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WATER QUALITY AND MACROINVERTEBRATES ASSESSMENT OF ENIONG CREEK, AKWA IBOM STATE, NIGER DELTA, NIGERIA

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ABSTRACT

The water quality and macroinvertebrates from the downstream section of Eniong Creek were studied for six months, between August 2022 and January 2023 in three sampling stations/ points; to evaluate the ecological integrity and its suitability for habitation of aquatic organisms. Water samples were collected using sterilized plastic bottles and 250 mL reagent bottles for biochemical oxygen demand, and analyzed in accordance with the standard examination methods. Water temperature, dissolved oxygen (DO), transparency (TP), total dissolved solids (TDS), electrical conductivity (EC), and pH were measured *in situ* while water samples were collected for determination of phosphate (PO_4^-), nitrate (NO_3^-), sulphate (SO_4^-), total suspended solids (TSS), and biochemical oxygen demand (BOD_5) in the laboratory. Macroinvertebrates samples were collected using the modified kick sampling technique, and identified using standard keys. The results showed that mean values for pH, DO, BOD_5 in stations I, II, and PO_4^-, TSS in all the stations were deviated from the permissible limits. Three phyla, comprising of six classes of macroinvertebrates were recorded. Phylum Mollusca constituted the highest percentage composition (221 individuals, 87.0 %), while Annelida was the lowest (16 individuals, 6.3 %). Maximum species (115, 45.3 %) was obtained in point III while minimum (62, 24.4 %) was in point II. The study reveals that the economic activities couples with climate changes and seasonal factor influenced the concentrations of certain water parameters and macroinvertebrates assemblages. Thus, the creek should be protected by regulating the level of economic activities within the watershed, in order to improve water quality, maintaining the ecological integrity, and socio-economic services they provide.

Keywords: Water quality, macroinvertebrates, eniong creek, niger delta.

INTRODUCTION

Niger Delta is uniquely bestowed with unique and productive freshwater systems; among them are the Eniong creek and the adjoining river. The creek provides habitats for some aquatic dependent species of plants and animals such as shrimps, insects, reptiles, fishes, planktons and other invertebrate's organisms (Akankali and Idongesit, 2019). Over the years, the system have been experiencing remarkable rates of qualitative and quantitative degradation, closely linked to anthropogenic activities such as lumbering, transportation, land used for agriculture, fishing, indiscriminate dumping of wastes, and other economic activities within the watershed, coupled with the rapid population growth of the area (Akankali and Idongesit, 2019). The interaction of these activities potential could introduce some chemical stressors, ranging from pesticides, metals, fertilizer, petroleum products and many other form

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of pollutants into the water body that prompt physiological stress on the inhabitant biota (Relyea and Hoverman, 2006;Thrush et al., 2008; Wagenhoff et al., 2011; Rasmussen et al., 2012; Liess and Von Der Ohe, 2015).

More so, the effect of one stressor may reduce the fitness of water quality, and potentially reducing the abundant of pollution sensitive organisms (Ephemeroptera, Plecoptera and Trichoptera) while the effect of other stressor may increase the fitness of pollution tolerant taxa such as Tubifex species, Chironomus species, Physa species and Bulinus species (Wagenhoff et al., 2011; Rasmussen et al., 2012; Liess and Von Der Ohe, 2015; Jonah et al., 2022).The biological communities in aquatic ecosystems have been found alone with the physicochemical characteristics as the based approach to check the ecological integrity of aquatic systems (Bere and Nyamupingidza, 2013; Patang et al., 2018; Anyanwu et al., 2019; Aliu et al., 2020; Emana and Bubie, 2021; Jonah et al., 2022).Studies (Santos and Ferreira, 2020; Anyanwu et al., 2021b) reported that the ecological effects of human activities in aquatic ecosystems can be predicted by the assessment of the inhabitant macroinvertebrate species.

Macroinvertebrates provide direct impacts of pollution in aquatic environments, since they lived for a long time in an area, and quickly response to ecological changes of natural habitat, and some are sessile so they give a true representation of the environmental conditions (Bate and George, 2021). Numerous species of macroinvertebrates ranging from annelids, arthropods, coelenterates, mollusks, and chordates are found in diverse water bodies, where they also play a fundamental role in the allotment of nutrients (Mann, 2000). Physical structure of habitats such as sediment substrate, particle size, and flow regime rather than pollutants also determines the community structure of benthic invertebrate fauna (Buss et al., 2004; Petersen and Friberg, 2009). The alteration of optimum physical and chemical parameters of aquatic systems could pose negative impacts on the abundances and distribution of invertebrate fauna which linked to anthropogenic activities (Cheng et al., 2005; Ugwumba and Esenowo, 2020; Jonah et al. 2022). The positioned of market and other economic activities along the downstream sections of the creek could pose negative impacts on the ecosystem. The aim of this study is to report the ecological integrity of Eniong creek, Akwa Ibom State, Nigeria using physicochemical characteristics and macroinvertebrate assemblages based approach.

MATERIAL AND METHODS

Study Area and Sampling Stations

The downstream section of Eniong creek is located between Ibiono and Itu L.G. Area, Akwa Ibom State, Nigeria, within Latitude 5^{o}12’20 North and Longitude 7^{o}58’27 East (Figure 1).The water body drained from Nkana Ikpe in Ini L.G. Area, transverse through Ikpanya community in Ibiono Ibom L.G. Area, Obot Akpabio to Asang Eniong and empties into the main cross river as a confluence (Akankali and Idongesit, 2019). The study area is characterized by a tropical climate change of long wet season between March and October and short dry season (November – February).

The creek is commonly called black water attributed to it colour; receives pollutants from point and non-point sources within the watershed, and the nearby settlements. The anthropogenic activities observed include, road construction, indiscriminate dumping of domestic wastes, farming, selling of food-stuff and transportation of goods. For the purpose of this study, three study points were chosen along the water body, using accessibility, and anthropogenic activities as criteria. The point I was after the

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incomplete bridge, and close to residential settlement with higher anthropogenic activities. Point II was located at the middle, 4 km off station I, and beside the local market and residential settlement with higher anthropogenic activities. Point III is located downstream, 7 km off point II, and very close to the main stream of cross river with minimal human activities.

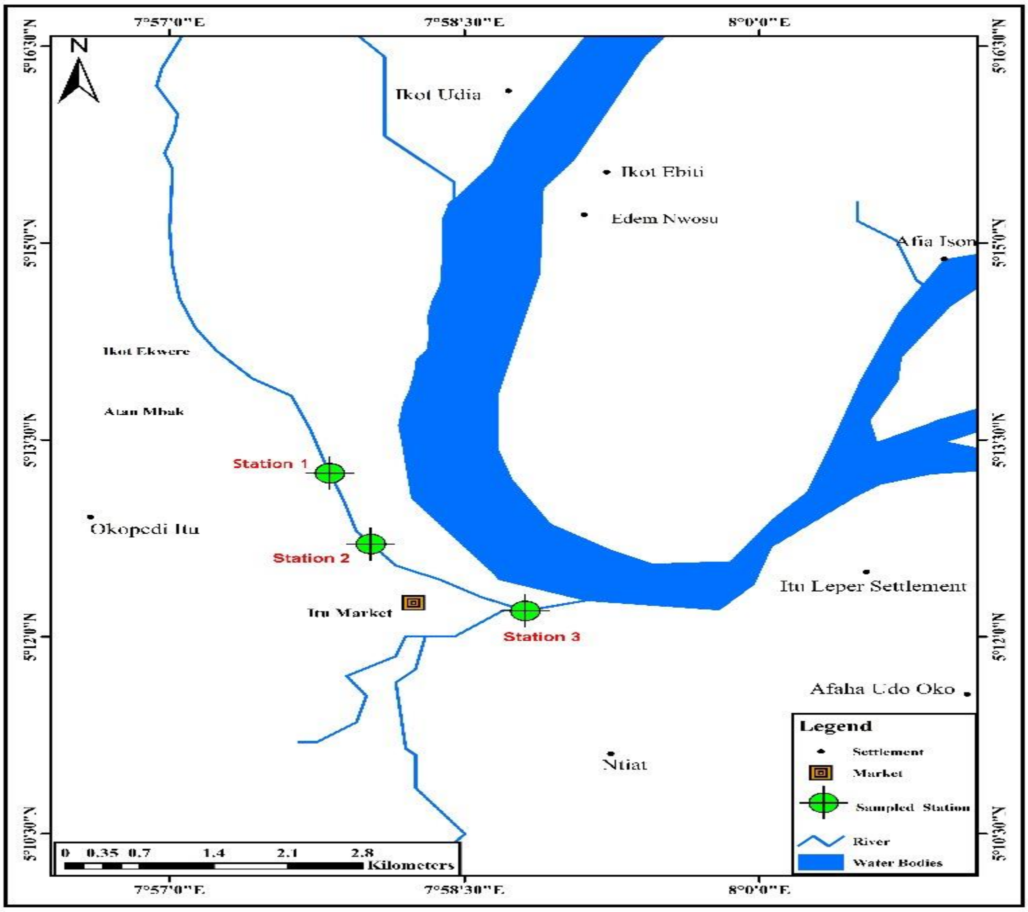

Figure 1: Map of Eniong Creek with Sampling Stations

Samples Collection and Analysis

Samples (water samples and macroinvertebrates) were collected on monthly basis from August 2022 and January 2023.The water samples for physicochemical analysis were collected using sterilized plastic bottles (one litre), for biochemical oxygen demand 250 mL reagent bottles with glass stopper was used. Parameters like Temperature, DO, Transparency (TR), TDS, EC, and pH level were determined at the sampling points while PO_4^-, NO_3^-, SO_4^-, TSS, and BOD_5 were determined *ex-situ* in accordance with APHA (2005) methods.

The macroinvertebrates were collected using the modified kick sampling technique described by Jonah et al., (2020b) and Anyanwu et al., (2021b).The macroinvertebrates at the river banks were swept with hand net. The residues retained on the net were sorted, and stored in a glass bottles, and preserved with 10% formalin, and transferred to the laboratory for proper identification. The identification was made to the genus level using taxonomic keys such as Macan (1959), Merritt et al. (2008), and Umar et al. (2013). Macroinvertebrates species were sorted into different taxonomic groups, counted and recorded based the sampling locations.

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Statistical Analysis

All data were summarized in Microsoft excel for statistically analysis using One-way ANOVA on SPSS- version 20.The source of significant difference at $P < 0.05$ of water parameters among the stations was determined with Tukey pairwise posthoc test. The biotic indices were determines using PAST statistical package (Hammer et al., 2001).

RESULTS

Hydro-Parameters

The average and range values are summarized in Table 1. The pH value ranged between 4.2 and 8.9; the minimum and maximum values were obtained in point III (August / December 2022). The water temperature value obtained across the stations was slightly differed; ranging from 22.5 to 28.3 ^{o}C. The minimum level was obtained in point I (August, 2022) while the maximum were in points II and III (November 2022).

Table1: Average value and standard error of physicochemical parameters from the downstream section of Eniong Creek

Parameters	Point **I** **X±S.E.M**	Point **II** **X±S.E.M**	Point **III** **X±S.E.M**	**Statistical significance**	**Standard limit ****
pH*	$5.63±0.04^{a}$ (4.8 – 6.2)	$5.32±0.08^{a}$ (4.5 – 6.5)	$7.28±0.09^{b}$ (4.2 – 8.9)	$P< 0.05$	6.5 – 8.5
Temp. ^{o}C	25.64±0.34 (22.5 – 27.6)	26.85±0.57 (23.2 – 28.1)	26.13±0.52 (24.0 – 28.3)	$P> 0.05$	Ambient
DO mg/L*	$3.16±0.32^{a}$ (2.24 – 5.61)	$2.88±0.52^{b}$ (2.10 – 4.35)	$5.23±0.19^{c}$ (3.15 – 5.33)	$P< 0.05$	> 6.0
TR (cm)	$41.0±0.23^{a}$ (34 – 78)	$40.0±0.31^{a}$ (35 – 55)	$67.0±0.33^{b}$ (49 – 82)	$P< 0.05$	NI
EC (µS/cm)	$127.65±2.34^{a}$ (88.4 – 164.53)	$126.23±2.14^{a}$ (120.4 – 195.73)	$104.52±2.23^{b}$ (92.8 - 148)	$P< 0.05$	NI
TDS (mg/L)	$63.85±3.43^{a}$ (45.2 – 82.4)	$68.23±2.21^{a}$ (61.2 – 95.9)	$52.6±2.31^{a}$ (45.4 – 75.5)	$P>0.05$	NI
PO_4^- (mg/L)*	$5.16±2.12^{a}$ (3.45 – 6.53)	$5.75±3.43^{a}$ (4.66 – 6.25)	$3.75±2.51^{b}$ (2.13 – 4.51)	$P< 0.05$	< 3.5
NO_3^-(mg/L)	$5.53±0.63^{a}$ (3.42 – 7.25)	$5.26±0.54^{a}$ (3.64 – 9.62)	$3.34±0.34^{b}$ (2.42 – 3.85)	$P< 0.05$	< 9.0
SO_4^-(mg/L)	$65.82±0.42^{a}$ (33.6 – 76.4)	$54.31±0.80^{b}$ (46.2 – 66.2)	$48.42±0.35^{c}$ (25.9 – 56.4)	$P< 0.05$	< 100
TSS (mg/L)*	$18.73±4.44^{a}$ (10.7 – 32.74)	$11.82±5.65^{b}$ (10.4 – 32.5)	$9.55±6.34^{b}$ (6.82 – 25.5)	$P<0.05$	< 0.25
BOD_5(mg/L)*	$4.25±0.56^{a}$ (3.5 – 5.22)	$4.63±0.46^{a}$ (2.32 – 5.18)	$2.12±0.64^{b}$ (2.53 – 4.72)	$P< 0.05$	< 3.0

**Average values either exceeded or below the acceptable limits of NESREA-2011; NI = Not indicated.*

The DO ranged from 2.10 to 5.61 mg/L; the highest was in stations I and III (January, 2023) when compare with station II while the lowest was recorded in stations I (August, 2022) and II (September 2022). ANOVA test showed significant variations ($F = 15.4$; $P < 0.05$) between the mean values. The value of water transparency were from 34to 82 cm; the highest was obtained in point III (January 2023) while the lowest were recorded between August and October 2022 in points I and II. The mean value in station III was statistically ($F = 19.0$; $P < 0.05$) difference from stations I and II. The EC and TDS were on the same trend; the lowest value (88.4 µS/cm) of EC and TDS (45.2 mg/L) were obtained in point I (June 2022) while the maximum values (195.73 µS/cm) and (95.9 mg/L) were recorded in station II (December 2022) respectively. The mean value of EC in points I and II were statistically ($F = 41.35$; $P <0.05$) difference from point III.

The PO_4^- level was from 2.13 to

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6.53 mg/L while NO_3^- was from 2.42 to 7.25 mg/L; minimum level of these parameters were obtained in point III (October 2022 and January 2023) while maximum were in I and II (August /September) respectively. The highest mean value of PO_4^- (5.16 and 5.75 mg/L) and NO_3^-(5.53 and 5.26 mg/L) was obtained in points I and II. ANOVA showed significant variations for PO_4^- in station III (F = 13.8; $P < 0.05$) and NO_3^- in stations I and II (F = 5.53; $P < 0.05$). The mean levels of PO_4^- across the sampling points exceeded the standard limit (3.5 mg/L) while NO_3^- was within the limit (9.0 mg/L).The TSS obtained was from 6.82 to 32.74 mg/L; the minimum was in point III (January, 2023) and maximum in stations I (32.74 mg/L) and 2 (32.5mg/L) in August 2022. The maximum mean level (18.73 mg/L) was obtained in station I while the lowest (9.55 mg/L) was in station III. The mean value recorded in station I was significantly (F = 53.6; $P < 0.05$) higher than others. The BOD values recorded ranged from 2.32 to 5.22 mg/L; the minimum was obtained in point II (January, 2023) while maximum were in stations I (5.22 mg/L) and II (5.18 mg/L) in August and September 2022 respectively. The mean value in stations I and II were significantly (F = 42.3; $P <$ 0.05) difference from station I; the values exceeded the standard limit (3.0 mg/L) except station III.

Table 2: Composition, abundance, distribution and diversity indices of macroinvertebrates species in downstream section of Eniong Creek

Phylum/ Class	Species	Station I	Station II	Station III	Total
Mollusca					
Class: Bivalvia	*Sphaerium corneum*	2	8	3	13
	Anodonta anatine	10	21	35	66
	Anodonta cygnea	9	6	4	19
Class: Gastropoda	*Melanoides tuberculata*	31	-	12	43
	Omphiscola glabra	6	3	5	14
	Pachymelania fusca	7	13	46	66
	Sub-total	**65**	**51**	**105**	**221**
Arthropoda					
Class: Crustacea	*Sudanonautes africanus*	4	-	-	4
Class: Insecta	*Simulium larvae*	3	2	-	5
Class: Malacastraca	*Macrobrachium vollenhoveni*	-	-	8	8
	Sub-total	**7**	**2**	**8**	**17**
Annalida					
Class: Clitellata	*Limnodrilus hoffmeisteri*	5	9	2	16
	Sub-total	**5**	**9**	**2**	**16**
Number of taxa		**9**	**7**	**8**	**10**
Number of individuals		**77**	**62**	**115**	**254**
Percentage composition (%)		**30.3**	**24.4**	**45.3**	**100**
Margalef's index values		**1.842**	**1.454**	**1.475**	
Shannon-wiener index values		**1.569**	**1.722**	**1.852**	
Pielou evenness index values		**0.6**	**0.799**	**0.707**	

Macroinvertebrates Composition

The study recorded a total abundance of 254 macroinvertebrates individual species from the three sampling stations (Table 2). The species recorded were belonging to three phyla, comprising of six classes of macroinvertebrates fauna. The highest species abundance (115 individuals, 45.3 %) was obtained in station III and the least (62, 24.4 %) in station II. Phylum Mollusca constituted the highest percentage composition (221

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individuals, 87.0 %), followed by Arthropoda (17 individuals, 6.7 %) and the least was Annelida (16 individuals, 6.3 %). The percentage composition between the six classes recorded was in the following order: Gastropoda (123 individuals, 48.4 %) > Bivalve (98 individuals, 38.6 %) > Clitellata (16 individuals, 6.3 %) > Malacastraca (8 individuals, 3.2 %) > Insecta (5 individuals, 1.9 %) > Crustacean (4 individuals, 1.3 %).

The class Clitellata (*Limnodrilus hoffmeisteri*), Malacastraca (*Macrobrachium vollenhoveni*), Insecta (*Simulium larvae*), and Crustacean (*Sudanonautes africanus*) were represented by one species each. The species *Pachymelania fusca and Anodonta anatina* were the highest occurring organisms, accounted for 66 (25.9 %) followed by *Melanoides tuberculata* (43, 16.9 %). Higher number of species was recorded in January 2023 (73, 28.74 %), followed by December 2022 (67, 26.38 %), November 2022 (58, 22.83 %), October (24, 9.45 %), August (19, 7.48 %) while the lowest was recorded in September (13, 5.12 %) respectively.

The macroinvertebrate biodiversity indices values are shown in Table 2. The Shannon-Wiener diversity index ranged between 1.569 and 1.852 with highest value in station III while the lowest was in station I. The Margalef's species richness index is also low, ranging between 1.454 and 1.842 with the maximum in point I while the least was in point II. Pielou's evenness index ranged from 0.6 (station I) to 0.799 (station II).

DISCUSSION

The findings revealed that the values of some hydro-parameters were influenced by level of anthropogenic activities and runoffs from the market. The pH values were varied between stations and the months; the extreme low values recorded during the wet month (August) suggest impact of surface runoff, moving more acidic dissolved substance and other pollutants into the water body (Jonah et al., 2020b). The recommended pH value for a healthy aquatic environment is between 6.5 and 8.5 (Shanks et al., 2013).The elevated value for pH in December 2022 in point III could associate with high photosynthesis by aquatic plants and low water levels (Yusuf, 2020). The ranged values recorded are similar with the findings reported previously by Akankeli and Idongesit (2019) in the disturbed sections of water body.

Water temperature is a critical parameter; it directly influenced the solubility of dissolved oxygen, growth and feeding habits of certain biota, reproduction and other biological activities (Halim et al., 2018).The value obtained across the stations was slightly differed among the stations. The intense precipitation in the area could be responsible for the lowest values in August 2022 while the higher value in November 2022 suggest absent of the above-mentioned factor, and possible higher air temperature diffused into the water body. The values recorded were within the optimum range (24 – 30 ^{o}C) set by NESREA (2011).

The elevated values of DO recorded in January 2023 suggest high photosynthetic events by green plants and low actions by aerobic bacteria. The cold harmattan wind which increases wave action during the dry months (December 2022 and January 2023) possible could contribute to the elevated value of DO recorded in January, 2023. The low values recorded in August and September 2022 attributed to flooding, increased surface runoffs, accumulation of organic wastes and low water transparency. The values across the stations were below 6.0 mg/L set as limit by NESREA (2011). Studies (Ukpatu et al., 2018; Jonah et al., 2020a, 2020b, 2020c) avowed that depletion of DO concentrations in water occurs due to the actions by microbes on organic

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pollutant and other materials in the water. During the process, more DO are used by aerobic bacteria to breakdown the organic matters (Mahre et al., 2007) while low transparency impaired photosynthesis by macrophytes which oxygen is released as a by-product. The extreme low means values in stations I and II when compare with III ascribe to combine effects of anthropogenic activities within the watershed (Jonah et al., 2022). The functioning and survival of biological communities may be adversely affected at DO concentrations lower than 5 mg/L while death of most fishes can occur at DO level lower than 2 mg/L (Chapman, 1996; Anyanwu and Mbekee, 2020; Jacob et al., 2023).

The transparency expresses the degree of clarity of water body; aquatic productivity and energy flow in aquatic trophic level are determined by the clarity of water. Higher value obtained in point III (January 2023) attributed to impact of surface runoffs, shifted more suspended materials and non-dissolvable particles from the watershed, land used coupled with the indiscriminate released of solid wastes into the water.

The EC and TDS were on the same trend with the lowest value in point I (June 2022) and the maximum values in station II (December 2022) respectively. The lowest values of these parameters in June attributed to dilution factor due to higher rainfall while the highest values in December suggest absent of the above-mentioned factor, leading to increase in the concentration of dissolved materials in the water (Jonah et al., 2020b). The highest mean value in both parameters recorded in stations I and II indicate moderate pollution from diverse sources, including effluent from domestic activities at the watershed.

The ranged values of PO_4^- and NO_3^- were higher when compare with the values (0.08 - 0.29 mg/L and 1.06 – 1.92 mg/L) reported previously by Akankali and Idongesit (2019). Similarly, Seiyaboh et al. (2017) in Sagbama Creek, Niger Delta Nigeria reported NO_3^- level (0.12 – 0.13 mg/L) lower when compare to the values reported in this study. The highest mean values of these parameters in points I and II suggest discriminate released of pollutants from the market into the system, in addition to occupant of the watershed, coupled with surface runoff and other seasonal factors. The mean levels of PO_4^- across the sampling points exceeded the standard limit (3.5 mg/L) while NO_3^- was within the limit (9.0 mg/L).The elevated values of TSS in August could be attributing to flooding from the terrestrial zone. The maximum mean level in station I suggest to dilution from lower cross river water. All the values were above the standard limit (0.25 mg/L).

The level of BOD is imperative since it does indicate the wellbeing and self-purification status of freshwater bodies (Anyanwu et al., 2021a). The higher value recorded in August and September in stations I and II could link with abundant of organic matter in the water (Jonah et al., 2019). The maximum average value (4.63 mg/L) recorded in point II indicating high level of organic and inorganic pollutants emanating by anthropogenic activities.The values exceeded the standard limit (3.0 mg/L) except station III. High BOD is capable of decreasing DO contents and adversely affecting the aquatic biota (George et al., 2020); according to Al-Sulaiman and Khudair (2018), BOD is a pointer to potential pollution problems.

The macroinvertebrates fauna obtained were varied across the stations. All were pollution tolerant species; capable of surviving in unstable ecosystems (Mariantika and Retnaningdyah, 2014) and become more abundant (Kucuk 2008). The 254 individual species recorded is lower when compare with the 374 species reported by Onyena (2019) in Creeks around Lagos Lagoon, Nigeria. The highest species abundance obtained in station III could be

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attributed to differences in environmental variables. The highest abundant of Mollusca suggest their ability to withstand severe polluted water body. The findings corroborate with the findings reported by Anyanwu and Jerry (2017) and Onyena (2019). The authors recorded dominance of Mollusca in Ikwu River, Umuahia, Southeast and Creeks around Lagos Lagoon, Nigeria respectively.

The abundant of Gastropod and Bivalve can be traced to physiological adaptation to organic pollution, favourable sediment type, and organic debris (decaying organic matters) which serves as food (Adeogun and Fafioye, 2011; Jonah et al., 2020b).The low species of Clitellata, Malacastraca, Insecta, and Crustacean could line to water quality characteristics (Bate and George, 2021). Studies (Edegbene et al., 2015; Jonah et al., 2020a) have proven aquatic macroinvertebrates as bio-indicator of pollution; and species recorded in this study belong to pollution tolerant group, which further expressed the level of perturbation and devastation of the ecosystem. The low species recorded across the points is an indication of imbalance in the ecosystem resulting from instability in the water associated with miscellaneous anthropogenic activities in the water and adjoin environment. The species *Pachymelania fusca and Anodonta anatina* were the highest occurring organisms, accounted for 66 (25.9 %) followed by *Melanoides tuberculata* (43, 16.9 %).However, the abundant of *Pachymelania fusca* (Gastropod) and *Anodonta anatine* (Bivalve) suggest availability of food and environmental preference (Adeogun and Fafioye, 2011; Jonah et al., 2020b).

Higher number of species was recorded in January 2023 (73, 28.74 %), followed by December 2022 (67, 26.38%), November 2022 (58, 22.83 %), October (24, 9.45 %), August (19, 7.48 %) while the lowest was recorded in September (13, 5.12 %) respectively. The temporal trend of species recorded coincides with the finding of Anyanwu et al., (2021b). The authors recorded the abundant of species during the dry months while lowest were recorded in the wet months attributed to seasonal prevailing water conditions. The low species recorded in August and September 2022 suggests high surface runoffs, low water transparency, higher nutrients concentration, low dissolved oxygen concentration and rise in water levels.

The Shannon-Wiener diversity index value was highest in station III while the lowest was in station I, indicating perturbation. The index expressed that values < 1 indicated heavily polluted conditions, 1 to 2 indicated moderate polluted conditions while > 3 indicated stable environmental conditions (Mason, 2002). The Margalef's species richness index values recorded indicates environmental instability. The maximum level in point I when compared with I and II is because the index focuses on the richness and taxonomic composition rather than community abundance (Meng et al., 2020). Pielou's evenness index ranged from 0.6 (station I) to 0.799 (station II) which could link to environmental variability and seasonal factor.

CONCLUSION

The study reveals that the economic activities within the watershed couples with climate changes and seasonal factor influenced the concentrations of pH, DO, PO_4^-, TSS, BOD_5, which further impaired the abundance of macro-invertebrates species. Most macroinvertebrates recorded were pollution tolerant species; the bio-indices showed that the creek is moderately polluted owing to human activities. The diversity and richness of species is influence by the habitat / substrate heterogeneity. Thus, the creek should be protected by regulating the level of economic / human activities within the watershed in order to improve the water

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quality, maintaining the ecological integrity and socio-economic services they provide.

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CONFLICT OF INTEREST

The authors declare that there is no any conflict of interests regarding the publication of this manuscript. In addition, plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy have been completely observed by the authors.

AUTHORS CONTRIBUTION

UEJ designed the study; UEJ / IKE collected samples and analyzed the data while interpretation the results were done by UEJ and UIE. Writing, reading and approving the final manuscript was by all authors.

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