

# Linking physico-chemical parameters and macroinvertebrates for water quality assessment of Kakamega and the East Usambara montane ecosystems in Kenya

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## Abstract

Sub-Saharan freshwater biodiversity is impacted by changes in land use and climate change. To evaluate the relationship between physico-chemical variables and macroinvertebrate community assemblages in Kakamega and the East Usambara Mountains forests, we explored benthic macroinvertebrate community structure in relation to physico-chemical conditions of the water along an anthropogenic stress gradient. Sampling was done in six selected streams during the rainy and dry seasons between April 2017 and November 2019. At Kakamega, 367 macroinvertebrate individuals were identified belonging to 22 families, while at Usambara, 456 individuals belonging to 25 families were identified. Spearman's correlation revealed significant interactions in species diversity, evenness, and richness between macroinvertebrates and several physico-chemical parameters (pH, conductivity, total dissolved substances, salinity and temperature). At Usambara, species richness, evenness and diversity was significantly negatively correlated with conductivity, tds, salinity, temperature and pH. We demonstrated that macroinvertebrate indices can be used as a quick evaluation tool of water quality in response to stream systems in the region as well as help in pointing out early warnings to help mitigate and reduce threats to stream biodiversity from anthropogenic activities.

## KEYWORDS

aquatic macroinvertebrates, biomonitoring, diversity, ecosystem, freshwater

## Résumé

La biodiversité des eaux douces en Afrique subsaharienne est affectée par les changements dans l'utilisation des terres et le changement climatique. Pour évaluer la relation entre les variables physico-chimiques et les assemblages de communautés de macroinvertébrés dans les forêts de Kakamega et des monts Usambara orientaux, nous avons exploré la structure des communautés de macroinvertébrés benthiques en relation avec les conditions physico-chimiques de l'eau le long d'un gradient de stress anthropogénique. L'échantillonnage a été réalisé dans six cours d'eau sélectionnés pendant les saisons des pluies et les saisons sèches entre avril 2017 et novembre 2019. À Kakamega, 367 macroinvertébrés appartenant à 22 familles ont été identifiés,

tandis qu'à Usambara, 456 individus appartenant à 25 familles ont été identifiés. La corrélation de Spearman a révélé des interactions considérables en termes de diversité, de régularité et de richesse des espèces entre les macroinvertébrés et plusieurs paramètres physico-chimiques (pH, conductivité, substances dissoutes totales, salinité et température). À Usambara, la richesse, la régularité et la diversité des espèces présentaient une corrélation considérablement négative avec la conductivité, les substances dissoutes totales, la salinité, la température et le pH. Nous avons démontré que les indices de macroinvertébrés peuvent être utilisés comme un outil d'évaluation rapide de la qualité de l'eau en réponse aux systèmes de cours d'eau de la région et qu'ils peuvent aider à détecter les alertes précoces afin d'atténuer et de réduire les menaces que les activités anthropogéniques font peser sur la biodiversité des cours d'eau.

## 1 | INTRODUCTION

Throughout the world, freshwater ecosystems occupy less than 0.01% of the planet's total surface water but they support more than 100,000 species—among them are fish, mammals, birds as well as insects. However, these freshwater ecosystems now face increasingly serious threats due to the effects on water quality of expanding human populations, agricultural activities, industrial pollution and climate change (Dudgeon et al., 2006). In a river ecosystem, water quality is influenced by several human activities including, land use, settlement patterns, farming and industrial activities as well as the threat of climate change (Mandaville, 2002; Patang et al., 2018). Globally, freshwater biodiversity threats can be grouped into five categories: water pollution; overexploitation; habitat destruction; flow modification and invasive species (Dudgeon et al., 2006). Understanding the ecological status of freshwater and its biological implications is vital for proper management and conservation of the resources and biodiversity for sustainable utilisation of the resources and reduction of pressure on the ecosystem (Ojija & Laizer, 2016).

Biomonitoring using benthic macroinvertebrates has been found to be very reliable as compared to using other organisms (Duran, 2006): (a) as they are ubiquitous in river systems amid different habitats and thus are exposed to the various habitat disturbances at these locations (Balachandran et al., 2013; Deborde et al., 2016; Lenat et al., 1980); (b) as they exhibit a sedentary behaviour and respond to pollution, hence making them good indicators of localised conditions (Hellawell, 1986; Li et al., 2010; Reece & Richardson, 1999; Uherek & Pinto Gouveia, 2014); (c) as they are known to have long life cycles when compared with other groups and respond to environmental stress thus creating an easy way of analysing potential threats like habitat loss and other disturbances (Gaufin, 1973; Lenat et al., 1980); (d) they can be impacted by environmental disturbances in the various aquatic ecosystems, and differ in their tolerance to pollution (Basset et al., 2004; Rosenberg & Resh, 1994). Macroinvertebrates can therefore be divided into three distinctive groups: (Appendix A) (i) those that are highly pollution sensitive, comprised of groups that require good quality water to survive with high

levels of dissolved oxygen—among them are stoneflies (Plecoptera), caddisflies (Trichoptera) and mayflies (Ephemeroptera); (ii) moderately pollution sensitive organisms: These organisms can survive in lower quality habitats and can withstand some levels of pollution in their environment—among the group are the dragonflies and damselfly (Odonata) and crane fly (Diptera; Tipulidae); (iii) those organisms that are pollution tolerant and capable of tolerating very low levels of dissolved oxygen—among this group are the aquatic earthworms (Annelida; Oligochaeta, leeches), midge larva (Diptera; Chironomidae) and backswimmers (Notonectidae) (Ojija & Laizer, 2016).

Within the aquatic ecosystem, physico-chemical properties play a vital role in the determination of macroinvertebrates' abundance, species richness and species composition (García et al., 2008). Macroinvertebrate community structure is known to be affected by increased concentration of pollutants in the waterways. The geomorphic, hydrologic, riparian zone and water quality are major factors influencing the macroinvertebrate community assemblages. The Kakamega and the East Usambara mountain forests are undergoing severe degradation of biodiversity and habitat loss from fragmentation and land conversion (Collinge, 1996).

Recent reports show that the use of macroinvertebrates to assess water quality has gained increasing acceptance in Sub-Saharan Africa, including studies examining the downstream effects of tea plantations on water quality (Biervliet et al., 2009), the assessment of the ecological integrity of river ecosystems (Ollis et al., 2006), the assessment of water quality using macroinvertebrates (Tamiru, 2019) and examining trends in nutrient concentrations, land use patterns and the structure of benthic macroinvertebrates (Kilonzo et al., 2014). However, in Kenya and Tanzania, the application of these monitoring tools is rather limited. Until recently, assessments of aquatic ecosystems involved solely the physico-chemical analysis of waters but the condition of the macroinvertebrate community is now recognised as a critically important variable in such studies (Stevenson et al., 1996; Suriano et al., 2011). Combining the analysis of physico-chemical and biological variables has provided a more accurate way of evaluating the health of aquatic ecosystems (Lobo & Callegaro, 2000; Rău et al., 2014; Selvanayagam & Abril, 2015). This study examines

the relationship between the benthic macroinvertebrate communities and physico-chemical variables in order to determine the factors which influence the distribution and richness of the communities. The objectives of the study were to characterise the distribution and assemblage structures of benthic macroinvertebrates to identify the main environmental variables associated with changes in benthic macroinvertebrate community composition. Twelve sites covering six streams were sampled during the dry and wet seasons in 2017–2019 at Usambara and Kakamega. The results enabled us to identify environmental variables that act as drivers of benthic macroinvertebrate community composition to support the basis of this study.

## 2 | MATERIALS AND METHODS

### 2.1 | Study area

The study areas comprised the Kakamega forest and the East Usambara mountains forest (Figure 1). The Kakamega Forest is within Kakamega County, Kenya. It forms part of the eastern-most

fragment of the Guineo-Congolian rainforest, which once stretched from the West African coast across Central Africa to Uganda and Kenya. It is the only surviving rainforest in Kenya. The forest offers a unique sanctuary for a high diversity of endemism (plants, birds and insects), as well as a vital watershed for some of the rivers that flow into Lake Victoria. The forest serves as a backbone for the people living around it, majority of whom rely on it for their livelihoods including medicinal plants, building materials and wild vegetables. (<https://www.kenyasafari.com/kakamega-forest-reserve.html>, <https://www.africa-expert.com/about-kenya/national-parks/kakamega-forest/>). The communities in the study area are low income and fully dependent on water flowing from the forest for survival. They also have limited access to healthcare due to the cost implications and hence the need to monitor the water quality to ensure that it is safe for human consumption.

The East Usambara Mountains forests found in Mkinga District, Tanga Region of Tanzania. The forests range from the lowlands up into the highlands of the Usambara mountains. The East Usambara Mountains Forests form part of the well-known Eastern Arc Mountains as well as the Coastal Forests of Kenya and Tanzania. The



FIGURE 1 Map of Kakamega and East Usambara mountain forests sampling stations, showing sampling sites along the six streams.

forests have extraordinarily high levels of endemism, with seven endemic vertebrates and 35 Eastern Arc strictly endemic vertebrates despite their small size. The forests also act as an important fuel-wood source, building materials, food and water for the residents. The people in Tanga rely on the ecosystem for their water. However, the forest's biodiversity has been impacted by fires, expansion of agricultural land, logging, mining, commercial firewood collection and hunting (UNESCO, <https://en.unesco.org/biosphere/africa/east-usambara>, BirdLife International (2023) Important Bird Areas factsheet: East Usambara Mountains). The rivers flowing from these forests are continuously being polluted due to human activities, thus affecting the aquatic ecosystem. The application of benthic macroinvertebrates to determine the safety of water for human consumption takes advantage of the ethno knowledge among the communities. Three streams were selected at each location (Kakamega, Usambara) and two sampling points (upper and lower) were established along a segment of the streams. The locations were selected because no other study had been undertaken previously to check the water quality of the rivers using macroinvertebrates as bioindicators.

## 2.2 | Macroinvertebrates sampling (Sass5 kick-sampling methodology)

Macroinvertebrate's collection was done along the streams from 2017 to November 2018 at each of the Kakamega ( $n=6$ ) and Usambara ( $n=6$ ) sampling sites. Samples were obtained using the Sass5 kick-sampling protocol (Dickens & Graham, 2002). The SASS net, a modified version of the Kick net with a 500- $\mu\text{m}$  mesh, was used in this sampling protocol. Various biotopes were tested inside a recommended time limit or potential area coverage (Dickens & Graham, 2002). The stones-in-current (SIC) and bedrock were searched (or 'kicked') for 2–5 min. In a similar manner, a search was conducted for bedrock and stones-out-of-current (SOOC) for 1 min. The SIC and SOOC samples were then combined into a 'Stones' (STONES) sample. Further sampling of the river vegetation covering 2 m of stream vegetation (SIC and SOOC) were searched and this was considered as the 'Vegetation' (VEG) sample; the gravel, sand and mud (GSM) sample was filtered after stirring and hand-picking of the macroinvertebrates done for 1 min.

The collected macroinvertebrates were later transferred in 70% alcohol and taken to the laboratory for identification.

## 2.3 | Physico-chemical assessment of the water quality

Conductivity, salinity, pH, total dissolved substances and temperature were measured using the PC Testr 35 (Eutech/Oakton Instruments, Singapore) in situ during sampling. Other site information recorded included coordinates using a Garmin GPS device, and all the other information was recorded including any human activities such as washing, bathing, domestic water collection and animal

watering. Physico-chemical assessment of the water quality was done using WHO (2008) guidelines, which are universally acceptable, that is conductivity  $<400\mu\text{s/cm}$  and TDS  $<1200\text{ mg/L}$  (Chikodzi et al., 2017). Salinity levels were also assessed using Guidelines for Fresh and Marine Waters National Water Quality Management Strategy, Australian Water Quality (Mcevoy et al., 2003) and salinity 0–1000 mg/L Environmental Protection Agency (EPA).

## 2.4 | Macroinvertebrates identification

All the samples were taken to the Biosystematics Research and Information Laboratory at ICIPE for identification. Macroinvertebrates sorting and identification was done to family under a stereomicroscope at  $10\times 40$  magnification with the help of available keys presented in Guides to Freshwater Invertebrates of Southern Africa (Arimoro, 2009; Harrison, 2009; Lowe, 2009; Schael 2009). A Practical Guide to the Identification of African Freshwater Snails (Frandsen et al., 1980), and the Freshwater Snails of Florida ID Guide (Thompson, 2019). After carrying out the identification process, scoring was done using SASS 5 procedure (Dickens & Graham, 2002). The ASPT was given as the average of the tolerance scores of all the macroinvertebrate families found and ranges from 1 to 15 (Appendix A). The index values are into five categories: SASS score  $>6.9$  for Unmodified Natural condition,  $5.8 < \text{SASS score} < 6.9$  for largely natural with few modifications (GOOD condition);  $4.9 < \text{SASS score} < 5.8$  for Moderately modified (FAIR Condition);  $4.3 < \text{SASS score} < 4.9$  for largely modified (POOR condition); and SASS indices  $< 4.3$  for seriously/critically modified (VERY POOR condition) (Tambala et al., 2016).

## 2.5 | Statistical data analyses

Analyses were done using the Free Statistic Kingdom Calculator Version 4.0. software. First, the measured physico-chemical parameters were summarised into descriptive statistics and presented as mean values and standard error (SE). To examine the mean difference of physico-chemical parameters between the streams, we used analysis of variance (ANOVA) and repeated measure ANOVA among the sampling sites. Tukey's post hoc tests were used to show the direction of the detected differences. Testing for normality was done using the Shapiro–Wilk test and homogeneity of variance test by use of Levene's test. Since the values of some of the environmental variables were found not normally distributed, a non-parametric Kruskal–Wallis test followed by post hoc Dunn's test. The determination of independence or association between the variables was done using Chi-square test. The correction of the level of significance of the means of physico-chemical variables was done using the Bonferroni correction procedure. Relationship among physico-chemical parameters and macroinvertebrate indices were analysed using Spearman rank correlation. Results with  $p < 0.05$  were considered statistically significant.

## 2.6 | Macroinvertebrates analyses

Hill numbers was used to quantify macroinvertebrate diversity of an assemblage and to compare diversity among sampling sites using the iNEXT package for interpolation/extrapolation of species diversity <https://chao.shinyapps.io/iNEXTOnline> (Hill, 1973; Hsieh et al., 2016). Hill numbers are denoted by a diversity order  $q$ , taxa richness ( $q=0$ ), asymptotic estimation for Shannon diversity ( $q=1$ ) and the Inverse Simpson index ( $q=2$ ) (Chao et al., 2014). To evaluate the sampling effort between the assemblages, the asymptote richness estimator was used. Modelling the operation of each assembly was done by adjusting to the three commonly used models: the geometric model (Motomura, 1932), the log series model (Fisher et al., 1943) and the log-normal distribution (Magurran, 2005; Preston, 1948). The Kolmogorov–Smirnov goodness of fit test (Magurran, 2005; Sokal & Rohlf, 1969) was conducted in order to evaluate the fitness of the species abundance distribution using [https://mean-rarity.shinyapps.io/rshiny\\_app1/](https://mean-rarity.shinyapps.io/rshiny_app1/), an interactive online application (Roswell & Dushoff, 2020).

The beta diversity determination was done using the Berger–Parker index expressed as a percentage (Berger & Parker, 1970; Caruso et al., 2007). In order to evaluate the diversity of macroinvertebrates, the Sorensen similarity index was applied (Chao et al., 2005). To measure the distribution of individuals among species, Pielou's evenness was used (Pielou, 1966, 1975). Lastly, the Spearman's rank correlation was used to visualise the beta diversity for attraction or repulsion and the Average Score per Taxon (ASPT) index used to measure the biological status (Dickens & Graham, 2002).

## 3 | RESULTS

### 3.1 | Physico-chemical parameters among the streams

Mean pH values recorded at Msi S01 ( $8.3 \pm 0.17$ ) and Msi S02 were higher as compared to other sites Mul S01 ( $6.7 \pm 0.17$ ) and

Kis S01 ( $6.8 \pm 0.06$ ) (Figure 2); mean highest conductivity and salinity were significantly higher at Muz S01 at  $498 \pm 10.31 \mu\text{S/cm}$ ,  $282 \pm 8.50 \text{ mg/L}$  Mat S02, respectively, while (Figure 3), lowest salinity levels were recorded at Mul S02 ( $43.3 \pm 3.43$ ). Mean temperatures varied across the stream with mean highest recorded at Muz S02 ( $29.6 \pm 0.27^\circ\text{C}$ ), mean lowest total dissolved substances were recorded at Ise S02 ( $52 \pm 3.58 \text{ mg/L}$ ) and the highest measured at Muz S01 ( $347 \pm 21.55 \text{ mg/L}$ ).

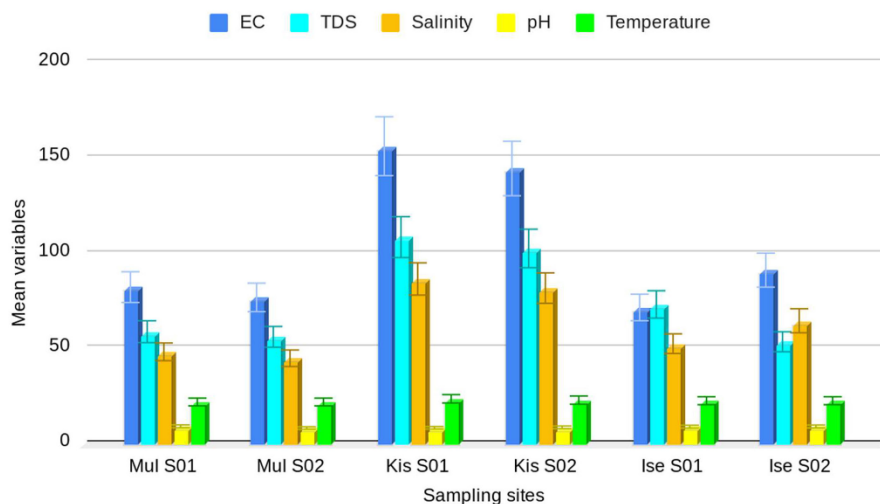
### 3.2 | Relationship between physico-chemical and species indices

At Kakamega, the Spearman correlation analysis (Figure 4a), showed that macroinvertebrate community indices were significantly affected by environmental variables. Macroinvertebrate diversity was significantly negatively correlated with tds ( $r=-0.71$ ,  $p=0.05$ ), salinity ( $r=-0.26$ ,  $p=0.05$ ) and temperature ( $r=-0.44$ ,  $p=0.05$ ): Shannon diversity significantly negatively correlated with conductivity and pH ( $r=-0.09$ ,  $p=0.05$ , and  $r=-0.03$ ,  $p=0.05$  respectively). Macroinvertebrate species evenness was significantly negatively correlated with Tds ( $r=-0.09$ ,  $p=0.05$ ), pH ( $r=-0.09$ ,  $p=0.05$ ) and ASPT ( $r=-0.43$ ,  $p=0.05$ ).

There was a positive correlation between richness, pH, tds, salinity, temperature and ASPT ( $r=0.03$ ,  $p=0.05$ ,  $r=0.66$ ,  $p=0.05$ ,  $r=0.74$ ,  $p=0.05$  and  $r=0.09$ ,  $p=0.05$  respectively). A strong negative correlation was observed between pH, salinity, conductivity, tds ( $r=-0.09$ ,  $p=0.05$ ,  $r=-0.14$ ,  $p=0.05$ ,  $r=-0.31$ ,  $p=0.05$ ), respectively, while temperature strongly positively correlated with EC, tds and salinity ( $r=0.74$ ,  $p=0.05$ ,  $r=0.74$ ,  $p=0.05$  and  $r=0.94$ ,  $p=0.05$ ) respectively.

At Usambara, species richness was significantly negatively correlated with conductivity, tds, salinity, temperature and ASPT ( $r=-0.54$ ,  $p=0.05$ ,  $r=-0.54$ ,  $p=0.05$ ,  $r=0.49$ ,  $p=0.05$ ,  $r=-0.64$ ,  $p=0.05$  and  $r=-0.37$ ,  $p=0.05$  respectively). Shannon diversity was significantly negatively correlated with pH ( $r=-0.76$ ,  $p=0.05$ ), whereas species evenness significantly negatively correlated with pH and temperature ( $r=-0.15$ ,  $p=0.05$  and  $r=-0.06$ ,  $p=0.05$

**FIGURE 2** Physico-chemical properties (mean  $\pm$  SE), ( $n=6$ ); conductivity, total dissolved substances (TDS), salinity, pH and temperature of the water quality recorded at Kakamega: Mul S01, Mul S02, Kis S01, Kis S02, Ise S01 and Ise S02.



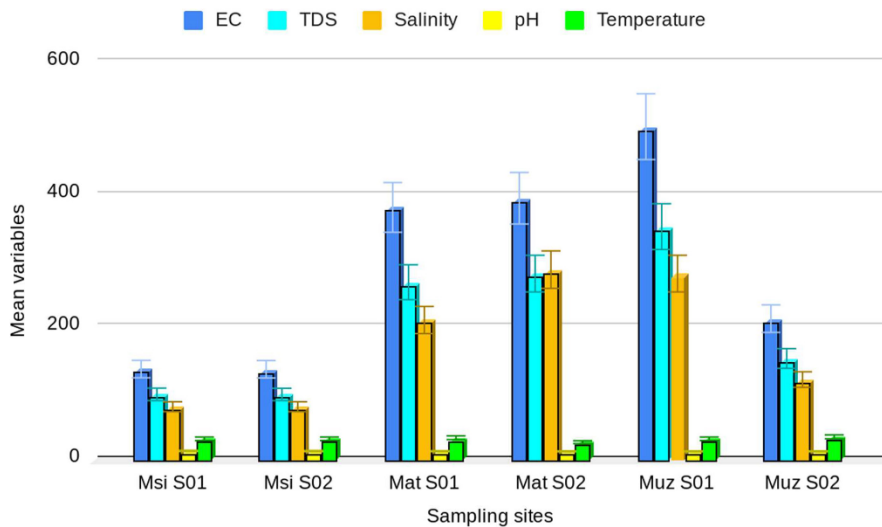


FIGURE 3 Physico-chemical properties (mean  $\pm$  SE), ( $n=6$ ); conductivity, total dissolved substances (TDS), salinity, pH and temperature of the water quality recorded at Usambara: Msi S01, Msi S02, Mat S01, Mat S02, Muz S01 and Muz S02.

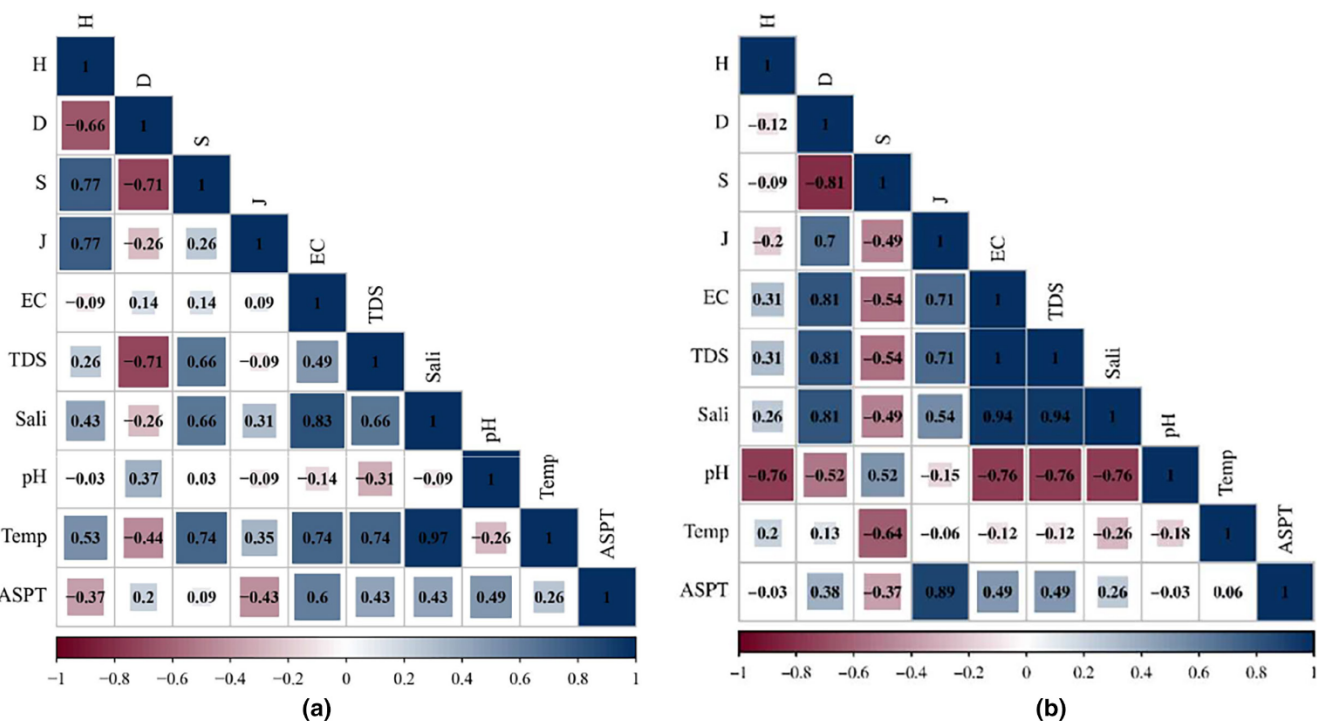


FIGURE 4 (a, b) Correlogram of Spearman's rank correlations, showing correlations between physico-chemical variables and between physico-chemical variables and macroinvertebrate indices. Shannon's index (H), Simpsons index (D), taxa richness (S), Pielou's evenness (J), conductivity (EC), total dissolved substances (TDS), salinity (Sali), pH, temperature (Temp) and Average Score Per Taxon (ASPT). Significant correlations ( $p < 0.05$ ).

respectively). ASPT was significantly negatively correlated with pH ( $r = -0.03$ ,  $p = 0.05$ ). There was a negative correlation between pH and conductivity, tds and salinity ( $r = -0.76$ ,  $p = 0.05$ ) respectively. Temperature had a negative correlation with conductivity, tds, salinity and pH ( $r = -0.12$ ,  $p = 0.05$ ,  $r = -0.12$ ,  $p = 0.05$  and  $r = -0.18$ ,  $p = 0.05$ ) respectively (Figure 4b).

The average score per Taxa (ASPT) was shown to differ among streams and the highest recording was at Muz S01 5.2 and Muz S02 5.0, Msi S02 recorded the lowest ASPT of 3.0 (Table 1). A general look at the sites indicated that ASPT was slightly higher upstream

apart from Ise S02 which recorded 4.4 downstream with upstream having 4.2.

### 3.3 | Sorensen similarity index

The Sorensen similarity index value ranges between 0 and 1, with a value close to 1 indicating a higher level of similarity between two sites' assemblages, whereas a value of 0 indicates no similarity (Chao et al., 2006; Magurran, 2005). The Multiple Site

**TABLE 1** Detailed information of sampling stations at Kakamega and the East Usambara mountain forests (S01 denotes upstream and S02 denotes downstream).

River's name	Sampling station	Coordinates	Site description
Muleche	Mu S01	N 00.25390 E 034.84945	Muddy site with steep terrain, a major route into the forest for both animals and humans.
	Mu S02	N 00.25559 E 034.84877	Slow moving and muddy water and located near a bridge with heavy signs of hoof prints and droppings.
Isecheno	Ise S01	N 00.22615 E 034.85809	A highly degraded area located near farmland, a lot of sedimentation from the agricultural activities around.
	Ise S02	N 00.22572 E 034.85839	Site with very low water levels, a heavy presence of human activities including washing, animal watering, serves as the main water collection point for the community.
Kisaina	Ki S01	N 00.22375 E 034.85358	A degraded site with heavy agricultural activities.
	Ki S02	N 00.22291 E 034.85245	Site with big boulders, vegetation destroyed by animal grazing at the site.
Msimbazi	Ms S01	S 04.86164 E 038.79493	Cultivation done to the riverbanks hence increased sedimentation at the site.
	Ms S02	S 04.85930 E 038.79561	Sand harvesting activities were recorded at the site, a major water collection area because of its proximity to the settlements.
Matemboni	Ma S01	S 04.89047 E 038.77475	Site with highly reduced water levels owing to habitat destruction at the site.
	Ma S02	S 04.89129 E 038.77408	Site located near farmland and with a lot of human activities.
Muzi	Mu S01	S 04.87952 E 038.74216	Site with fast-flowing waters and located some short distance away from the forest and settlements.
	Mu S02	S 04.87564 E 038.74463	The site was located on the road and served as a crossing point with no bridge, abstraction activities at the site observed.

Similarity Index was used to assess the similarity between macroinvertebrate assemblages of Kakamega and Usambara sampling sites. The Sorensen's index value, 1 was recorded at the Usambara and Kakamega indicating a high degree of similarity between the assemblages.

### 3.4 | Rarefaction/extrapolation curves and the rank abundance plot

Rarefaction and extrapolation plots based on sample size were constructed using the number of individuals as the measure of sampling effort. At Kakamega, the reference sample size (number of individual macroinvertebrates) was 367, and the observed species richness, Shannon diversity and Simpson diversity (Hill numbers for  $q=0, 1, 2$ ) for the reference sample were, 22.0, 11.69 and 8.17 respectively (solid line). The sample size for the Usambara site was 456, and the corresponding observed Hill numbers were 25.0, 16.90 and 13.18 respectively (solid line).

At Usambara, the rarefaction and extrapolation curve seem to have reached an asymptote (Figure 5b), whereas the one for Kakamega would require more sampling effort to achieve an asymptote. The asymptotic richness estimator suggested a possibility that more than 45 species could be recorded at this scale.

### 3.5 | Rank abundance plot

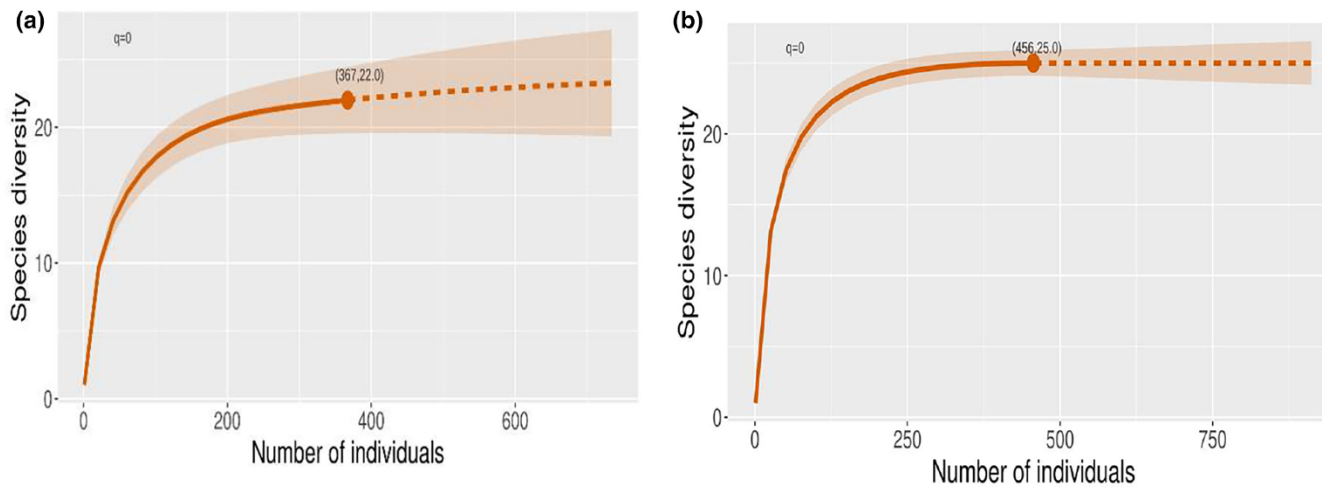
See Figure 6.

## 4 | DISCUSSION

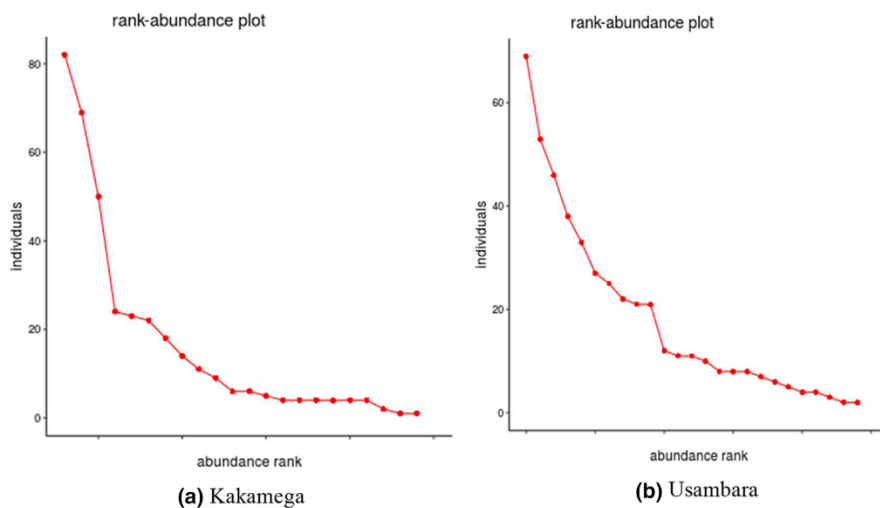
In order to have a better understanding of the expected impact of environmental factors on species of freshwater ecosystems, our study is the first attempt to investigate the response of macroinvertebrate community structure to environmental variables in Kakamega and East Usambara regions. The findings suggest that intricate interactions between physico-chemical properties influence a stream's ecological status and water quality.

### 4.1 | Vulnerabilities of benthic macroinvertebrates

During the study, Kakamega recorded a total of 22 families belonging to seven Orders, while Usambara recorded 25 families belonging to 10 Orders. The study presented slightly lower macroinvertebrates taxa as compared to those reported in some Kenyan and Tanzania rivers: Subukia River (32 taxa, Mbaka & Aura, 2022); Kilimanjaro rivers, Tanzania (48 taxa, Elias et al., 2014). These differences can



**FIGURE 5** (a, b) Sample size-based rarefaction and extrapolation curves showing the macroinvertebrates accumulation according to the number of sampled individuals from the six sites in Kakamega and six sites in Usambara. Rarefaction curves are represented in solid lines, extrapolation curves in dashed lines; shaded areas represent 95% confidence intervals based on a bootstrap method with 200 replications.



**FIGURE 6** (a, b) Observed rank-abundance distributions for the macroinvertebrates samples from Kakamega and Usambara. Kakamega ( $N=367$ ,  $S=22$ ), Kolmogorov-Smirnov test ( $D=0.24$ ,  $p=0.00187$ ). Usambara ( $N=456$ ,  $S=25$ ) and Kolmogorov-Smirnov test ( $D=0.24$ ,  $p=0.000776$ ). The Kakamega community (a) shows dominance by a small number of species with a long 'tail' of rare species, while the Usambara community shows dominance by a small number of species with a long 'tail' of rare species; fewer species of intermediate rarity are also shown (b). The two plots assumed a log series distribution (Fisher et al., 1943).

be associated with variations of major factors influencing macroinvertebrates distribution among them riparian vegetation type, seasonal variability and substrates (Bossley & Smiley Jr, 2019; Duan et al., 2008; Stark & Phillips, 2009). According to Parmesan (2006), the analyses indicated that interactions between environmental and biological factors have the potential to result in the species extinction. Moreover, global warming combined with extreme weather conditions like floods, droughts and heat waves, could cause conditions that are higher than what many species typically experience in their natural habitats (Davis et al., 2005; Parmesan, 2006). Spearman's correlation analysis (Figure 4a) showed that the species richness was positively correlated with TDS, salinity and temperature, while Shannon's diversity index was significantly negatively correlated with conductivity and pH at the Kakamega stations. At the Usambara sites, richness was significantly and negatively correlated with TDS,

salinity, temperature and ASPT. Our study results (Figure 4a) indicated that temperature had a significant negative correlation with pH, at Kakamega and significantly was negatively correlated with conductivity, TDS, salinity and pH at Usambara suggesting its influence on macroinvertebrate species composition (Figure 4b). Our results at Kakamega were peculiar and differed much from those reported in Bird and Day (2016) who reported that physico-chemical gradients did not correlate with species richness.

## 4.2 | Physico-chemical variables

During this study, there were variations in water quality among the selected streams and the sampling stations at Kakamega and the East Usambara. The ecology of aquatic macroinvertebrates



has been regarded as fundamentally influenced by pH (Thomsen & Friberg, 2002; Yuan, 2004). According to a study done by Yuan (2004), pH values below 5 or higher than 9 may pose a threat to macroinvertebrate community composition and abundance. Further, Tamiru (2019) demonstrated that macroinvertebrate diversity, abundance and biomass is reduced by alkaline water. The pH values recorded in this study fall within the acceptable range of natural water and are tied to high diversity of some macroinvertebrate communities (pH 6.5–8.5) based on some standards (Environmental Protection Agency of Ireland [EPA], 2001; Rodier et al., 2009; U.S. Environmental Protection Agency [USEPA], 1980; WHO, 2017). The recorded results show similarity to those of Venkatesharaju et al. (2010) and Minaya et al. (2013) with pH ranges of 6.7–8.3. This could be attributed to increased erosion, agricultural runoffs among other anthropogenic activities along the streams. pH was also slightly higher at Msi S01 and Msi S02 sites as compared to other sites (Hamner et al., 2006; Sharma et al., 2014) with similar results recorded in the Ganges River. These results may be due to domestic activities done at the sites including washing and bathing using detergent resulting in higher alkalinity (Alavaisha et al., 2019).

Variability in mean conductivity measures was recorded during the study with Usambara values higher than those at Kakamega. Conductivity refers to how much current water can conduct and is a result of high concentrations of ions as well as nutrient load; it could also be associated with total dissolved substances (Golterman, 1975; Wetzel, 1983). The conductivity values recorded show that all waters were within the acceptable range for natural water, (Appendix B: Table B1) which ranges from 0.5 to 1500  $\mu\text{S}/\text{cm}$  (Rodier et al., 2009).

According to Golterman (1975) and Singh et al. (2010), TDS acts as a degree of dissolved substances and could be influenced by surface runoffs. TDS could contribute in ecosystem disturbance through pollution, asphyxiation of some gill-breathing organisms by clogging gills and water clarity (Rejsek, 2002; Rodier et al., 2009; Rosewarne et al., 2013; Swinkels et al., 2014).

Records show Ise S02 recorded the lowest mean TDS readings while Muz S01 recorded the highest; this could be connected to the accelerated human activities along the river continuum. All sampling sites within the Kakamega and Usambara streams recorded acceptable levels of TDS for aquatic ecosystem functioning.

The highest mean values of salinity were recorded at Mat S02. According to Mcevoy et al. (2003), salinity is defined as the driver of ecosystem structure; several studies show species decline with salinity levels greater than 1000 mg/L. The levels recorded during the study were quite low and thus can support the biodiversity, a negative correlation between salinity and species diversity at Kakamega was recorded while a negative correlation was observed between species richness and salinity at Usambara. Diversity is largely limited by the ability of each species to tolerate salinity stress, known as halotolerance. A negative correlation between species richness and salinity has been observed in many saline ecosystems around the world (Hammer, 1986; Williams et al., 1990). Salinity levels of 1–3, have been known to exhibit reduced species richness and abundance and a change in species composition in macroinvertebrate assemblages (Boix et al., 2007).

Although only a weak link between global warming and biodiversity has been established, if aquatic macroinvertebrates can be observed for a significant amount of time, it is generally agreed that they will likely show strong responses to future global warming (Burgmer et al., 2007; Hogg & Williams, 1996). Temperature recordings were found to vary across the various points with the mean lowest recorded at Mul S01 and the highest recorded at Muz S02. This was expected due to the degradation of the riparian vegetation and forest cover within the area. Vegetation cover reduces solar radiation, hence decreasing the temperature reaching the water (Eady et al., 2013; Li et al., 2012; Yan et al., 2016).

### 4.3 | Macroinvertebrates' diversity indices and level of sampling effort

According to a study by Soberón and Llorente (1993), Moreno and Halfpeter (2001) and Willott (2001), the number of individuals collected is viewed as a preferable proportion for examining sampling effort than the number of trapping periods given the former accommodates variations in species richness and diversity, trap efficiency, temporal as well as assemblages variations. At Usambara, the flattened extrapolation curve shows it has reached the asymptote (Figure 5b), while the increasing extrapolation (Figure 5a), suggested a high number of rare species in the reference sample and expected growth in identified species (Table 2). Further, a labour-intensive sampling programme will be required to cover the entire diversity of macroinvertebrates at Kakamega to achieve the asymptote. Our study puts into focus the already mentioned usefulness of the rarefaction/extrapolation method that makes possible the precise comparisons of macroinvertebrate diversity in different aquatic ecosystems, with regard to rare species. This study also brings into focus the use of the Hill diversities which only requires minor modifications to the diversity metrics that ecologists are accustomed to, which is capable of estimating the sample coverage while data collection is ongoing, and hence assist in determining which communities require more or less sampling (Rasmussen & Starr, 1979).

### 4.4 | Species abundance distribution (SAD) plots

Ecological disturbance as a concept covers wide range of subjects that should be considered beyond pollution. Among these are habitat loss

TABLE 2 Macroinvertebrates taxa and associated diversity indices were calculated using the iNext package.

Location	# of individuals	S.obs	S.Chao1	se.Chao1
Kakamega	367	22.0	23.99	2.42
Usambara	456	25.0	25.0	1.45

Note: Species diversity was used to calculate the indices.

Abbreviations: S.obs, observed richness; S.Chao1, index of estimated richness; se.Chao1, standard error for Chao1.

and fragmentation that can impact community assemblages. Species abundance distribution (SAD) marks the distribution of abundances of all species within a sample or ecological community. SAD may be taken as a rapid assessment tool (Hill et al., 1995). The SAD has been applied in many activities in monitoring, for example, in the monitoring of fish communities for management of fisheries (Ambak & Mohsin, 1986) and for measuring the fungi communities recovery following managed fire disturbance (Persiani & Maggi, 2013). The performance of the rare species is vital for management and conservation and the analysis of SAD over time allows conservationists to observe rare species abundance changes in relation to the other species in the community. SADs in disturbed communities have been seen to follow distributions close to that of log series. Our study agrees with the findings (Hill et al., 1995) (Figure 6a,b).

#### 4.5 | Macroinvertebrates biodiversity indices and water quality

According to the Berger–Parker index (Table 1), Odonata dominated the two study locations, accounting for more than 50% of the taxa collected followed by Diptera. These two groups are very important with their potential for biomonitoring river/stream health as the fluctuation of the Odonata may signal the decline in water quality, whereas the high number of Diptera could be a result of their ability to inhabit different environments regardless of the level of degradation (Adler & Courtney, 2019). In our study, we can attribute it to human anthropogenic activities.

The presence of the macroinvertebrate communities at degraded sites was marked by either absence of any sensitive taxa (or the presence of few); the dominance of only a few taxa; and larger numbers of pollution-tolerant taxa (Elias et al., 2014; Lyimo, 2012). The highest obtained values of Pielou's evenness index were recorded at Kakamega. The results are in agreement with those by Ojija whose evenness score was ( $J'$  0.82) (Ojija & Kavishe, 2016). We conclude that the waters of the Kakamega and Usambara streams had deteriorated, with similar findings already reported in other studies (Imorou et al., 2019). The Sorensen Similarity Index for all the sites in Kakamega and Usambara returned a value of 1 indicating a comparatively higher similarity in the macroinvertebrates assemblages.

Positive correlations were seen between species richness, diversity and ASPT at Kakamega with the assumption that they were similarly affected by changes in river water quality (Kartikasari, 2013), whereas a negative correlation was recorded between species richness, diversity and ASPT at the Usambara locations (Figure 4b). The macroinvertebrate structure in water courses are sensitive to the changes in the environmental conditions (Dallas, 2007; Yan et al., 2016). A high abundance of tolerant macroinvertebrates and low diversity of sensitive taxa in impacted streams studies have been recorded in several studies (Hall et al., 2001; Walsh et al., 2001). Consequently, the impact on species distribution and changes in ecological processes in the ecosystem comes as a result of alteration of the water quality (Lock et al., 2011; Ollis et al., 2006).

Anthropogenic disturbances are the main contributing factor to water quality deterioration which eventually impacts the macroinvertebrate assemblages (Shimba et al., 2018). Vegetation degradation due to overgrazing, sedimentation from run-offs, water abstraction, other unforeseen conditions (e.g. the absence of a bridge at Muz SO2 where people and vehicles were crossing through the open river) and domestic water usage including washing and bathing with detergents tamper with the macroinvertebrate assemblage by directly affecting the pH and TDS of the stream, eventually interfering with the habitat or physiology of the macroinvertebrates based on the time and quantity of exposure.

Human anthropogenic activities such as irrigation, clear felling and the release of industrial effluents in many aquatic ecosystems may result in altering several factors including stream flow patterns, channel morphology, physico-chemical parameters and changes in community composition resulting into loss of certain species and increases of others (Mwangi, 2014). During the study, it was concluded that increased and uncontrolled activities along the streams were contributing significantly to increased sedimentation due to habitat destruction and unsustainable land-use practices along the two regions and may have been the contributing factor for the poor water quality in the selected streams.

## 5 | CONCLUSIONS

In our study, we examined the natural drivers of macroinvertebrate diversity in the Kakamega and the Usambara regions. Analysis shows the physico-chemical variables had an influence on the overall macroinvertebrate diversity in the two regions. High-intensity human anthropogenic disturbances, such as agricultural runoff, adversely impacted the aquatic ecosystem and affected macroinvertebrate community structure in the Kakamega and the Usambara regions. Thus, the macroinvertebrate community diversity structure in Kakamega and Usambara should be strengthened by improving the ecological environment and controlling environmental pollution (non-point source pollution) in the watershed. Understanding the relative effects of these environmental variables is a necessary step in determining the required activities for proper river/stream management and conservation.

### Recommendations

Watershed land-use intensity is a key component influencing macroinvertebrates community structure. Consequently, future exploration ought to focus on the effect of land use and other anthropogenic stressors on macroinvertebrates or contrast future outcomes with those of this study to investigate the succession of the macroinvertebrate communities assemblages in the Kakamega and the East Usambara region.

## AUTHOR CONTRIBUTIONS

Joseph G. Ndungu and Mary W. Gikungu led the study, Rose M. Marubu, John B. Ochola, Nixon B. Onyimbo, Caroline W. Muriuki Mary W. Gikungu and Joseph G. Ndungu conceptualised, designed and performed this study; Joseph G. Ndungu conducted field studies and analysed data; Joseph G. Ndungu, Mary W. Gikungu, Rose M. Marubu, John B. Ochola, Nixon Onyimbo, Subramanian Sevgan, Caroline W. Muriuki and Kalist E. Komu participated in writing, reviewing and editing the draft article.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

Data are available from the corresponding author upon reasonable request.

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## APPENDIX A

## Photographic illustrations of specimens (highly pollution sensitive, moderately pollution tolerant and pollution tolerant)

## Group 1. Highly pollution-sensitive organisms



## Group 2. Moderately pollution-sensitive organisms



## Group 3. Pollution-tolerant organisms



## APPENDIX B

TABLE B1 Means ( $\pm$ SD), maximum and minimum values of physico-chemical variables recorded at all the sites.

Region	Site	Values	Conductivity ( $\mu$ S)	TDS (mg/L)	Salinity (mg/L)	pH	Temperature ( $^{\circ}$ C)
Kakamega	Mul S01	Minimum	60	53	35	7.2	20
		Mean	80.7 $\pm$ 6.13	57.3 $\pm$ 1.32	46.7 $\pm$ 3.81	7.6 $\pm$ 0.14	20.4 $\pm$ 0.23*
		Maximum	104	61	59	8.0	21.1
	Mul S02	Minimum	50	52	35	6.0	19.6
		Mean	75.3 $\pm$ 5.46*	54.6 $\pm$ 1.92	43.3 $\pm$ 3.43	6.7 $\pm$ 0.17*	20.4 $\pm$ 0.024
		Maximum	90	57	54	7.0	21
	Kis S01	Minimum	114.4	88	73	6.7	20.8
		Mean	154.7 $\pm$ 10.14	107 $\pm$ 5.12	85 $\pm$ 4.07	6.8 $\pm$ 0.06*	22 $\pm$ 0.42
		Maximum	190	121	96	7.0	23
	Kis S02	Minimum	89	78	70	6.7	20
		Mean	143 $\pm$ 13.44	101 $\pm$ 8.53	80.2 $\pm$ 3.38	7 $\pm$ 0.13	21.4 $\pm$ 0.46
		Maximum	173	134	90	7.6	22.6
	Ise S01	Minimum	60	65.3	39	6.8	20
		Mean	70 $\pm$ 3.07	71.6 $\pm$ 1.83	51 $\pm$ 4.27	7.4 $\pm$ 0.28	21 $\pm$ 0.36
		Maximum	80	76.5	67	8.5	22
Ise S02	Minimum	68	40	55	6.8	20.2	
	Mean	89.6 $\pm$ 6.30	52 $\pm$ 3.58	63 $\pm$ 2.91	7.5 $\pm$ 0.23	21 $\pm$ 0.22	
	Maximum	113.3	60	70	8.2	21.8	
East Usambara	Msi S01	Minimum	106	73	65	7.8	25.8
		Mean	132 $\pm$ 7.86	93.8 $\pm$ 8.88	75 $\pm$ 3.68	8.3 $\pm$ 0.17	26.5 $\pm$ 0.74
		Maximum	157	130	89	8.7	27.8
	Msi S02	Minimum	123	89	64	7.8	25.9
		Mean	131.5 $\pm$ 2.89	93.6 $\pm$ 3.50	74.8 $\pm$ 3.76	8 $\pm$ 0.09*	26.6 $\pm$ 0.31
		Maximum	140	110	89	8.4	27.8
	Mat S01	Minimum	312	220	189	7.4	26.9
		Mean	376 $\pm$ 16.42	263 $\pm$ 12.32	206 $\pm$ 7.68	7.8 $\pm$ 0.11*	28.2 $\pm$ 0.32*
		Maximum	422	298	234	8.0	28.8
	Mat S02	Minimum	371	238	253	7.2	20
		Mean	390 $\pm$ 4.88	276 $\pm$ 9.76	282 $\pm$ 8.50	7.6 $\pm$ 0.14	21.5 $\pm$ 0.39
		Maximum	403	298	302	8.0	22.4
	Muz S01	Minimum	468	295	249	7.0	25.9
		Mean	498 $\pm$ 10.31	347 $\pm$ 21.55	276 $\pm$ 9.32	7.6 $\pm$ 0.20	26.6 $\pm$ 0.31
		Maximum	536	412	300	8.2	27.8
Muz S02	Minimum	189	126	85	7.2	28.8	
	Mean	208 $\pm$ 9.32	148 $\pm$ 6.72	116 $\pm$ 12.96	7.6 $\pm$ 0.09*	29.6 $\pm$ 0.27	
	Maximum	242	169	156	7.8	30.5	

Note: Kakamega ( $n=6$ ),  $F$ -statistic value = 20.0363,  $p$ -value = <0.0001, Kruskal–Wallis test  $\chi^2=147.39$ ,  $p<0.001$ ; and The East Usambara ( $n=6$ ),  $F$ -statistic value = 9.8246,  $p$ -value = <0.0001, Kruskal–Wallis test  $\chi^2=143.18$ ,  $p<0.001$ .

\*Significant differences obtained through Tukey's post hoc comparison test ( $p<0.05$ ).