a tropical climate whose weather patterns is influenced by of the western Indian Ocean Monsoon winds. There are two tropical monsoon seasons, the Southeast monsoon (SEM) prevailing from April to September, which is cooler compared to the Northeast monsoon (NEM) that is characterized by dry weather and sets from October to March (Nyamora *et al.*, 2018; Nyamora *et al.*, 2023). The wet seasons are experienced between April and October but long rains usually begin towards the end of March with the peak occurring in April or May in case of delays and then start declining through August and September when the dry period beckons. Short rains are then witnessed between October and November.

However, of late this is no longer the trend as rain of unpredictable heights can be experienced at any time of the year (Nyakeya *et al.*, 2024). Many studies have been concentrated in the north coast unlike the south coast hence the current study will be undertaken in the southern region of the Kenyan coast. This region receives the highest mean annual rainfall of slightly above 1,016 mm. It experiences temperature range of between 20°C and 35°C. This

study will be carried out in 12 sampling stations in the South Coast estuary spread out among 5 sub-estuaries with each delineated with specific sampling sites depending on distinct ecological characteristics: Mapu, Mwena (3 stations), Mkurumudzi (2), Ramisi (3), and Uмба (3) estuarine ecosystems in the south coast of Kenya, the Western Indian Ocean (WIO) region (Fig. 1).

The area is characterized by one of the largest mangrove habitats (the Vanga-Funzi system covering 6,980 ha). Some of the common mangrove species found in this area include *Rhizophora mucronata* and *Avicenia marina*, plus other seven more species, which supports a disarray of biodiversity. Other critical habitats include sea-grass meadows and coral reefs. These systems form an important ecological and socio-economic zone for the coastal people. These systems' integrity definitely determines the productivity of the inshore waters and those of the continental shelf areas.



**Figure 1. Map of the study area (Source: Authors).**

According to the 2019 Census, Kwale County in which the study area is situated has a total population of 866,820 people with annual growth pegged at 3.1% (KNBS, 2019). Much of this population (17%) is concentrated along the coastal parts bordering the Indian Ocean hence impacting the coastal ecosystems and marine habitats via different human induced activities to support their livelihoods. Further, it is projected that this population may increase to 969,442 (i.e. 10.6%) by the year 2027, meaning much more degradation of both riverine and estuarine systems if sustainable management plans are not put into place (Nyakeya *et al.*, 2024).

The Mkurumudzi River basin, covers an area of 230 km<sup>2</sup> and is located 50 km South of Mombasa City, Kenya. The river traverses about 40 km right from Shimba Hills National Reserve down to Gazi Bay in the Indian ocean where it supports a vast forest of mangroves in the estuary. It is an important river in the region that support a number of commercial activities such as mining by the Base Titanium Limited Company and irrigation of sugar cane farms mainly run by Kwale International Sugar Company (KISCOL). It also provides water for domestic use, watering of livestock apart from irrigating small-scale farms in the area (Nyakeya *et al.*, 2024). Of significance also is that it regulates the micro-climate of this semi-arid region. The basin is characterized by a sub-humid climate, and experiences short rains of 800 mm between the months of October and December and long rains between March and May of about 1300 mm.

The river experiences a mean evaporation of 2170 mm year<sup>-1</sup>, with an aridity index of 0.55 (Katuya, 2014). It enjoys warm temperatures during the months of November to April with mean temperature of 27.0°C, whereas colder months record an average temperature of 25.0°C. In the event of low rainfall, the river is recharged by groundwater making it a permanent river. Agriculture is the major economic activity that takes place whereby such crops as sugarcane, maize, beans, cowpeas, millet and sorghum, okra, cassava) are grown. Livestock husbandry, commercial mining (Base Titanium Ltd., Ukunda, Kenya), commercial farming of sugarcane, commercial

mining, tourism activities associated with the sea and the Hills National Reserve, and fishing (in the river and mainly in the Indian Ocean) are other anthropogenic activities of importance.

Ramisi River starts from Chenze Ranges with many first order ephemeral streams feeding it and traverses a mixed terrain before it flows to the Indian Ocean at Ramisi estuary. It supports an extensive mangrove ecosystem near Funzi Island. The river's salinity characteristics are as a result of in-filtration by brackish geothermal water mainly from Mwananyamala hot springs. Although the underground infiltration makes it somehow saline, it is utilized for irrigation of agricultural crops in the basin. The river supports a mixture of biodiversity including several crocodiles that are distributed along the river continuum depending on the season of the year.

The Uмба River on the other hand, is a transboundary river that flows through Tanzania and Kenya. Its source is in the tectonic type of mountain called Usambara in Mkinga, Tanzania, which stands at an altitude of 2,000 m above sea level. This river discharges its waters to the Indian Ocean on the Kenyan part at a small town known as Vanga, near the boundary of Kenya and Tanzania. It traverses a vast area of about 200 km long, carrying with it terrigenous sediments into the estuary. Its total catchment is approximately 8,000 km<sup>2</sup>. The river is threatened by numerous anthropogenic activities, but because of its unique biodiversity and significant ecological role it plays for both countries, a Transboundary Conservation Area (TBCA) extending from Diani, Kwale County in Kenya at an altitude of 39°00' E, 4°25' S to Tanga in Tanzania (i.e., 39°40' E, 5°10' S) has been proposed and is yet to be unveiled any time soon. The distance between Diani and Mkinga in Tanzania is 120 km. This conservancy shall include a narrow stretch of the coastline in the two countries, covering an estimated area of 2,500 km<sup>2</sup>. The TBCA is important because of its contiguous interrelated marine and terrestrial ecosystems with common socio-economic status.

River Mwena traverses about 180 km<sup>2</sup> from its source to the Indian Ocean. It is one of the least studied rivers in the coast of Kenya. It is highly influenced by the anthropogenic activities right from its source because of high population pressure. High water abstraction is prevalent and during the dry seasons its levels reduce drastically. On the other hand, River MAPU acted as the reference point for this study due to its pristine nature owing to the fact that it is surrounded by thick macrophytes and least influenced by anthropogenic activities.

### Sampling sites selection and description

The sampling stations were purposively selected taking into considerations different hydrological and ecological factors. Anthropogenic activities along the gradient of each river and urbanization, and at the estuaries where they (rivers) discharge their waters into the ocean were also considered. Therefore, all the sampling sites were located downstream just before the rivers empty their waters into the ocean and immediately after (i.e., at the estuarine). Accessibility was also another factor that was taken into account and, therefore, stations before the ocean mainly at designated bridges were given priority. The latitudes and longitudes of each sampling station was measured using a Geographical Position System (GPS), Gemina (US made). Mapu river was used as the reference point due to the fact that it is minimally impacted. Sampling was done both during the rainy and dry seasons.

### Sampling design

A mixed sampling design was employed in this study whereby both probability and non-probability designs were applied. Purposive sampling design which is a non-probability design was used to settle on the sampling stations owing to the predetermined (known) factors such as the hydrology, anthropogenic activities along the gradient of each river, urbanization and accessibility and at the estuaries where respective rivers discharge their waters into the ocean. Therefore,

the study sites included coastal regions combined with urban and estuarine systems or areas, more so sites impacted by agricultural, urban and freshwater inputs as well as industrial/domestic wastewater effluents. Based on the above criteria, the selected sampling sites were visited purposively in each month for sampling for a period of one year. Probability sampling design i.e. simple random design was then employed to collect *Rhagovelia spp.*, the macroinvertebrates FFG of the predator group from pools, runs and riffles.

### Sample Collection

#### *Macro-invertebrate sampling and laboratory processing*

*Rhagovelia spp.* which belongs to macroinvertebrate FFG of predators were collected in triplicates at random locations in each of the selected sites with a Surber sampler (0.09 m<sup>2</sup>, 250" mesh size). Samples were kept alive in cold corked vials preserved using ethanol (70% v/v) until analyzed in the laboratory. In the field all samples were stored live in cooler boxes, transported to KMFRI Mombasa laboratory in darkness. In the laboratory, samples were immediately transferred into the deep freezer after being sorted and identified up to the lowest levels following macroinvertebrate identification keys for marine (Richmond, 1997; Branchet *et al.*, 2008) and freshwater (Gerber and Gabriel, 2002) ecosystems. They were counted, weighed, and frozen at -20°C until analyzed for organochlorine pesticides.

#### Analysis of pesticides

The OCPs detection were performed using a TSQ Vantage Triple-Stage Quadrupole Mass Spectrophotometer (Thermo Electron) equipped with a heated electrospray ionization probe (HESI-II). Separation, detection, identification and quantification of target analyses followed the same methods described by Wille *et al.* (2011). The identification and quantification of OCPs was performed with a Agilent Technologies 6890N gas chromatograph with an electron capture detector

(GC–ECD) using a 30 m 0.25 mm i.d. capillary column coated with 5% phenyl-substituted dimethylpolysiloxane phase (0.25 mm film thickness). Automatic split less injections of 2  $\mu\text{L}$  was applied and the total purge rate was adjusted to 50 ml  $\text{min}^{-1}$ . Hydrogen was used as the carrier gas at a constant pressure of 40 kPa at 100°C, while nitrogen made-up gas at a rate of 60 ml  $\text{min}^{-1}$ . Injector and detector temperatures was 280°C and 320°C, respectively. Oven temperature was calibrated as follows: 70°C for 1 minute, raised at 40°C  $\text{min}^{-1}$  to 170°C, then raised at 1.5°C  $\text{min}^{-1}$  to 230°C for 1 minute and at 30°C  $\text{min}^{-1}$  to 300°C with a final hold of 5 minutes.

#### Quality assurance and quality control

To ensure quality assurance/quality control (QA/QC) is adhered to, the analytical methods were ensured by the use of a standard reference material (SRM 1941b – organics in marine sediment) purchased from the National Institute of Standards and Technology (USA). This was done in duplicate and average recovery of analytes was obtained. The analytes recovery was achieved through spiked blanks and matrices. Analytes in procedural blanks were subtracted from the samples. Laboratory check solutions were routinely injected into GC–ECD and GC–MS to confirm instrument accuracy and precision. Calibration of the instruments were performed using a nine-level analytical curve. Quantification of analytes followed the internal standard procedure and the surrogate recoveries were acceptable.

#### Statistical Analysis

Data were presented as means ( $\pm\text{SD}$ ) after testing for normality and homogeneity of variances, using Levene's and Shapiro-Wilk tests (Levene, 1960; Lina *et al.*, 2015). Analysis of variance (ANOVA) was used to test for significant differences among sampling stations. Tukey *Post hoc* Multiple Comparison test was applied to determine which sites differed significantly from one other. All the analysis was done using the 64-bit R Software version 4.3.0 (R-core team, 2023). All the observed differences were considered statistically significant at  $p < 0.05$ .

## Results

Figure 2 depicts the mean concentration values of a) heptachlor, b) H\_hepoxide, c) Cis\_chlor-dane d) T\_nonachlor, e) HCB and f) mirex pesticides in macroinvertebrate FFGs for *Rhagovelia* species sampled in the South Coast estuarine ecosystems of Kenya. Heptachlor pesticides in *Rhagovelia* species registered a mean concentration value of  $5.54 \pm 2.04 \text{ ng g}^{-1} \text{ dw}$  in the twelve sampled stations; while the lowest ( $2.87 \pm 0.15 \text{ ng g}^{-1} \text{ dw}$ ) value was observed at station RB and the highest ( $9.03 \pm 0.33 \text{ ng g}^{-1} \text{ dw}$ ) at ME. Analysis of variance (ANOVA) for the mean concentration of heptachlor pesticides in *Rhagovelia spp.* sampled among the twelve sites demonstrated that they differed significantly ( $F = 157.16$ ,  $df = 11$ ,  $p = < 2.2e-16$ ). In addition, *post hoc* Tukey test inferred a significant difference in the mean concentration of heptachlor pesticides for *Rhagovelia* species among stations ME ( $9.03 \pm 0.33 \text{ ng g}^{-1} \text{ dw}$ ), MG ( $3.77 \pm 0.25 \text{ ng g}^{-1} \text{ dw}$ ), MKE ( $4.12 \pm 0.31 \text{ ng g}^{-1} \text{ dw}$ ), MM ( $6.89 \pm 0.18 \text{ ng g}^{-1} \text{ dw}$ ), MAPU ( $6.34 \pm 0.25 \text{ ng g}^{-1} \text{ dw}$ ), RB ( $2.84 \pm 0.15 \text{ ng g}^{-1} \text{ dw}$ ), RE ( $4.38 \pm 0.29 \text{ ng g}^{-1} \text{ dw}$ ), and UL ( $5.21 \pm 0.45 \text{ ng g}^{-1} \text{ dw}$ ). Conversely, stations ME and MMJ; MG and RM; MM and UE; and RE and ULB did not show any significant differences in the mean concentrations of heptachlor pesticides for *Rhagovelia spp.* at  $p < 0.05$ .

Additionally, H\_hepoxide pesticides mean concentration levels in *Rhagovelia spp.* samples sampled along the sampling stations during the study period ranged from  $3.92 \pm 0.26$  to  $10.09 \pm 0.35 \text{ ng g}^{-1} \text{ dw}$  for stations ME and UE respectively with a mean concentration of  $7.04 \pm 1.82 \text{ ng g}^{-1} \text{ dw}$  (Fig. 2b). The mean concentration values in H\_hepoxide for *Rhagovelia spp.* samples were significantly different among the twelve sampled stations ( $F = 106.71$ ,  $df = 11$ ,  $p = 2.2e-16$ ). When the means were subjected to *post hoc* Tukey test, it revealed that many of the stations were statistically different from one another (i.e. ME,  $3.92 \pm 0.26 \text{ ng g}^{-1} \text{ dw}$ ; MG,  $7.65 \pm 0.28 \text{ ng g}^{-1} \text{ dw}$ ; MKE,  $7.44 \pm 0.21 \text{ ng g}^{-1} \text{ dw}$ ; MM,  $6.37 \pm 0.25 \text{ ng g}^{-1} \text{ dw}$ ; MMJ,  $8.23 \pm 0.40 \text{ ng g}^{-1} \text{ dw}$ ; MAPU,  $4.92 \pm 0.34 \text{ ng g}^{-1} \text{ dw}$ ; RM,  $5.94 \pm 0.40 \text{ ng g}^{-1} \text{ dw}$ ; UE,  $10.09 \pm 0.35 \text{ ng g}^{-1} \text{ dw}$ ; and UL,

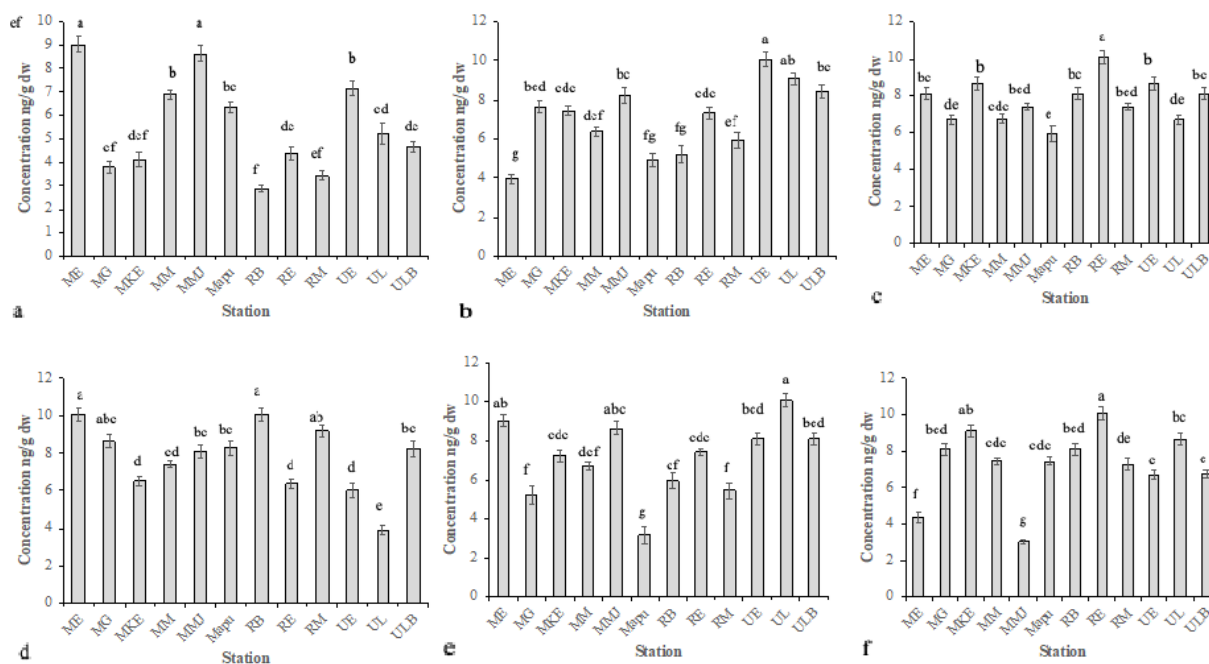
9.06 ± 0.32 ng g<sup>-1</sup> dw). Stations MKE and RE; MMJ and ULB; and MAPU and RB on the other hand did not differ statistically.

Cis\_chlordane concentration in *Rhagovelia spp.* ranged from 5.94 ± 0.40 to 10.09 ± 0.35 ng g<sup>-1</sup> dw with a mean of 7.71 ± 1.2 ng g<sup>-1</sup> dw (Fig. 2c). Sampling station RE recorded the highest concentration (10.09 ± 0.35 ng g<sup>-1</sup> dw) whereas MAPU had the least (5.94 ± 0.40 ng g<sup>-1</sup> dw). A significant difference between the sampling stations was observed (F = 27.83; df = 11; p = 2.2e-16). A *post hoc* Tukey test on the other hand, showed that stations ME (8.09 ± 0.33 ng g<sup>-1</sup> dw), MG (6.69 ± 0.22 ng g<sup>-1</sup> dw), MKE (8.64 ± 0.34 ng g<sup>-1</sup> dw), MM (6.73 ± 0.25 ng g<sup>-1</sup> dw), MMJ (7.41 ± 0.18 ng g<sup>-1</sup> dw), MAPU (5.94 ± 0.40 ng g<sup>-1</sup> dw) and RE (10.09 ± 0.35 ng g<sup>-1</sup> dw) differed significantly at p < 0.05. Stations ME, RB and ULB; MG and UL; MKE and UE; and MMJ and RM were not significantly different.

The mean concentration level of HCB in *Rhagovelia spp.* samples was 7.08 ± 1.92 ng g<sup>-1</sup> dw with station UL recording the highest value of 10.09 ± 0.35 ng g<sup>-1</sup> dw and MAPU station recorded the lowest (3.16 ± 0.42 ng g<sup>-1</sup> dw) (Fig. 2d). Analysis of variance (ANOVA) on the mean concentration residues of HCB pesticides in *Rhagovelia spp.* samples yielded a significant variation among the sampled stations (F = 77.79, df = 11, p < 2.2e-16). Tukey's HSD pairwise mean comparisons on the concentration values of HCB pesticides in *Rhagovelia* species showed a significant difference in stations ME (9.03 ± 0.33 ng g<sup>-1</sup> dw), MG (5.21 ± 0.45 ng g<sup>-1</sup> dw), MKE (7.23 ± 0.30 ng g<sup>-1</sup> dw), MM (6.69 ± 0.22 ng g<sup>-1</sup> dw), MMJ (8.64 ± 0.34 ng g<sup>-1</sup> dw), MAPU (3.16 ± 0.42 ng g<sup>-1</sup> dw), RB (5.94 ± 0.40 ng g<sup>-1</sup> dw), UE (8.09 ± 0.32 ng g<sup>-1</sup> dw) and UL (10.09 ± 0.35 ng g<sup>-1</sup> dw). In contrast, such stations as MG (5.21 ± 0.45 ng g<sup>-1</sup> dw) and RM (5.44 ± 0.41 ng g<sup>-1</sup> dw); MKE (7.23 ± 0.30 ng g<sup>-1</sup> dw) and RE (7.41 ± 0.18 ng g<sup>-1</sup> dw); and UE (8.09 ± 0.32 ng g<sup>-1</sup> dw) and ULB (8.09 ± 0.33 ng g<sup>-1</sup> dw) were statistically not different at p < 0.05.

Mirex was yet another pesticide that was found in the *Rhagovelia spp.* during the study period in the South Coast estuarine ecosystems of Kenya (Fig. 2f). Its concentration level in all the sampled stations ranged between 3.03 ± 0.13 ng g<sup>-1</sup> dw at MMJ station and 10.09 ± 0.35 ng g<sup>-1</sup> dw at RE station; and a mean value of 7.26 ± 1.94 ng g<sup>-1</sup> dw. There was no significant difference that was registered in the concentration levels of mirex pesticides in *Rhagovelia spp.* sampled in the twelve sampling stations (F = 102.37, df = 11, p = 2.2e-16). Furthermore, Tukey's HSD pairwise mean comparisons test revealed that there existed significant differences in the means of mirex concentrations of *Rhagovelia spp.* in such stations as ME (4.38 ± 0.29 ng g<sup>-1</sup> dw); MG (8.09 ± 0.33 ng g<sup>-1</sup> dw); MKE (9.08 ± 0.33 ng g<sup>-1</sup> dw); MM (7.45 ± 0.19 ng g<sup>-1</sup> dw); MMJ (3.03 ± 0.13 ng g<sup>-1</sup> dw); RE (10.09 ± 0.35 ng g<sup>-1</sup> dw); RM (7.30 ± 0.33 ng g<sup>-1</sup> dw); UE (6.73 ± 0.25 ng g<sup>-1</sup> dw) and UL (8.64 ± 0.34 ng g<sup>-1</sup> dw) at p < 0.05. On the contrary, stations MG (8.09 ± 0.33 ng g<sup>-1</sup> dw) and RB (8.09 ± 0.33 ng g<sup>-1</sup> dw); MM (7.45 ± 0.19 ng g<sup>-1</sup> dw) and MAPU (7.44 ± 0.21 ng g<sup>-1</sup> dw); and UE (6.73 ± 0.25 ng g<sup>-1</sup> dw) and ULB (6.75 ± 0.22 ng g<sup>-1</sup> dw) did not differ significantly.

*p,p'*DDE, *o,p'*DDE, *o,p'*\_DDD, *p,p'*\_DDD, *o,p'*\_DDT and *p,p'*\_DDT concentration levels in macro-invertebrate FFGs for *Rhagovelia* species are shown below (Fig. 3). *p,p'*\_DDE concentrations in *Rhagovelia* species among the twelve stations were also measured. The lowest mean of 3.92 ± 0.26 ng g<sup>-1</sup> dw was observed in station RE and the highest (10.09 ± 0.35 ng g<sup>-1</sup> dw) in station MM (Fig. 3a). A mean concentration of 7.20 ± 1.55 ng g<sup>-1</sup> dw was recorded. There was a significant difference in the mean concentration of *p,p'*\_DDE pesticides in *Rhagovelia* species sampled in the twelve stations (F = 66.17, df = 11, p = 2.2e-16). Further, *post hoc* Tukey's HSD pairwise mean comparisons displayed a significant difference in the mean concentration of *p,p'*\_DDE pesti-



**Figure 2. Spatial mean ( $\pm$  SD) concentrations in a) heptachlor, b) H\_hepoxide, c) Cis\_chlordane d) T\_nonachlor, e) HCB and f) mirex pesticides in *Rhagovelia* species (predator) sampled in the twelve stations at the South Coast estuarine systems of Kenya. Superscript letters represent mean differences among the sampling stations obtained by performing Tukey's HSD pairwise mean comparisons. ME: Mwena Estuary; MG: Mkurumudzi Gazi; MKE: mkurumudzi Estuary; MM: Mwena Manda; MMJ: Mwena Majoreini; RB: Ramisi Bridge; RE: Ramisi Estuary; RM: Ramisi Mwachande; UE: Uмба Estuary; UL: Uмба Lenjo; ULB: Uмба Lunga-lunga Bridge.**

cides in *Rhagovelia* spp. among stations ME ( $6.64 \pm 0.23 \text{ ng g}^{-1} \text{ dw}$ ), MKE ( $7.41 \pm 0.18 \text{ ng g}^{-1} \text{ dw}$ ), MM ( $10.09 \pm 0.35 \text{ ng g}^{-1} \text{ dw}$ ), MMJ ( $8.09 \pm 0.32 \text{ ng g}^{-1} \text{ dw}$ ), and RE ( $3.92 \pm 0.26 \text{ ng g}^{-1} \text{ dw}$ ). There was no statistical difference among stations ME ( $6.64 \pm 0.23 \text{ ng g}^{-1} \text{ dw}$ ), MG ( $6.21 \pm 0.33 \text{ ng g}^{-1} \text{ dw}$ ), RM ( $6.22 \pm 0.27 \text{ ng g}^{-1} \text{ dw}$ ) and ULB ( $6.02 \pm 0.37 \text{ ng g}^{-1} \text{ dw}$ ); MKE ( $7.41 \pm 0.18 \text{ ng g}^{-1} \text{ dw}$ ) and UL ( $7.41 \pm 0.18 \text{ ng g}^{-1} \text{ dw}$ ); and MMJ ( $8.09 \pm 0.32 \text{ ng g}^{-1} \text{ dw}$ ), MAPU ( $8.09 \pm 0.32 \text{ ng g}^{-1} \text{ dw}$ ), RB ( $8.23 \pm 0.40 \text{ ng g}^{-1} \text{ dw}$ ), and UE ( $8.09 \pm 0.33 \text{ ng g}^{-1} \text{ dw}$ ).

For *o,p'*-DDE pesticides in macroinvertebrate for *Rhagovelia* spp. sampled in the South Coast estuarine ecosystems of Kenya, its mean concentration ranged between  $5.12 \pm 0.46 \text{ ng g}^{-1} \text{ dw}$ , station ULB and  $10.09 \pm 0.35 \text{ ng g}^{-1} \text{ dw}$ , station MKE; and its overall mean was  $7.15 \pm 1.64 \text{ ng g}^{-1} \text{ dw}$  (Fig. 3b). There existed a significant difference in the mean concentration of *o,p'*-DDE pesticides in macroinvertebrate for *Rhagovelia* species among the sampled stations ( $F = 67.46$ ,  $df = 11$ ,  $p = < 2.2e-16$ ). A *post*

*hoc* Tukey test further confirmed significant differences between different mean in *o,p'*-DDE pesticides in macroinvertebrates for *Rhagovelia* spp. for the following stations: ME ( $8.64 \pm 0.34 \text{ ng g}^{-1} \text{ dw}$ ), MG ( $8.09 \pm 0.33 \text{ ng g}^{-1} \text{ dw}$ ), MKE ( $10.09 \pm 0.35 \text{ ng g}^{-1} \text{ dw}$ ), MMJ ( $6.95 \pm 0.17 \text{ ng g}^{-1} \text{ dw}$ ), and MAPU ( $4.41 \pm 0.28 \text{ ng g}^{-1} \text{ dw}$ ). There was no significant difference, however, reported on stations: ME ( $8.64 \pm 0.34 \text{ ng g}^{-1} \text{ dw}$ ) and MM ( $8.64 \pm 0.34 \text{ ng g}^{-1} \text{ dw}$ ); MG ( $8.09 \pm 0.33 \text{ ng g}^{-1} \text{ dw}$ ), RB ( $7.15 \pm 0.30 \text{ ng g}^{-1} \text{ dw}$ ), RE ( $7.44 \pm 0.21 \text{ ng g}^{-1} \text{ dw}$ ), and UL ( $7.15 \pm 0.30 \text{ ng g}^{-1} \text{ dw}$ ); MMJ ( $6.95 \pm 0.17 \text{ ng g}^{-1} \text{ dw}$ ) and UE ( $6.89 \pm 0.18 \text{ ng g}^{-1} \text{ dw}$ ); and MAPU ( $4.41 \pm 0.28 \text{ ng g}^{-1} \text{ dw}$ ), RM ( $5.21 \pm 0.45 \text{ ng g}^{-1} \text{ dw}$ ) and ULB ( $5.12 \pm 0.46 \text{ ng g}^{-1} \text{ dw}$ ).

The highest concentration of *o,p'*DDD pesticides in *Rhagovelia* spp. was observed at station ME,  $9.03 \pm 0.33 \text{ ng g}^{-1} \text{ dw}$  while the lowest mean was recorded at station MG ( $2.85 \pm 0.16 \text{ ng g}^{-1} \text{ dw}$ ) (Fig. 3c). The mean concentration value for all the sampled stations was  $5.98 \pm 0.28 \text{ ng}$

$\text{g}^{-1}$  dw. The concentrations of *o,p'*DDD levels in *Rhagovelia spp.* were significantly different in all the stations ( $F = 199.19$ ,  $df = 11$ ,  $p = < 2.2\text{e-}16$ ). To test whether each of the station means were statistically different, post hoc Tukey test revealed that the concentrations of *o,p'*DDD in *Rhagovelia spp.* at stations ME, MG, MKE, RB, and UE differed significantly at  $p < 0.05$ . Stations ME, MM and MMJ; MG, Mapu and RM; RB, RE and ULB; and UE and UL on the other hand were statistically similar.

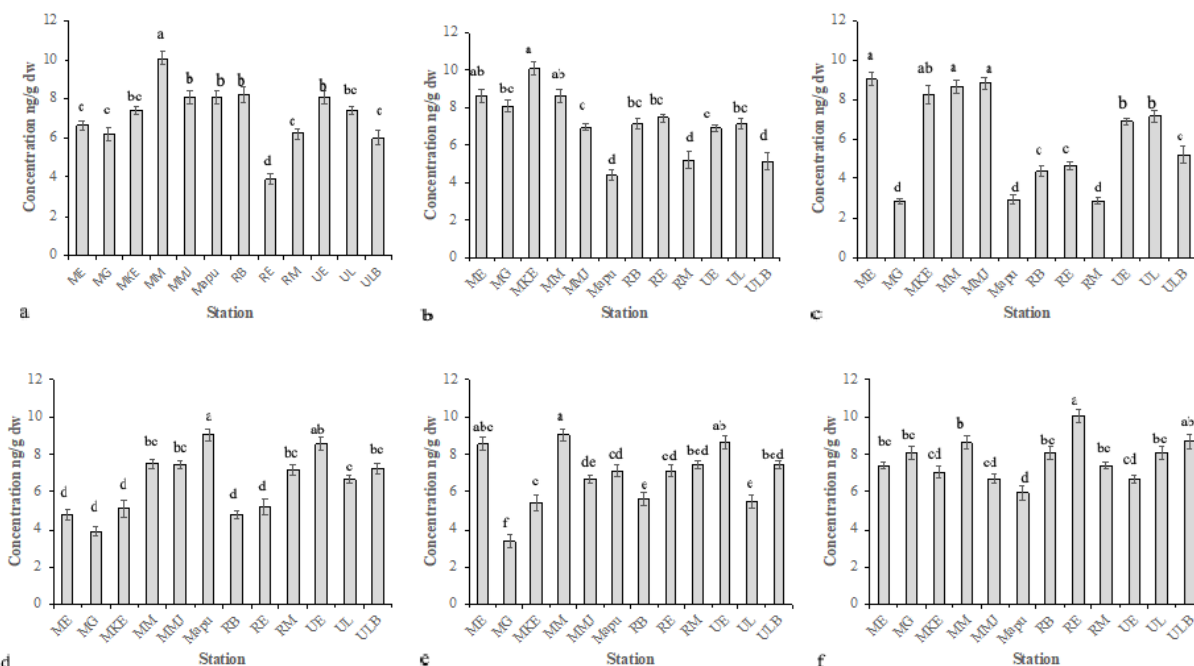
The residues of *p,p'*DDD concentration in *Rhagovelia spp.* varied from  $3.92 \pm 0.26 - 9.03 \pm 0.33$   $\text{ng g}^{-1}$  dw for stations MG and MAPU respectively (Fig. 3d). The average concentration of *p,p'*DDD for *Rhagovelia spp.* sampled in the twelve stations was  $6.46 \pm 1.63$   $\text{ng g}^{-1}$  dw. The concentration of *p,p'*DDD in *Rhagovelia spp.* differed significantly ( $F = 73.61$ ,  $df = 11$ ,  $p = < 2.2\text{e-}16$ ) among the twelve sampled stations. A post hoc Tukey test analysis displayed a significant difference in the different mean concentrations of *p,p'*DDD in *Rhagovelia spp.* sampled at stations MAPU ( $9.03 \pm 0.33$   $\text{ng g}^{-1}$  dw), UE ( $8.55 \pm 0.35$   $\text{ng g}^{-1}$  dw), and UL ( $6.67 \pm 0.22$   $\text{ng g}^{-1}$  dw) at  $p < 0.05$ . The rest of the remaining stations did not differ statistically (i.e. ME ( $4.81 \pm 0.28$   $\text{ng g}^{-1}$  dw), MG ( $3.92 \pm 0.26$   $\text{ng g}^{-1}$  dw), MKE ( $5.13 \pm 0.46$   $\text{ng g}^{-1}$  dw), RB ( $4.80 \pm 0.21$   $\text{ng g}^{-1}$  dw) and RE ( $5.21 \pm 0.45$   $\text{ng g}^{-1}$  dw); MM ( $7.51 \pm 0.26$   $\text{ng g}^{-1}$  dw), MMJ ( $7.44 \pm 0.21$   $\text{ng g}^{-1}$  dw), RM ( $7.18 \pm 0.29$   $\text{ng g}^{-1}$  dw) and ULB ( $7.23 \pm 0.30$   $\text{ng g}^{-1}$  dw).

Station MM recorded the highest mean concentration value ( $9.03 \pm 0.33$   $\text{ng g}^{-1}$  dw) for *o,p'*DDT pesticide in *Rhagovelia spp.* while MG had the lowest ( $3.38 \pm 0.38$   $\text{ng g}^{-1}$  dw); the mean concentration value was  $6.84 \pm 1.62$   $\text{ng g}^{-1}$  dw (Fig. 3e). ANOVA revealed that the mean concentration values for *o,p'*DDT pesticide in *Rhagovelia* species differed statistically across stations ( $F = 86.93$ ,  $df = 11$ ,  $p = < 2.2\text{e-}16$ ). Tukey's HSD pairwise mean comparisons for *o,p'*DDT pesticide in *Rhagovelia* species across different stations revealed that there was significant differences between stations ME ( $8.55 \pm 0.35$   $\text{ng g}^{-1}$  dw), MG ( $3.38 \pm 0.38$   $\text{ng g}^{-1}$  dw), MKE ( $5.44 \pm 0.41$   $\text{ng g}^{-1}$  dw), MM ( $9.03 \pm 0.33$   $\text{ng g}^{-1}$  dw), MMJ ( $6.71 \pm 0.21$   $\text{ng g}^{-1}$  dw),

MAPU ( $7.15 \pm 0.30$   $\text{ng g}^{-1}$  dw) and RM ( $7.44 \pm 0.21$   $\text{ng g}^{-1}$  dw). Alternatively, stations MKE ( $5.44 \pm 0.41$   $\text{ng g}^{-1}$  dw), RB ( $5.67 \pm 0.36$   $\text{ng g}^{-1}$  dw) and UL ( $5.51 \pm 0.34$   $\text{ng g}^{-1}$  dw); MAPU ( $7.15 \pm 0.30$   $\text{ng g}^{-1}$  dw) and RE; and RM ( $7.44 \pm 0.21$   $\text{ng g}^{-1}$  dw) and ULB ( $7.44 \pm 0.21$   $\text{ng g}^{-1}$  dw) were statistically not different at  $p < 0.05$ .

The highest concentration of *p,p'*DDT pesticides in *Rhagovelia spp.* sampled in the South Coast of Kenya among the twelve stations occurred at station RE ( $10.09 \pm 0.35$   $\text{ng g}^{-1}$  dw) whereas the lowest was recorded at MAPU ( $5.94 \pm 0.40$   $\text{ng g}^{-1}$  dw) with a mean of  $7.75 \pm 1.11$   $\text{ng g}^{-1}$  dw (Fig. 3f). There was significant difference ( $F = 27.36$ ,  $df = 11$ ,  $p = < 2.2\text{e-}16$ ) in the mean concentration of *p,p'*DDT pesticides in *Rhagovelia spp.* among the sampling stations. In addition, Tukey's HSD pairwise mean comparisons for *p,p'*DDT pesticides in *Rhagovelia spp.* revealed significant differences among stations ME ( $7.41 \pm 0.18$   $\text{ng g}^{-1}$  dw), MKE ( $7.07 \pm 0.31$   $\text{ng g}^{-1}$  dw), MM ( $8.64 \pm 0.34$   $\text{ng g}^{-1}$  dw), MAPU ( $5.94 \pm 0.40$   $\text{ng g}^{-1}$  dw), RE ( $10.09 \pm 0.35$   $\text{ng g}^{-1}$  dw) and ULB ( $8.71 \pm 0.38$   $\text{ng g}^{-1}$  dw). In contrast, stations ME ( $7.41 \pm 0.18$   $\text{ng g}^{-1}$  dw), MG ( $8.09 \pm 0.32$   $\text{ng g}^{-1}$  dw), RB ( $8.09 \pm 0.33$   $\text{ng g}^{-1}$  dw), RM ( $7.41 \pm 0.18$   $\text{ng g}^{-1}$  dw) and UL ( $8.09 \pm 0.32$   $\text{ng g}^{-1}$  dw); and MKE ( $7.07 \pm 0.31$   $\text{ng g}^{-1}$  dw), MMJ ( $6.73 \pm 0.25$   $\text{ng g}^{-1}$  dw) and UE ( $6.69 \pm 0.22$   $\text{ng g}^{-1}$  dw).

The mean concentration of HCN,  $\alpha$ -HCH,  $\gamma$ -HCH and  $\beta$ -HCH pesticides in macroinvertebrate FFGs for *Rhagovelia spp.* as recorded in different stations is illustrated in Figure 4. The mean concentration of HCN pesticide in the *Rhagovelia spp.* sampled along different sampling stations in South Coast estuarine systems of Kenya was  $7.57 \pm 1.71$   $\text{ng g}^{-1}$  dw; and ranged from  $3.32 \pm 0.19$  to  $10.09 \pm 0.35$   $\text{ng g}^{-1}$  dw (Fig. 4a). There existed significant statistical differences ( $F = 96.39$ ,  $df = 11$ ,  $p = < 2.2\text{e-}16$ ) in the mean concentration of HCN pesticide for *Rhagovelia* species samples across the sampling stations. post hoc Tukey test showed a significant difference among the means of concentration levels in HCN pesticides for *Rhagovelia* species (ME,  $3.32 \pm 0.19$   $\text{ng g}^{-1}$  dw; MG,  $7.26 \pm 0.22$   $\text{ng g}^{-1}$  dw; MKE,  $8.09 \pm 0.33$   $\text{ng g}^{-1}$  dw; MMJ,  $9.03 \pm 0.33$   $\text{ng g}^{-1}$  dw; MAPU,  $5.99 \pm 0.35$



**Figure 3. Mean ( $\pm$ SD) spatial variation in the concentration of a)  $p,p'$ -DDE, b)  $o,p'$ -DDE, c)  $o,p'$ -DDD, d)  $p,p'$ -DDD, e)  $o,p'$ -DDT and f)  $p,p'$ -DDT pesticides in macroinvertebrate FFGs for *Rhagovelia* species in estuarine systems of South Coast, Kenya. The superscript letters represent mean differences among the stations obtained by performing Tukey's HSD pairwise mean comparisons. ME: Mwena Estuary; MG: Mkurumudzi Gazi; MKE: mkurumudzi Estuary; MM: Mwena Manda; MMJ: Mwena Majoreini; RB: Ramisi Bridge; RE: Ramisi Estuary; RM: Ramisi Mwachande; UE: Uмба Estuary; UL: Uмба Lenjo; ULB: Uмба Lunga-lunga Bridge.**

ng  $g^{-1}$  dw; RB,  $7.43 \pm 0.30$  ng  $g^{-1}$  dw; RM,  $8.07 \pm 0.27$  ng  $g^{-1}$  dw; UE,  $6.69 \pm 0.22$  ng/g and ULB,  $10.09 \pm 0.35$  ng  $g^{-1}$  dw). However, there was no statistical difference in the mean concentrations of HCN pesticides for *Rhagovelia* species among stations MKE ( $8.09 \pm 0.33$  ng  $g^{-1}$  dw), MM ( $8.64 \pm 0.34$  ng  $g^{-1}$  dw), RE ( $8.09 \pm 0.33$  ng  $g^{-1}$  dw) and UL ( $8.09 \pm 0.32$  ng  $g^{-1}$  dw).

The concentration values for  $\alpha$ -HCH pesticides in *Rhagovelia* species among the sampling stations was  $6.7 \pm 1.9$  ng  $g^{-1}$  dw with the values ranging between  $3.32 \pm 0.19$  ng  $g^{-1}$  dw and  $9.08 \pm 0.33$  ng  $g^{-1}$  dw (Fig. 4b). There was a significant difference in mean concentration levels of  $\alpha$ -HCH pesticides for *Rhagovelia* species among the sampling stations at  $p < 0.05$  level for the twelve stations ( $F = 34.32$ ,  $df = 11$ ,  $p = < 2.2e-16$ ). *post hoc* Tukey test results revealed that stations ME ( $9.08 \pm 0.33$  ng  $g^{-1}$  dw), MG ( $5.05 \pm 0.44$  ng  $g^{-1}$  dw), MKE ( $7.20 \pm 0.30$  ng  $g^{-1}$  dw), MM ( $6.67 \pm 0.22$  ng  $g^{-1}$  dw), MMJ ( $8.55 \pm 0.35$  ng  $g^{-1}$  dw), MAPU ( $6.42 \pm 0.29$  ng  $g^{-1}$  dw), RE ( $3.32 \pm 0.19$  ng  $g^{-1}$  dw),

UL ( $5.21 \pm 0.45$  ng  $g^{-1}$  dw) and ULB ( $4.38 \pm 0.29$  ng  $g^{-1}$  dw) differed significantly with station ME recording the highest mean concentration ( $9.08 \pm 0.33$  ng  $g^{-1}$  dw) and RE the least ( $3.32 \pm 0.19$  ng  $g^{-1}$  dw). Stations ME and RB; MKE and UE; MMJ and RM did not differ statistically (ME = RB; MKE = UE; and MMJ = RM).

$\gamma$ -HCH concentrations in *Rhagovelia* species among the sampling stations registered a mean of  $6.7 \pm 2.22$  ng  $g^{-1}$  dw with values ranging from  $2.74 \pm 0.18$  ng  $g^{-1}$  dw, MAPU station to  $9.45 \pm 0.51$  ng  $g^{-1}$  dw at station MMJ (Fig. 4c). The mean concentration residues of  $\gamma$ -HCH pesticides in *Rhagovelia* species differed significantly among the sampling stations ( $F = 120.90$ ,  $df = 11$ ,  $p = < 2.2e-16$ ). Multiple pairwise comparison Tukey test indicated that stations ME ( $4.18 \pm 0.27$  ng  $g^{-1}$  dw), MG ( $9.03 \pm 0.33$  ng  $g^{-1}$  dw), MKE ( $7.18 \pm 0.29$  ng  $g^{-1}$  dw), MAPU ( $2.74 \pm 0.18$  ng  $g^{-1}$  dw), RB ( $5.21 \pm 0.45$  ng  $g^{-1}$  dw) and UE ( $8.64 \pm 0.34$  ng  $g^{-1}$  dw) were statistically different at  $p < 0.05$ . On the other hand, stations MG ( $9.03 \pm 0.33$  ng  $g^{-1}$  dw), MM ( $9.03 \pm 0.33$

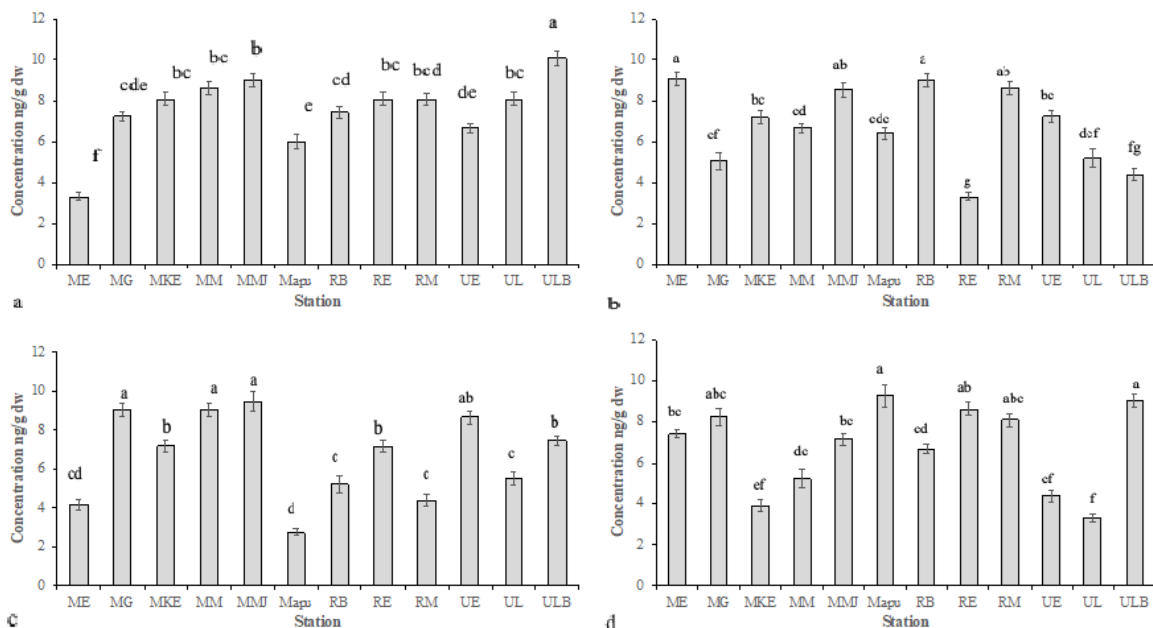
ng g<sup>-1</sup> dw), MMJ (9.45 ± 0.51 ng g<sup>-1</sup> dw); MKE (7.18 ± 0.29 ng g<sup>-1</sup> dw), RE (7.15 ± 0.30 ng g<sup>-1</sup> dw), ULB (7.44 ± 0.21 ng g<sup>-1</sup> dw); and RB (5.21 ± 0.45 ng g<sup>-1</sup> dw), RM (4.38 ± 0.29 ng g<sup>-1</sup> dw) and UL (5.51 ± 0.34 ng g<sup>-1</sup> dw) were statistically not significant.

The mean concentration of β-HCH pesticides in *Rhagovelia* species on the other hand ranged from 3.32 ± 0.19 ng g<sup>-1</sup> dw (station UL) – 9.28 ± 0.55 ng g<sup>-1</sup> dw at Mapu station with a mean of 6.8 ± 2.08 ng g<sup>-1</sup> dw (Fig. 4d). There was a significant difference observed in the mean concentration residues of β-HCH pesticides in *Rhagovelia* species among the sampling stations (F = 147.48, df = 11, p = < 2.2e-16). *post hoc* comparisons using Tukey HSD test on the mean concentration values of beta\_HCH pesticides in *Rhagovelia* species denoted that stations ME (7.44 ± 0.21 ng g<sup>-1</sup> dw), MG (8.23 ± 0.40 ng g<sup>-1</sup> dw), MKE (3.92 ± 0.26 ng g<sup>-1</sup> dw), MM (5.21 ± 0.45 ng g<sup>-1</sup> dw), MAPU (9.28 ± 0.55 ng g<sup>-1</sup> dw), RB (6.69 ± 0.22 ng g<sup>-1</sup> dw), RE (8.64 ± 0.34 ng g<sup>-1</sup> dw) and UL (3.32 ± 0.19 ng g<sup>-1</sup> dw) were different statistically (p < 0.05). Conversely, stations ME (7.44 ± 0.21 ng g<sup>-1</sup> dw) and MMJ

(7.15 ± 0.30 ng g<sup>-1</sup> dw); MG (8.23 ± 0.40 ng g<sup>-1</sup> dw) and RM (8.09 ± 0.32 ng g<sup>-1</sup> dw); MKE (3.92 ± 0.26 ng g<sup>-1</sup> dw) and UE (4.38 ± 0.29 ng g<sup>-1</sup> dw) were statistically similar.

## Discussion

The analysis conducted on the macroinvertebrates FFG of *Rhagovelia spp.* data revealed significant temporal effects on various OCPs levels. The small or low p-values (p < 2.2e-16) associated with each factor indicated a high degree of statistical significance, providing strong evidence against the null hypothesis of “no significant effect” of stations on OCPs concentrations. Further significant differences noted by different OCPs concentrations from *Rhagovelia spp.* samples among the sampling stations could be explained by the localized sources of pesticides or environmental conditions. Some of the associated sources of OCPs concentrations could vary from agricultural practices to urbanization and probably due to industrial effluents. This could occur through surface run-off and sub-surface infiltration into estuarine systems. Equally, the



**Figure 4.** Mean (±SD) spatial variation in the concentration of a) HCN, b) α-HCH, c) γ-HCH and d) β-HCH pesticides in macroinvertebrate FFGs for *Rhagovelia* species in estuarine systems of South Coast, Kenya. The superscript letters represent mean differences among the sampling stations obtained by performing Tukey's HSD pairwise mean comparisons. ME: Mwena Estuary; MG: Mkurumudzi Gazi; MKE: mkurumudzi Estuary; MM: Mwena Manda; MMJ: Mwena Majoreini; RB: Ramisi Bridge; RE: Ramisi Estuary; RM: Ramisi Mwachande; UE: Uмба Estuary; UL: Uмба Lenjo; ULB: Uмба Lunga-lunga Bridge.

temporal distribution was attributed to climatic factors and the seasonal anthropogenic occurrences related to crop pest control and management.

Therefore, the bioassay of benthic macroinvertebrates' body concentrations of OCPs can be utilized to explain the state of environmental perturbation because they play a key role in measuring the bioavailability of a given contaminant in the environment (Solà & Prat, 2006; Peter, *et al.*, 2018). The *Rhagovelia* spp showed that irrespective of the varying conditions of different sampling sites, it could easily bioconcentrate the OCPs although in different concentration levels hence a recommendable candidate for toxicological studies. Even though this study utilized the entire body of the organism owing to laborious work involved in separating different body tissues due to the bioassay organism's body size, internal composition and distribution of contaminants among body organs/tissues is not homogeneous because the distribution patterns is both pollutant and species-specific or broadly, taxon-specific (Hare *et al.*, 2003). This study, therefore, corroborated well with Hare (1992) who averred that FFGs of macroinvertebrates readily bind OCPs contaminants on the surface of their exoskeleton and body organs hence detecting them in the entire body (Franzle, 2015). This is the strategy this present study adopted whereby the entire body of the macroinvertebrate FFG of the *Rhagovelia* spp. was utilized thus offering the best environmental solution (Pastorino *et al.*, 2020a) as far as the OCPs contamination is concerned because they were bioavailable in all sampling sites.

The spatial patterns displayed by the OCPs could have been induced by a number of physicochemical water quality attributes or the environmental factors. The bioavailability of a given contaminant such as that of pesticides can be influenced by such parameters as the water pH, conductivity, temperature, TDS, redox potential, salinity and total organic content, and is the percentage of the total sum of pesticides that is available in time and space for adsorption by an organism (Tessier & Turner, 1995; Peltier *et al.*, 2008). The *Rhagovelia* spp. samples from

all the twelve sampling sites had OCPs although in different levels, which demonstrates the robustness of using macroinvertebrate FFGs as bioindicators of environmental quality. Similarly, due to their intimate relationship with sediments which act as sinks for pollutants, they easily bioconcentrated the OCPs. Same reasons have been advanced where it is widely believed that macroinvertebrates are good indicators of pollution because they are bottom dwellers, which make them more efficient to bioaccumulate pollutants (Nyakeya *et al.*, 2022). Their ability to bioconcentrate toxicants also depends on the geochemical background of the sediments (Turner, 1995).

*Rhagovelia* species falls under the predator FFG of macroinvertebrates hence a high probability of having predated on other FFGs thus increased chances of biomagnification. Depending on the level of macroinvertebrate FFGs, there are different pathways through which OCPs can find their way into the body. Filterers can access them via gills and the nutritional requirements such as filtration in the water column, grazer-scrapers through foraging on periphyton and phytoplankton, collector-gatherers by collection and gathering of fine particulate matter, shredders via feeding on coarse particles of organic matter deposited in/on sediments, and lastly predators through preying on other invertebrates (Mebane *et al.*, 2020). OCPs are among hydrophobic contaminants often detected in aquatic organisms as *Rhagovelia* spp. and can be magnified by trophic interactions, beginning with those at the base of the food web. Therefore, OCPs may have been sorbed to existing algae cells consumed by the grazing macroinvertebrates/zooplankton which in turn might have been preyed upon by *Rhagovelia* spp. in the next trophic level. During sorption or grazing, OCPs are portioned to lipid rich organs and tissues leading to their bioaccumulation (Bard, 1999). There is high efficiency experienced during the OCPs transfer from one trophic level to another resulting to biomagnification at each level (Larsson *et al.*, 2000). Therefore, the concentration levels witnessed in different stations could be ex-

plained by transfer of OCPs to *Rhagovelia* spp. via other macroinvertebrate FFGs at the lower trophic levels.

According to Dallinger and Rainbow (1993) the concentration of toxicants in microbenthic invertebrates are proportional to the pollutant uptake, transport, utilization, and excretion, and varies with each taxa, genus and/or species. The concentration of OCPs in *Rhagovelia* spp. could have been bioconcentrated or bioaccumulated depending on the bioavailability of the different pesticides from dissolved and particulate organic matter and the ability of the OCP escaping from the macroinvertebrate (Franzle, 1995). Moreover, the variations in OCPs concentrations that was witnessed from one station to another could be as a result of sex, size and age. This is in agreement with Pastorino *et al.* (2020b) who opined that in a temporal and spatial scale, the amounts of OCPs among the group of species/taxa of macroinvertebrates taking refuge in a given ecosystem is likely to vary on the basis of early history strategies such as size, age, sex, and developmental stage of the individuals. In addition, Pros (1981) and Hare (1992) confirmed that related taxa, up to species level but under the same genus, and inhabiting a homogeneous system could bioconcentrate different levels of OCPs.

## Conclusion and recommendations

The aim of this study was to assess the distribution of OCPs in macroinvertebrates FFGs of *Rhagovelia* spp. in the tropical estuarine ecosystems of South Coast, Kenya. Sixteen OCPs were recorded from *Rhagovelia* spp. sampled in all the twelve study sites, with varying concentration levels. The ANOVA results underscored the multifaceted nature of environmental dynamics, with 'station' exerting significant influences on OCPs concentrations in *Rhagovelia* spp. The observed interaction effect further accentuated the complexity of environmental processes. Overall, these results provided valuable insights into the factors influencing OCPs levels in *Rhagovelia* spp.

and can be used to guide future research and environmental management strategies. These findings offer valuable insights for environmental monitoring and management efforts, emphasizing the need for targeted interventions to mitigate chemical exposures and safeguard environmental health. It is on the foregoing basis that the null hypothesis, which stated that there is no significant difference in the distribution of OCPs by aquatic macroinvertebrates FFG of *Rhagovelia* spp. between the sampling stations was rejected. The statistical analysis revealed that each station played a crucial role in determining the levels of OCPs in *Rhagovelia* spp. due to both environmental factors, early life history strategies of the tested bioassay organism, and different sources of OCPs as influenced by anthropogenic activities. The study recommends for the application of macroinvertebrate FFG of *Rhagovelia* spp. in biomonitoring of estuarine ecosystems. Further research may delve into elucidating specific drivers behind spatial variations in OCPs concentrations from *Rhagovelia* species to facilitate informed decision-making for sustainable environmental stewardship. However, to fully understand the impacts of OCPs in the environment we strongly recommend for the use of all/different FFGs of macroinvertebrates such as grazers, collector-gatherers, filterers and shredders in order to bring out the general behavior of these pesticides along the food web.

## Ethical approval

The authors complied with the provisions of KMFRI research policy that spells out the code of conduct for researchers. KMFRI is a state corporation established in 1979 by the science and technology act, cap 250 of the laws of Kenya. The act was repealed in 2013 by the science, technology and innovation act no. 28, which recognizes KMFRI as a national research institution under section 56, fourth schedule. Further, the study was approved by the Institutional Scientific and Ethics Review Committee (ISERC) of Kisii University, Ref. No. KSU/ISERC/OO11/7/24.

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